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ORIGINAL ARTICLE

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

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

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

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Introduction

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

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

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

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

Patients and methods

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

Patient selection

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

Study design and treatments

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

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

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

Assessments

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

Safety and tolerability

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

Pharmacokinetic study design and analytical studies

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

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

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

Pharmacokinetic analyses

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

Results

Patient characteristics and disposition

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

Escalation, DLT, and MTD

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

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

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

Safety and tolerability

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

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

Table 1 Demographic characteristics

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

H2 antagonists for all subsequent cycles with no further incidents.

Efficacy

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

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

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

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

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

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

Pharmacokinetics

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

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

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

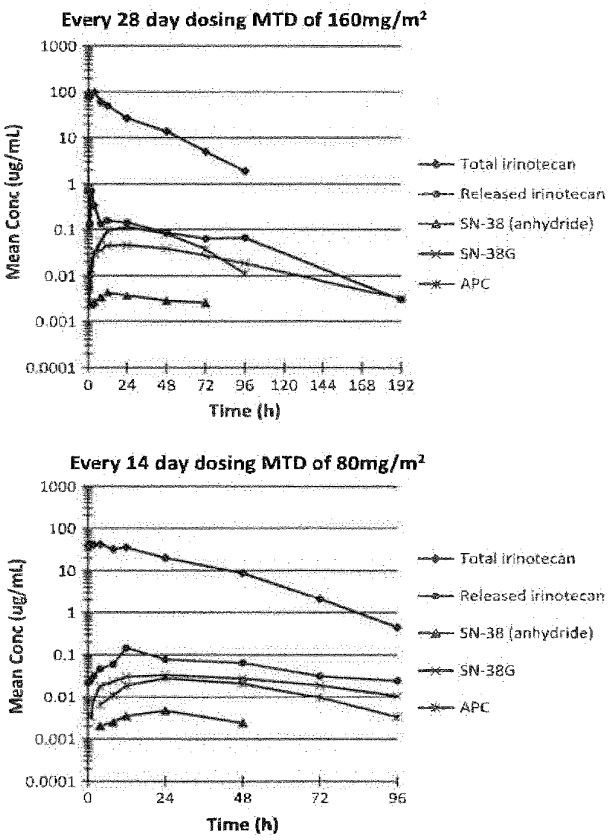

Fig. 1 Mean concentration versus time plot total irinotecan, released irinotecan, SN-38, and SN-38G at MTD for 28-day dosing (160 mg/m^2) and 14-day dosing (80 mg/m^2). NPC concentrations in plasma were below the lower limit of detection

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

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

NPC concentrations in plasma were below the lower limit of detection

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

Discussion

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

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

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

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

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

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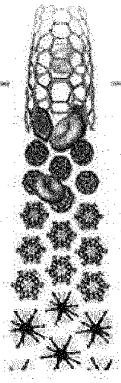

Lipid-based nanoformulation of irinotecan: dual mechanism of action allows for combination chemo/angiogenic therapy

A number of studies have outlined the antiangiogenic effects of cytotoxic agents when administered frequently at low doses. These studies suggest that the effect of the cytotoxic agent is on the vasculature within the tumor and it is assumed that there is little or negligible cytotoxicity. Liposomal drug delivery systems have the ability to provide a dual mechanism of activity where tumor accumulation can deliver high local concentrations of the drug at the site of action with concomitant slow release of the drug from carriers in the blood compartment that results in antivascular effects, similar to that achieved when dosing frequently at low levels. Although this dual mechanism of activity may be linked to other lipid nanoparticle formulations of anticancer drugs, this article summarizes the evidence supporting direct (cytotoxic) and indirect (antivascular) actions of a liposomal formulation of irinotecan.

KEYWORDS: antiangiogenic therapy cytotoxic therapy dual mechanism irinotecan liposome metronomic dosing nanoformulation vascular normalization

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Cancer, as with other life-threatening diseases, is influenced by multiple molecular mechanisms as well as host microenvironmental factors. Tumors typically have a high degree of heterogeneity and their growth is based on enhanced survival capacity, the ability to resist apoptosis and the ability to proliferate endlessly in the absence of growth signals or in the presence of antigrowth signals [1]. Heterogeneity increases the number and the diversity of cellular targets, while also being reflected in the multiple and diverse signaling pathways within each given cancer cell, hence there is a need for combining cytotoxics, cytostatics and biological agents to provide optimal treatment responses. Multimodality therapeutic approaches must also exploit the balance between efficacy and toxicity. There are several strategies that can address tumor heterogeneity [2]: the classic approach of combining agents that have proven to be active as single agents [3]; the development of drug combination products [4]; and the identification of drugs and/or therapeutic targets that exhibit pleiotropic mechanisms of action [5,6]. Our group has an interest in identifying strategies that target both the proliferating tumor cells as well as the tumor-associated blood vessels [7,8].

Angiogenesis is the growth of new blood vessels from pre-existing blood vessels in response to VEGF and angiopoietin family members [9]. Tumor blood vessels are structurally and functionally abnormal, a result of excessive endothelial cell proliferation and a lack of supporting structural elements brought about by an imbalance in pro- and anti-angiogenic factors. This leads to tortuous, dilated and saccular blood vessels with increased resistance to blood flow as well as an irregular blood supply [10]. Angiogenesis is a key factor necessary for tumor growth beyond microscopic size and also contributes to tumor cell stress, such as transient hypoxia. In addition, the erratic nature of blood flow contributes to poor drug distribution in tumors. Therefore, one type of strategy for treating cancer, involving the use of drugs targeting angiogenesis, is a strategy designed to limit tumor growth. An unexpected outcome of this treatment strategy, however, was tumor vascular normalization.

Normalization of blood vessels refers to the elimination of excess endothelial cells and immature and inefficient blood vessels; in essence, correcting the disorganized vasculature brought on by rapid angiogenesis within the tumor. Tumor vessel normalization may occur following anti-VEGF therapy, such as bevacizumab, and has also been reported for other therapeutics, including trastuzumab [11] and low dose continuous chemotherapy, and we have also recently demonstrated this using various liposomal chemotherapeutics [12]. Jain has postulated that this normalized vasculature would result in enhanced chemotherapeutic delivery, a consequence that would primarily impact the delivery/distribution of small molecular weight drugs [10]. What is interesting is how this effect would influence delivery of nanoscaled drug delivery systems, which localize in sites of tumor

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growth due to the enhanced permeability and retention (EPR) effect. In fact, a recent study has shown that vascular normalization actually contributes to decreases in the EPR effect and associated decreases in lipid nanoparticle delivery to tumors [13].

A number of drug delivery strategies targeting tumors or specific cell targets within tumors have been evaluated. These strategies have largely focused on delivering more drug to sites where they can exert their effect on tumor cell populations and decreased delivery to sites of toxicity. In recognition of the role of the tumor microenvironment, the normal cells and associated stromal elements that surround the tumor, efforts have shifted to targeting nontumor cells such as epithelial cells, fibroblasts, immune cells, and endothelial cells. An ideal therapeutic strategy would include targeting of both the tumor cells and some element of stroma. This article will focus on liposomal delivery systems that are designed to achieve therapeutic effects by virtue of both antivascular as well as antitumor mechanism of activity.

Targeting tumor vasculature

Antiangiogenic therapy, or targeting of the vasculature within tumors, was first proposed as a treatment in 1971 by Dr Judah Folkman [14] and has now grown to a major focal area in cancer therapy. Bevacizumab is a humanized monoclonal antibody that binds and inactivates all VEGF isoforms in circulation [15] thereby inhibiting binding to the VEGF receptors. In 2004, Bevacizumab became the first approved antiantiogenic therapy in combination with intravenous 5-fluorouracil/oxaliplatin in the first-line treatment of colorectal cancer. Subsequently, bevacizumab has also been approved in combination with carboplatin and paclitaxel in first-line treatment of non-small-cell lung cancer, and with IFN-α in metastatic kidney cancer. There are numerous studies examining combinations with other agents, including irinotecan in which the efficacy of irinotecan has been shown to be enhanced by bevacizumab addition [16,17]. There are a number of experimental antiangiogenic agents in various stages of clinical development, as listed in TABLE 1, including both antibodies and small molecules.

Metronomic dosing

Antiangiogenic therapy is a critically important area of research, yet it is important to note that these agents are not being developed as standalone therapeutics, but are currently being used or tested in combination with traditional chemotherapeutic agents, which may in turn have their own intrinsic antiangiogenic properties when administered continuously at low doses or as a low dose on a repeat basis. This metronomic dosing was pioneered by Robert Kerbel and refers to chemotherapy given at frequent intervals and at low doses with no prolonged drug-free breaks [18]. The doses are low enough to reduce toxic side effects [19], and are thought to damage the newly forming endothelial cells within tumors. It is believed that this effect on tumor-associated endothelial cells occurs at doses below those required to exert cytotoxic/cytostatic effects on proliferating tumor cells. Regardless, the pruning of new blood vessels within the tumor exerts antitumor effects and eventually the remaining blood vessels within the tumor have a structure that is more closely associated with normal blood vessels. The advantages to this type of therapy lie in the continued exposure of the endothelial cells to the chemotherapeutic agent rather than short bursts of high drug concentration, known to cause toxic effects.

Studies of metronomic dosing have been conducted on a number of different drugs, including temozolomide [20], gemcitabine [21] and topotecan [22]; however, early efforts were focused on cyclophosphamide, which has been postulated to induce apoptosis in tumor endothelial cells leading to the collapse of angiogenic vessels and ultimate suppression of tumor growth. Clinical studies of daily oral cyclophosphamide in combination with low-dose methotrexate for treatment of metastatic breast cancer have shown promising results [23,24]. Of note, each of these drugs are administered orally and therefore lend themselves well to metronomic scheduling. This type of scheduling, however, is quite challenging when using chemotherapeutics not amenable to oral dosing or extended continuous infusions.

More recently, studies of metronomic dosing have included irinotecan in a number of indications including glioma [25] and colorectal cancer [26,27]. In the clinical setting, these studies have required the use of continuous irinotecan infusion [27] by implantation of a central venous catheter with a programmable pump that was refilled weekly. Allegrini *et al.* [27] demonstrated in their clinical study that the angiogenesis associated thrombospondin-1 (TSP-1) was markedly increased in response to metronomic irinotecan (in this group, irinotecan was dosed at 1.4 mg/m^2/day, representing a 75% dose reduction over earlier identified infusion dose levels as published by Herben *et al.* [28]). At the tested doses, the regimens were not toxic and efficacy

Table 1. Experimental antiangiogenic agents.

Category	Inhibitor	Mechanism of action	Ref.
Antibody	Volociximab	Chimeric anti-$\alpha_5\beta_1$ that blocks $\alpha_5\beta_1$ binding to fibronectin and causes endothelial cell apoptosis	[49]
Antibody	IM-1C11	Chimeric anti-KDR that binds the VEGF receptor KDR, therefore preventing VEGF binding and subsequent endothelial cell proliferation	[50]
Antibody	Etaracizumab	Humanized monoclonal antibody targeting the $\alpha_v\beta_3$ integrin that inhibits the adhesive interations of endothelial cells	[51]
Small molecule	Cilengitide	Targets the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_1$	[52]
Small molecule	Sorafenib	Multikinase inhibitor (VEGF receptor-2, VEGF receptor-3, PDGF receptor); currently indicated for treatment of hepatocellular and renal cell carcinoma	[53–56]
Small molecule	Sunitinib	Multikinase inhibitor that targets VEGFR1, VEGFR2, VEGFR3, PDGF receptor, fms-like tyrosine kinase 3, c-kit and rearranged during transfection (RET)	[57–59]
Small molecule	Cediranib	Selective inhibitor of the VEGF pathway	[60–62]
Small molecule	Pazopanib	Multikinase inhibitor that blocks VEGF receptor-1, -2, -3, PDGF receptor and cytokine receptor	[63,64]
Small molecule	Vandetanib	Blocks both the VEGF receptor and EGF receptor pathways as well as inhibiting the RET receptor tyrosine kinase activity	[65,66]

results were similar to those of other schedules of third/fourth-line treatment in patients with metastatic colorectal cancer (typically <10%). While results were promising in this study in terms of effects on vasculature as measured by TSP-1 and VEGF levels, efficacy was not improved over existing options.

Allegrini's group has since continued research into metronomic dosing of irinotecan and is now looking at combination with semaxinib, an experimental inhibitor of the Flk-1/KDR VEGF receptor tyrosine kinase. A study by Bocci *et al.* demonstrated that metronomic dosing of irinotecan with semaxinib not only decreased colorectal cancer xenograft tumor vessel density and modulated both VEGF and TSP-1 expression, but also significantly inhibited tumor growth [29]. This has also been demonstrated with irinotecan alone in the U87 xenograft model of glioblastoma by Takano *et al.*, in which tumors were grown subcutaneously and irinotecan was administered intraperitoneally daily at a dose of 1 or 4 mg/kg for 21 days from the day of tumor cell inoculation [25]. Tumor growth was significantly inhibited in a dose-dependent manner without evidence of toxicity using this schedule, and both VEGF and hypoxia inducible factor (HIF)-1α were significantly reduced.

Lipid-based delivery systems

Liposomes are spherical structures made up of phospholipids and cholesterol. The lipids spontaneously adopt bilayer structures that are separated by aqueous channels. Following hydration of dried lipids with a selected buffer, large (>1 micron) structures are formed with multiple lipid bilayers and these structures need to be processed further to generate unilamellar structures that are small (50–200 nm) and encompass a central aqueous core. Therapeutic agents can be encapsulated in the hydrophobic (partitioning in the lipid bilayer) as well as hydrophilic (encapsulation in the aqueous core) region of the liposome and this makes them very versatile as drug carriers. Passively targeted liposomal drugs (i.e., relying on the pharmacokinetic properties of the carrier only) often achieve vast increases in delivery of the associated drug to sites of disease, such as cancer, when compared with administration of free drug due to a phenomenon known as the EPR effect. The EPR effect is due to the leaky vasculature present within tumors, which allows for the accumulation of macromolecules with a diameter of less than 600 nm [30,31]. Since tumors often lack draining lymphatics, the regionally localized liposomes are retained at the site for extended periods of time. The ability of lipid-based formulations to localize in sites of tumor growth via the EPR effect is enhanced when that lipid-based formulation is designed to exhibit extended circulation lifetimes.

Liposomes and other delivery systems, such as micelles or block copolymers, lend themselves well to functionalization, or modification of the surface with targeting ligands [32,33], antibodies (enhanced targeting) [34] or polyethylene glycol (prevention of aggregation) [35]. As indicated above, however, liposomes are able to passively accumulate in tumor tissue by virtue of the leaky vasculature found in tumors, even without such modifications. Those liposomal systems with surface modifications may be taken into cells, for example by receptor-mediated endocytosis. Alternatively, encapsulated drug may be released

in close proximity to target cells with subsequent cellular uptake, or there may be fusion of liposomes with cellular membranes, with release of liposomal contents into the cytosol. The mechanism of liposome-assisted drug delivery is very dependent on composition. Most formulations advanced toward clinical testing are simple ones (no associated targeting ligands), where drug release from the liposome is required for the drug to access target cells. There are many lipid-based drug delivery systems currently in clinical testing, and a number of clinically approved products as indicated in TABLE 2. This highlights the utility of this technology as a clinically advanced approach to achieve improved therapeutic effects.

Combined modality therapy: targeting tumor cells & vasculature

The combination of antiangiogenic agents and traditional chemotherapy offers the ability to target both the tumor cells and vascular components of tumors, and in many instances, use of the antiangiogenic drug prior to chemotherapy has been shown to enhance the efficacy of chemotherapy due to vascular normalization achieved by the antiangiogenic drug [36]. However, given the demonstrated successes of metronomic dosing, one could envision development of drug carrier formulations of drugs known to exhibit antiangiogenic effects when given metronomically. This was postulated by Ng *et al.* [37] and tested in studies recently published from our laboratory [9]. More specifically, the use of metronomic dosing of irinotecan in the aforementioned glioma research [25] offered a tantalizing glimpse at a single therapy that could be both antiangiogenic as well as cytotoxic. In this paper, Takano *et al.* utilized nonformulated irinotecan at either 1 or 4 mg/kg daily intraperitoneal injection over 21 days and compared this regimen to a more conventional dosing schedule using 10 or 40 mg/kg. The results indicated that while conventional dosing did result in inhibition of glioma growth, it was associated with systemic toxicity. Treatment with the metronomic regimen resulted in both inhibition of tumor growth without toxicity and additionally inhibited angiogenesis.

The principle of metronomic dosing lies in the maintenance of drug levels in plasma over time with no prolonged drug-free periods, as mentioned above. This is also achieved when using appropriately designed liposomal drugs; formulations that slowly release drug from the carrier, which is retained in the vascular compartment over extended time periods. The extended circulation lifetime is needed to achieve enhanced tumor drug delivery by the EPR effect. Importantly, it is well established that drug within the carrier is slowly released over time, a release process that occurs in the tumor compartment as well as the blood compartment. A major benefit to the use of liposomal formulations, when compared with metronomic dosing, is the removal of the need for dosing on a frequent basis. The extended circulation life of drugs administered in liposomal form has been shown to result in marked improvements in efficacy in xenograft tumor models for vincristine [38,39], doxorubicin [40–42], irinotecan [43,44] and other cytotoxic compounds [45,46]. It is thus curious as to whether some of the therapeutic benefits achieved through use of these formulations are due to antiangiogenic mechanisms, in addition to direct cytotoxic effects on the tumor cells.

This has indeed been shown to be the case in a newly developed liposomal formulation of irinotecan (FIGURE 1), Irinophore C™, currently in late preclinical development. Irinophore C is exemplary of the benefit in circulation longevity

Table 2. Approved and investigational liposomal anticancer drugs.

Product name	Drug	Indication	Ref.
DaunoXome®	Daunorubicin	HIV-related Kaposi's sarcoma	[67]
Myocet®	Doxorubicin	Combination therapy with cyclophosphamide in metastatic breast cancer	[68]
Doxil®/Caelyx®	Doxorubicin	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer	[68]
CPX-351	Cytarabine:daunorubicin	Acute myeloid leukemia and first relapse acute myeloid leukemia (currently in Phase II trial)	[69]
CPX-1	Irinotecan HCl:floxuridine	Colorectal cancer (completed Phase II testing)	[70]
Marqibo®	Vincristine sulfate	Acute lymphoblastic leukemia and melanoma (ongoing pivotal clinical trials)	[71]
NanoVNB®	Vinorelbine	Vinorelbine responsive malignancies (completed Phase I testing)	[72]

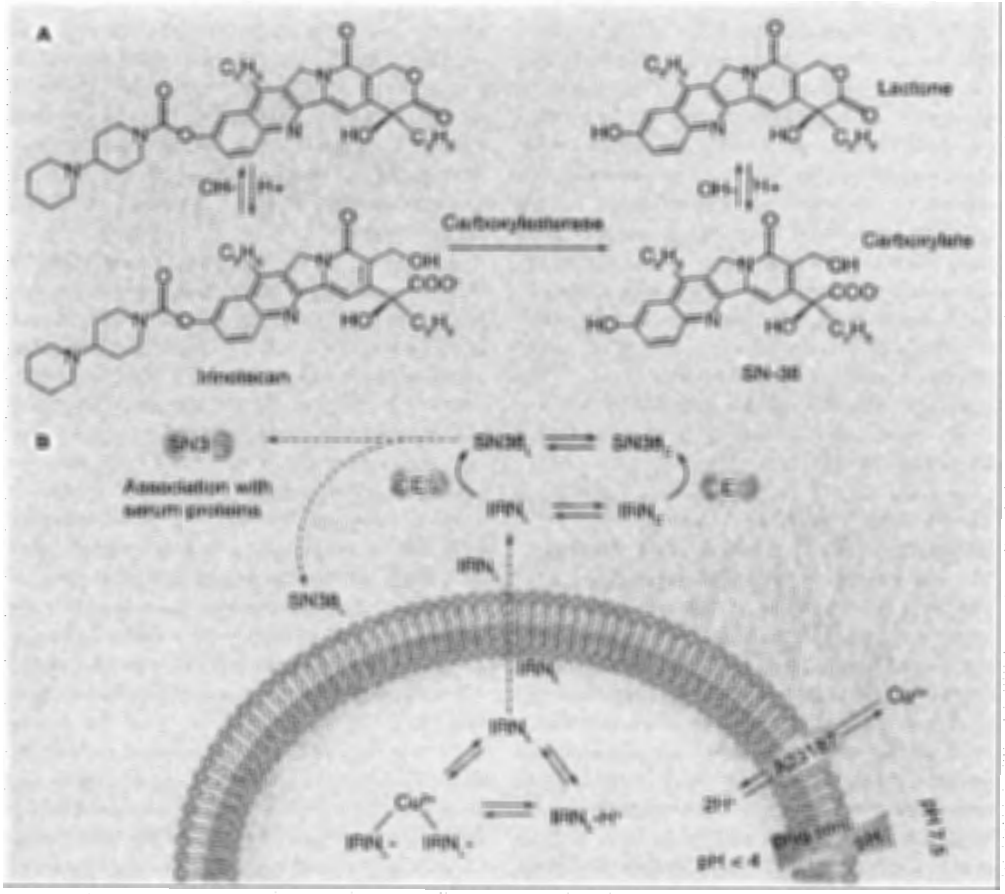

Figure 1. Liposomal encapsulation of irinotecan. (A) IRN and SN38 lactone and carboxylate conformations: conversion of the lactone to carboxylate form is favored at physiological pH; irinotecan conversion to SN38 is mediated by CES. **(B)** Representation of Irinophore C™ drug release and conversion: due to the low pH inside the liposomes, IRN is maintained in its lactone form (IRN_L). In addition to interacting with copper present in the formulation, it is anticipated that IRN_L may also interact with the inner and outer leaflet of the liposomal membrane, an interaction that is required for release of IRN_L. Once released, IRN_L is hydrolyzed through a pH-dependent reversible process to its carboxylate form (IRN_C). IRN is metabolized to SN38 by CES present in the liver (hCE1), the GI tract (hiCE) and tumor macrophages. The lactone form of SN38 ($SN38_L$) can also be hydrolyzed to its carboxylate form ($SN38_C$). It is possible that a fraction of $SN38_L$ may interact with the lipid carrier membrane or serum proteins; interactions that may decrease the rate of $SN38_L$ hydrolysis to $SN38_C$. CES: Carboxylesterase; IRN: Irinotecan.

afforded a drug by virtue of liposomal encapsulation in that the plasma area-under-the curve is improved 1000-fold following intravenous administration in mice [44]. Importantly, the carrier maintains irinotecan in the active lactone conformation [47] in contrast to administration of free irinotecan where the majority of drug is converted to the inactive carboxylate form at physiological pH. In addition, levels of the more active metabolite SN-38 (lactone) are increased up to 30-fold when irinotecan is administered in the Irinophore C formulation [44]. This is shown diagrammatically in Figure 2, in which the active form of the drug may be seen crossing from the blood vessel into the tumor interstitium.

In order to gain better understanding of the mechanism of action of antitumor activity of Irinophore C, the treatment-induced effects on the tumor microenvironment in a xenograft model of colorectal cancer were examined [6]. In this study, mice were treated once per week for 3 or 6 weeks with Irinophore C. Multimodality imaging techniques were used to assess hypoxia, cell density by Hoechst 33342 staining, K_{trans} (the volume transfer constant of a solute between the blood vessels and extracellular tissue compartment of the tumor), labeling of endothelial

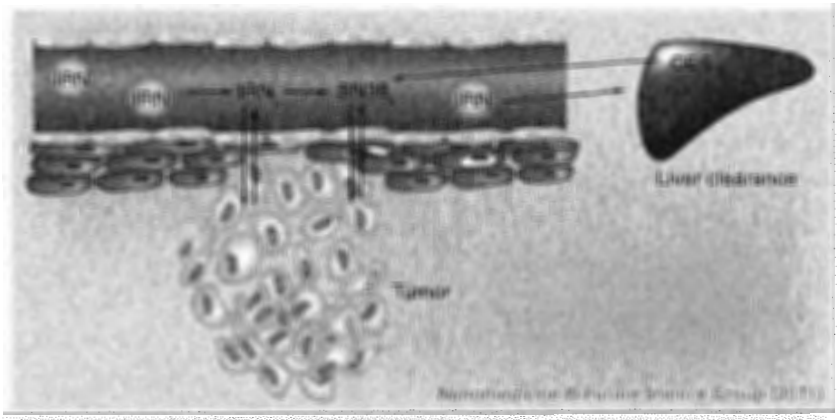

Figure 2. Representation of Irinophore C™ drug release and interaction with biological and tumor compartments. Following intravenous injections, it is known that appropriately designed lipid carriers are retained in the plasma compartment for extended periods compared with the free drug. Inevitably, a significant portion of drug-loaded carriers will accumulate in the liver and the spleen. This process will result in elimination of the carrier and associated drug from the plasma, while also contributing to irinotecan metabolism to SN38 via carboxylesterases of the liver and cells of the mononuclear-phagocytic system.
CES: Carboxylesterase; IRN: Irinotecan.

cells by CD31 staining and accumulation of second drug. It was shown that treatment, even at doses lower than that previously defined as an efficacious dose, resulted in inhibition of tumor growth. Noninvasive MRI revealed a decrease in K_{trans}, while cryosection staining showed higher perfusion of Irinophore C-treated tumors. Paradoxically, this study also demonstrated that tumor sections from Irinophore C-treated mice had a lower percentage of CD31-positive cells. This result, when combined with the finding that tumor hypoxia was decreased overall, led to the conclusion that Irinophore C given on a weekly schedule was acting comparably to metronomic drug dosing. Normalization of tumor vasculature occurred at the same time that the active drug component of the formulation was inhibiting the growth of tumor cells. To confirm the antiangiogenic mechanism of action, promoters and inhibitors of angiogenesis were assessed and it was shown that Irinophore C treatment caused downregulation of proangiogenic factors VEGF, IL-8 and HIF-1α while the antiangiogenic TIMP-1 and TSP-1 were upregulated (FIGURE 3) [6].

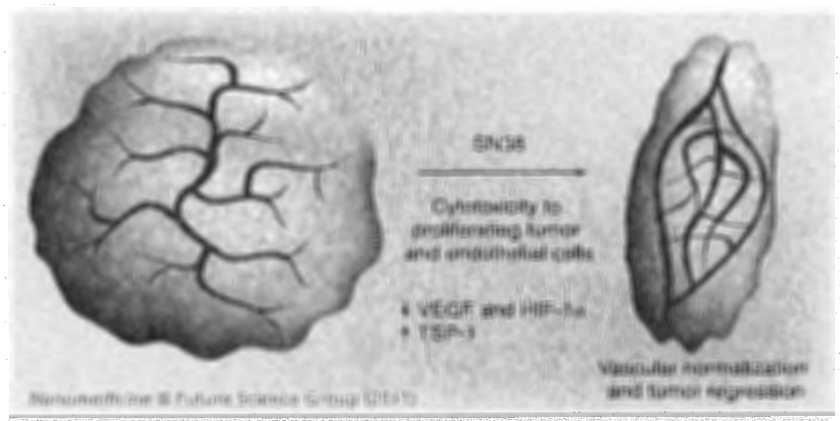

Figure 3. Tumor vasculature normalization. Extended exposure to SN38 provided by Irinophore C™ induces vascular normalization by a direct cytotoxicity on proliferating endothelial cells [12], and indirectly by triggering a reduction in VEGF and HIF-1α proangiogenic factors, and an increase in TSP-1 antiangiogenic factors [6]. Tumor vascular normalization may alter tumor metabolism and eventually increase tumor cell proliferation.
TSP-1: Thrombospondin.

A critical requirement of chemotherapy is the ability of a drug to reach the target tissue. In many instances, the tortuous nature of tumor vasculature prevents the optimal tissue distribution of systemically administered drug. Following vessel normalization, however, it is reasonable to assume that drug uptake in tumor tissue would be increased. This was indeed shown to be the case for mice pretreated with Irinophore C, in that approximately 1.5-fold higher levels of 5-fluorouracil and 2.7-fold higher levels of doxorubicin were found in tumor tissue as compared with saline pretreated controls [6].

Further validation of the concept of dual mechanism of action for liposomal formulations of cytotoxic drugs, specifically for Irinophore C, was provided by Verreault *et al.* [12]. This study demonstrated vascular normalization effects in an orthotopic glioblastoma model following intravenous administration of liposomal irinotecan (Irinophore C), doxorubicin (Caelyx®) or liposomal vincristine. This study assessed both efficacy and vascular function in stereotactically implanted glioblastoma cells in immunodeficient mice. Irinophore C treatment resulted in tumors with blood vessels that were morphologically more mature. In the subcutaneous model, Irinophore C restored the basement membrane architecture, increased the pericyte coverage and reduced blood vessel diameters, suggestive of a restoration of vessel architecture to a more normal state. In the more clinically relevant orthotopic model, Irinophore C treatment restored the basement membrane architecture and reduced the blood vessel diameters of the tumor vasculature, again suggesting a restoration of the vessel architecture to a more normal state. Irinophore C also increased the quantity of vessel staining in the center of tumors, suggesting a more homogenous distribution of blood across the entire tumor. Furthermore, the drop in K_{trans} values in the glioma model was interpreted as a decrease in vessel permeability consistent with the suggestion that Irinophore C treatment improved vascular

function in the tumor [12]. While a drop in K_{trans} is associated with decreased vessel permeability, we note that the tumor vasculature was more functional following Irinophore C treatment, which is of importance in glioma as only 25% (tumor center) – 75% (tumor periphery) of vasculature is typically functional in this tumor type [48]. The more functional blood vessels should improve the ability of injected compounds to extravasate at the tumor site, provided these compounds can normally cross the blood–brain barrier.

Future perspective

The results of these recent papers suggest that it is possible to recognize the benefits of combination therapy with a single, carefully designed therapeutic; one that targets the tumor cells directly with cytotoxic action and tumor-associated vascular endothelial cells for an antiangiogenic mechanism of action. Cancer therapy research has traditionally been conducted in 'silos' where one team researches cytotoxic effects and others research antiangiogenic effects or other microenvironment effects (for example). It is critically important to think outside of these silos and consider the multiple or combination therapeutic effects that may in fact be present when using a single therapeutic, and that perhaps this should be best considered in the context of rationally designed nanocarriers, such as liposomal or polymer-based carriers.

Financial & competing interests disclosure

The Irinophore C™ technology has been licensed from the BC Cancer Agency to Champions Oncology Inc. and as contributors to this work, some of the authors stand to benefit from future royalty payments that flow back to the BC Cancer Agency (DN Waterhouse, M Anantha and MB Bally). Work on Irinophore C has been supported by the Canadian Institutes of Health Research, the Terry Fox Research Institute, the Centre for Drug Research and Development, the National Cancer Institute of Canada, and the Cancer Research Society. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Targeting tumor vasculature

- Antiangiogenic therapy is of growing importance in treatment strategies for a number of cancer types targeting blood vessels within tumors. This can result in several outcomes:
  - Selective killing of tumor endothelial cells and subsequent starvation of the tumor.
  - Inhibition of proangiogenic factors, such as VEGF and HIF-1α.
  - Normalization of tumor vasculature and enhanced delivery of small molecular weight cytotoxic agents.
- Antiangiogenic therapies are typically paired with conventional chemotherapeutics in combination regimens due to lack of single agent activity.

Metronomic dosing

- Many traditional cytotoxic drugs can be administered in a metronomic fashion (multiple low doses) and act mechanistically as antiangiogenic agents.

Lipid-based delivery systems

- It is possible to mimic metronomic dosing by utilization of nanoformulations, such as liposomes, which have extended drug payout times, therefore reducing/eliminating the need for repeated dose administration.

Combined modality therapy: targeting tumor cells & vasculature

- Rationally designed nanoformulations of conventional chemotherapeutics are able to exert dual mechanisms of action: antiangiogenic effects exerted on the endothelial cells as well as direct cytotoxic effects exerted on the tumor cells.
- A liposomal irinotecan formulation has been shown to have multimechanistic activity:
  - Cytotoxic activity against tumor cells with demonstrated efficacy in a wide range of animal xenograft models.
  - Normalization of tumor vasculature.
  - Enhanced uptake of subsequently administered small molecular weight chemotherapeutic agent.

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

CAMPTOSAR (Irinotecan) Injection, intravenous infusion
Initial U.S. Approval: 1996

WARNING: DIARRHEA and MYELOSUPPRESSION
See full prescribing information for complete boxed warning.

- **Early and late forms of diarrhea can occur. Early diarrhea may be accompanied by cholinergic symptoms which may be prevented or ameliorated by atropine. Late diarrhea can be life threatening and should be treated promptly with loperamide. Monitor patients with diarrhea and give fluid and electrolytes as needed. Institute antibiotic therapy if patients develop ileus, fever, or severe neutropenia. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs.**
- **Severe myelosuppression may occur.**

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

CAMPTOSAR is a topoisomerase inhibitor indicated for:

- First-line therapy in combination with 5-fluorouracil and leucovorin for patients with metastatic carcinoma of the colon or rectum. (1)
- Patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy. (1)

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

- Colorectal cancer combination regimen 1: CAMPTOSAR 125 mg/m^2 intravenous infusion over 90 minutes on days 1, 8,15, 22 with LV 20 mg/m^2 intravenous bolus infusion on days 1, 8, 15, 22 followed by 5-FU intravenous bolus infusion on days 1, 8, 15, 22 every 6 weeks. (2.1)
- Colorectal cancer combination regimen 2: CAMPTOSAR 180 mg/m^2 intravenous infusion over 90 minutes on days 1, 15, 29 with LV 200 mg/m^2 intravenous infusion over 2 hours on days 1, 2, 15, 16, 29, 20 followed by 5-FU 400 mg/m^2 intravenous bolus infusion on days 1, 2, 15, 16, 29, 30 and 5-FU 600 mg/m^2 intravenous infusion over 22 hours on days 1, 2, 15, 16, 29, 30. (2.1)
- Colorectal cancer single agent regimen 1: CAMPTOSAR 125 mg/m^2 intravenous infusion over 90 minutes on days 1, 8, 15, 22 then 2-week rest. (2.2)
- Colorectal cancer single agent regimen 2: CAMPTOSAR 350 mg/m^2 intravenous infusion over 90 minutes on day 1 every 3 weeks. (2.2)

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

CAMPTOSAR Injection is available in three single-dose sizes:

- 2 mL-fill vial containing 40 mg irinotecan hydrochloride injection
- 5 mL-fill vial containing 100 mg irinotecan hydrochloride injection
- 15 mL-fill vial containing 300 mg irinotecan hydrochloride injection

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

- Hypersensitivity to CAMPTOSAR or its excipients (4)

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

- **Diarrhea and cholinergic reactions:** Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is usually transient and may be accompanied by cholinergic symptoms. Consider prophylactic or therapeutic administration of 0.25 mg to 1 mg of intravenous or subcutaneous atropine (unless clinically contraindicated). Late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR) can occur. Monitor and replace fluid and electrolytes. Treat with loperamide. Use antibiotic support for ileus and fever. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs. (5.1)
- **Myelosuppression:** Manage promptly with antibiotic support. Interrupt CAMPTOSAR and reduce subsequent doses if necessary. (5.2)
- **Patients with Reduced UGT1A1 Activity:** Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of CAMPTOSAR treatment. (5.3)
- **Hypersensitivity:** Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed. Discontinue CAMPTOSAR if this occurs. (5.4)
- **Renal Impairment/Renal Failure:** Rare cases of renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea. (5.5)
- **Pulmonary Toxicity:** Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have occurred. Interrupt for new or progressive dysnpnea, cough, and fever pending evaluation. If IPD diagnosed, discontinue and institute appropriate treatment as needed. (5.6)
- **Toxicity of the 5 Day Regimen:** CAMPTOSAR should not be used in combination with a regimen of 5-FU/LV administered for 4-5 consecutive days every 4 weeks outside of a clinical study. (5.7)
- **Pregnancy:** CAMPTOSAR can cause fetal harm when administered to a pregnant woman. (5.9)
- **Hepatic Impairment:** In clinical trials, CAMPTOSAR has not been administered to patients with serum bilirubin > 2.0 mg/dL, or transaminases > 3 times ULN if no liver metastases, or transaminases > 5 times ULN if liver metastases. With the weekly dosage schedule, patients with total bilirubin levels 1.0-2.0 mg/dL had greater likelihood of grade 3-4 neutropenia. (5.10)

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

Common adverse reactions (≥30%) observed in combination therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, mucositis, neutropenia, leukopenia (including lymphocytopenia), anemia, thrombocytopenia, asthenia, pain, fever, infection, abnormal bilirubin, alopecia. (6.1)

Common adverse reactions (≥30%) observed in single agent therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, alopecia. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc at 1-800-438-1985 or *www.pfizer.com* or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

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

- Strong CYP3A4 Inducers: Do not administer for at least 2 weeks prior to initiation of irinotecan therapy. (7.2)
- Strong CYP3A4 Inhibitors: Discontinue at least 1 week prior to starting irinotecan therapy and do not use during irinotecan therapy. (7.3)

----------USE IN SPECIFIC POPULATIONS----------

- **Nursing Mothers:** Discontinue nursing when receiving therapy with CAMPTOSAR. (8.3)
- **Geriatric Use:** Closely monitor patients greater than 65 years of age because of a greater risk of early and late diarrhea in this population. (8.5)
- **Patients with Renal Impairment**: Use caution and do not use in patients on dialysis. (8.6)
- **Patients with Hepatic Impairment**: Use caution. (2.1, 5.10, 8.7, 12.3)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 07/2012

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: DIARRHEA AND MYELOSUPPRESSION

- **Early and late forms of diarrhea can occur. Early diarrhea may be accompanied by cholinergic symptoms which may be prevented or ameliorated by atropine. Late diarrhea can be life threatening and should be treated promptly with loperamide. Monitor patients with diarrhea and give fluid and electrolytes as needed. Institute antibiotic therapy if patients develop ileus, fever, or severe neutropenia. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs.**
- **Severe myelosuppression may occur.**

1 INDICATIONS AND USAGE

- CAMPTOSAR Injection is indicated as a component of first-line therapy in combination with 5-fluorouracil (5-FU) and leucovorin (LV) for patients with metastatic carcinoma of the colon or rectum.

- CAMPTOSAR is indicated for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Colorectal Cancer Combination Regimens 1 and 2

Administer CAMPTOSAR as a 90-minute intravenous infusion followed by LV and 5-FU. The currently recommended regimens are shown in Table 1.

A reduction in the starting dose by one dose level of CAMPTOSAR may be considered for patients with any of the following conditions: prior pelvic/abdominal radiotherapy, performance status of 2, or increased bilirubin levels. Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients.

Table 1. Combination-Agent Dosage Regimens and Dose Modifications[a]

Regimen 1 6-wk cycle with bolus 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU	125 mg/m^2 intravenous infusion over 90 minutes, days 1,8,15,22 20 mg/m^2 intravenous injection bolus, days 1,8,15,22 500 mg/m^2 intravenous injection bolus, days 1,8,15,22		
		Starting Dose & Modified Dose Levels (mg/m^2)		
		Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR	125	100	75
	LV	20	20	20
	5-FU	500	400	300
Regimen 2 6-wk cycle with infusional 5-FU/LV (next cycle begins on day 43)	CAMPTOSAR LV 5-FU Bolus 5-FU Infusion[b]	180 mg/m^2 intravenous infusion over 90 minutes, days 1,15,29 200 mg/m^2 intravenous infusion over 2 hours, days 1,2,15,16,29,30 400 mg/m^2 intravenous injection bolus, days 1,2,15,16,29,30 600 mg/m^2 intravenous infusion over 22 hours, days 1,2,15,16,29,30		
		Starting Dose & Modified Dose Levels (mg/m^2)		
		Starting Dose	Dose Level -1	Dose Level -2
	CAMPTOSAR	180	150	120
	LV	200	200	200
	5-FU Bolus	400	320	240
	5-FU Infusion[b]	600	480	360

[a]Dose reductions beyond Dose Level 2 by decrements of ≈ 20% may be warranted for patients continuing to experience toxicity. Provided intolerable toxicity does not develop, treatment with additional cycles may be continued indefinitely as long as patients continue to experience clinical benefit.
[b]Infusion follows bolus administration.

Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients *[see Warnings and Precautions (5.10), Use in Specific Populations (8.7) and Clinical Pharmacology (12.3)]*.

Dose Modifications

Based on recommended dose levels described in Table 1, Combination Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 2, Recommended Dose Modifications for Combination Regimens. All dose modifications should be based on the worst preceding toxicity.

Table 2. 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 ↓ 2 dose levels	↓ 1 dose level
4 (≥10 stools/day > pretx)		↓ 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

2.2 Colorectal Single Agent Regimens 1 and 2

Administer CAMPTOSAR as a 90-minute intravenous infusion. The currently recommended regimens are shown in Table 3.

A reduction in the starting dose by one dose level of CAMPTOSAR may be considered for patients with any of the following conditions: prior pelvic/abdominal radiotherapy, performance status of 2, or increased bilirubin levels. Dosing for patients with bilirubin >2 mg/dL cannot be recommended because there is insufficient information to recommend a dose in these patients.

Table 3. Single-Agent Regimens of CAMPTOSAR and Dose Modifications

Regimen 1 (weekly)[a]	125 mg/m^2 intravenous infusion over 90 minutes, days 1,8,15,22 then 2-week rest		
	Starting Dose and Modified Dose Levels[c] (mg/m^2)		
	Starting Dose	Dose Level -1	Dose Level -2
	125	100	75
Regimen 2 (every 3 weeks)[b]	350 mg/m^2 intravenous infusion over 90 minutes, once every 3 weeks[c]		
	Starting Dose and 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.

Dose Modifications

Based on recommended dose-levels described in Table 3, Single-Agent Regimens of CAMPTOSAR and Dose Modifications, subsequent doses should be adjusted as suggested in Table 4, Recommended Dose Modifications for Single-Agent Schedules. All dose modifications should be based on the worst preceding toxicity.

Table 4: 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
	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
2 (1000 to 1499/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3 (500 to 999/mm^3)		↓ 50 mg/m^2	↓ 50 mg/m^2
4 (<500/mm^3)	Omit dose until resolved to ≤ grade 2, then ↓ 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
	↓ 25 mg/m^2	Maintain dose level	Maintain dose level
2 (4-6 stools/day > pretx)	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3 (7-9 stools/day > pretx)		↓ 50 mg/m^2	↓ 50 mg/m^2
4 (≥10 stools/day > pretx)	Omit dose until resolved to ≤ grade 2 then ↓ 50 mg/m^2		
Other nonhematologic[d] toxicities			
1	Maintain dose level	Maintain dose level	Maintain dose level
	↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
2	Omit dose until resolved to ≤ grade 2, then ↓ 25 mg/m^2	↓ 25 mg/m^2	↓ 50 mg/m^2
3		↓ 50 mg/m^2	↓ 50 mg/m^2
4	Omit dose until resolved to ≤ grade 2, then ↓ 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

2.3 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 Dosage and Administration (2.1 and 2.2) and Warnings and Precautions (5.3)]*. 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 1-4).

2.4 Premedication

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. A similar antiemetic regimen should be used with Camptosar in combination therapy.

Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms.

2.5 Preparation of Infusion Solution

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

CAMPTOSAR Injection 20 mg/mL is intended for single use only and any unused portion should be discarded.

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 mg/mL to 2.8 mg/mL. Other drugs should not be added to the infusion solution.

The solution is physically and chemically stable for up to 24 hours at room temperature and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C, 36° to 46°F), 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.

The CAMPTOSAR Injection solution should be used immediately after reconstitution as it contains no antibacterial preservative. 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 4 hours if kept at room temperature. If reconstitution and dilution are performed under strict aseptic conditions (e.g. on Laminar Air Flow bench), CAMPTOSAR

Injection solution should be used (infusion completed) within 12 hours at room temperature or 24 hours if refrigerated (2° to 8°C, 36° to 46°F).

2.6 Safe Handling

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.

2.7 Extravasation

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.

3 DOSAGE FORMS AND STRENGTHS

CAMPTOSAR Injection is available in three single-dose sizes:

- 2 mL-fill vial containing 40 mg irinotecan hydrochloride
- 5 mL-fill vial containing 100 mg irinotecan hydrochloride
- 15 mL-fill vial containing 300 mg irinotecan hydrochloride

4 CONTRAINDICATIONS

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

5 WARNINGS AND PRECAUTIONS

5.1 Diarrhea and Cholinergic Reactions

Early diarrhea (occurring during or shortly after infusion of CAMPTOSAR) is usually transient and infrequently severe. It may be accompanied by cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Bradycardia may also occur. Early diarrhea and other cholinergic symptoms may be prevented or treated. Consider prophylactic or therapeutic administration of 0.25 mg to 1 mg of intravenous or subcutaneous atropine (unless clinically contraindicated). These symptoms are expected to occur more frequently with higher irinotecan doses.

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. Grade 3-4 late diarrhea occurred in 23-31% of

patients receiving weekly dosing. In the clinical studies, the median time to the onset of late diarrhea was 5 days with 3-week dosing and 11 days with weekly dosing. Late diarrhea can be complicated by colitis, ulceration, bleeding, ileus, obstruction, and infection. Cases of megacolon and intestinal perforation have been reported. Patients should have loperamide readily available to begin treatment for late diarrhea. Begin loperamide at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normal. One dosage regimen for loperamide is 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. Monitor and replace fluid and electrolytes. Use antibiotic support for ileus, fever, or severe neutropenia. Subsequent weekly chemotherapy treatments should be delayed in patients until return of pretreatment bowel function for at least 24 hours without anti-diarrhea medication. Patients must not be treated with irinotecan until resolution of the bowel obstruction. If grade 2, 3, or 4 late diarrhea recurs, subsequent doses of CAMPTOSAR should be decreased *[see Dosage and Administration (2)]*.

Avoid diuretics or laxatives in patients with diarrhea.

5.2 Myelosuppression

Deaths due to sepsis following severe neutropenia have been reported in patients treated with CAMPTOSAR. 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. Manage febrile neutropenia promptly with antibiotic support *[see Warnings and Precautions (5.2)]*. Hold CAMPTOSAR if neutropenic fever occurs or if the absolute neutrophil count drops $<1000/mm^3$. After recovery to an absolute neutrophil count $\geq 1000/mm^3$, subsequent doses of CAMPTOSAR should be reduced *[see Dosage and Administration (2)]*.

When evaluated in the trials of weekly administration, the frequency of grade 3 and 4 neutropenia was 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). Patients who have previously received pelvic/abdominal irradiation are at increased risk of severe myelosuppression following the administration of CAMPTOSAR. Based on sparse available data, the concurrent administration of CAMPTOSAR with irradiation is not recommended.

Patients with baseline serum total bilirubin levels of 1.0 mg/dL or more also had a 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). 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.

5.3 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 (2)]*.

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.

5. 4 Hypersensitivity

Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed. Discontinue CAMPTOSAR if anaphylactic reaction occurs.

5. 5 Renal Impairment/Renal Failure

Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea.

5. 6 Pulmonary Toxicity

Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have occurred in patients receiving irinotecan (in combination and as monotherapy). Risk factors 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. In Japanese studies, a reticulonodular pattern on chest x-ray was observed in a small percentage of patients. New or progressive, dyspnea, cough, and fever should prompt interruption of chemotherapy, pending diagnostic evaluation. If IPD is diagnosed, irinotecan and other chemotherapy should be discontinued and appropriate treatment instituted as needed *[see Adverse Reactions (6.1)]*.

5.7 Toxicity of the 5 Day Regimen

Outside of a well-designed clinical study, CAMPTOSAR Injection should not be used in combination with a regimen of 5-FU/LV administered for 4-5 consecutive days every 4 weeks because of reports of increased toxicity, including toxic deaths. CAMPTOSAR should be used as recommended in Table 2 *[see Dosage and Administration (2)]*.

5.8 Increased Toxicity in Patients with Performance Status 2

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.

5.9 Pregnancy

CAMPTOSAR can cause fetal harm when administered to a pregnant woman. Irinotecan was embryotoxic in rats and rabbits at doses significantly lower than those administered to humans on a mg/m^2 basis. In rats, at exposures approximately 0.2 times those achieved in humans at the 125 mg/m^2 dose, irinotecan was embryotoxic and resulted in decreased learning ability and female fetal body weight in surviving pups; the drug was teratogenic at lower exposures (approximately 0.025 times the AUC in humans at the 125 mg/m^2 dose).There are no adequate and well-controlled studies of irinotecan in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with CAMPTOSAR.

5.10 Patients with Hepatic Impairment

The use of CAMPTOSAR in patients with significant hepatic impairment 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) *[see Dosage and Administration (2.1), Use in Specific Populations (8.7) and Clinical Pharmacology (12.3)]*.

6 ADVERSE REACTIONS

6.1 Clinical Studies Experience

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 clinical practice.
Common adverse reactions (≥30%) observed in combination therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, mucositis, neutropenia, leukopenia (including lymphocytopenia), anemia, thrombocytopenia, asthenia, pain, fever, infection, abnormal bilirubin, and alopecia.

Common adverse reactions (≥30%) observed in single agent therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, and alopecia.

Serious opportunistic infections have not been observed, and no complications have specifically been attributed to lymphocytopenia.

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 Dosage and Administration (2)]*.

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

Table 5. 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 every 6 weeks N=225		Bolus 5-FU/LV daily x 5 every 4 weeks N=219		Irinotecan weekly x 4 every 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	84.9	22.7	69.4	13.2	83.0	31.0
late	--	15.1	--	5.9	--	18.4
grade 3	--	7.6	--	7.3	--	12.6
grade 4	45.8	4.9	31.5	1.4	43.0	6.7
early						
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	0.9	0	3.2	0.5	0	0
Exfoliative dermatitis	19.1	0	26.5	0.9	14.3	0.4
Rash	43.1	--	26.5	--	46.1	--
Alopecia[b]						
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	9.3	0.9	5.0	0	9.0	0
Vasodilatation	5.8	1.3	2.3	0.5	5.8	1.7
Hypotension	9.3	--	11.4	--	5.4	--
Thromboembolic events[c]						

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

Adverse Event	Study 2			
	Irinotecan + 5-FU/LV infusional days 1&2 every 2 weeks N= 145		5-FU/LV infusional days 1&2 every 2 weeks N=143	
	Grades 1-4	Grades 3&4	Grades 1-4	Grades 3&4
TOTAL Adverse Events	100	72.4	100	39.2
GASTROINTESTINAL				
Diarrhea	72.4	14.4	44.8	6.3
late	--	10.3	--	4.2
grade 3	--	4.1	--	2.1
grade 4	28.3	1.4	0.7	0
Cholinergic syndrome[b]	66.9	2.1	55.2	3.5
Nausea	17.2	2.1	16.8	0.7
Abdominal pain	44.8	3.5	32.2	2.8
Vomiting	35.2	2.1	18.9	0.7
Anorexia	30.3	0.7	25.2	1.4
Constipation	40.0	4.1	28.7	2.8
Mucositis				
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 AND NUTRITIONAL				
Bilirubin	19.1	3.5	35.9	10.6
DERMATOLOGIC	10.3	0.7	12.6	0.7
Hand and foot syndrome	17.2	0.7	20.3	0
Cutaneous signs	56.6	--	16.8	--
Alopecia[c]				
RESPIRATORY				
Dyspnea	9.7	1.4	4.9	0

CARDIOVASCULAR	3.4	1.4	0.7	0
Hypotension	11.7	--	5.6	--
Thromboembolic events[d]				

[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. One of the 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 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%).

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 7 are based on the experience of the 304 patients enrolled in the three studies described in *CLINICAL STUDIES (14.1)*.

Table 7. 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 AND 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

Table 7. Adverse Events Occurring in >10% of 304 Previously Treated Patients with Metastatic Carcinoma of the Colon or Rectum[a]

[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 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 8 lists the grade 3 and 4 adverse events reported in the patients enrolled to all treatment arms of the two studies described in *CLINICAL STUDIES (14.1)*.

Table 8: 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 AND NUTRITIONAL				
Hepatic [c]	9	7	9	6
DERMATOLOGIC				
Hand and 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

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.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post approval use of CAMPTOSAR. 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.

Myocardial ischemic events have been observed following irinotecan therapy. Thromboembolic events have been observed in patients receiving CAMPTOSAR.

Symptomatic pancreatitis, asymptomatic pancreatic enzyme elevation have been reported. Increases in serum levels of transaminases (i.e., AST and ALT) in the absence of progressive liver metastasis have been observed.

Hyponatremia, mostly with diarrhea and vomiting, has 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.

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.

7 DRUG INTERACTIONS

7.1 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 (2)]*. 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.

7.2 Strong CYP 3A4 Inducers

Anticonvulsants and other strong inducers: 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 or other strong inducers such as rifampin and rifabutin has not been defined. Consideration should be given to substituting non-enzyme inducing therapies at least 2 weeks prior to initiation of irinotecan therapy.

St. John's wort: 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.

Dexamethasone, a moderate CYP3A4 inducer, does not appear to alter the pharmacokinetics of irinotecan.

7.3 Strong CYP 3A4 Inhibitors

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.

7.4 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.

7.5 Drug-Laboratory Test Interactions

There are no known interactions between CAMPTOSAR and laboratory tests.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D *[see Warnings and Precautions (5.9)]*

CAMPTOSAR can 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). Intravenous administration of irinotecan 6 mg/kg/day to rats and rabbits during the period of organogenesis resulted in increased post-implantation loss and decreased numbers of live fetuses. In separate studies in rats, this dose produced an irinotecan C_{max} and AUC of about 2 and 0.2 times, respectively, the corresponding values in patients administered 125 mg/m^2. In rabbits, the embryotoxic dose was about one-half the recommended human weekly starting dose on a mg/m^2 basis. Irinotecan was teratogenic in rats at doses greater than 1.2 mg/kg/day and in rabbits at 6.0 mg/kg/day. In separate studies in rats, this dose 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. In rabbits, the teratogenic dose was 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 this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with CAMPTOSAR.

8.3 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. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from CAMPTOSAR, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of

the drug to the mother.

8.4 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].

8.5 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 (12.3)* and *Adverse Reactions (6.1)]*. 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 Clinical Pharmacology (12.3) and Dosage and Administration (2)]*.

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].

8.6 Renal Impairment

The influence of renal impairment on the pharmacokinetics of irinotecan has not been

evaluated. Therefore, use caution in patients with impaired renal function. Irinotecan is not recommended for use in patients on dialysis.

8.7 Hepatic Impairment

Irinotecan clearance is diminished in patients with hepatic impairment 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. Therefore, use caution in patients with hepatic impairment. 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 (2.1), Warnings and Precautions (5.10) and Clinical Pharmacology (12.3)]*.

10 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.

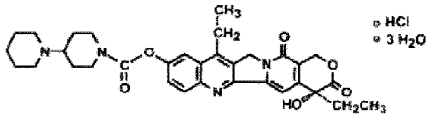
11 DESCRIPTION

CAMPTOSAR Injection (irinotecan hydrochloride injection) is an antineoplastic agent of the topoisomerase I inhibitor class.

CAMPTOSAR is supplied as a sterile, pale yellow, clear, aqueous solution. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol, NF, 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 empirical formula is $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$ and molecular weight is 677.19. It is slightly soluble in water and organic solvents. Its structural formula is as follows:



Irinotecan Hydrochloride

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

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.

12.2 Pharmacodynamics

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 Clinical Pharmacology (12.3)]*. 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.

12.3 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 9:

Table 9. 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.

Distribution

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

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 Precautions (5.3) and Dosage and Administration (2)]*. SN-38

glucuronide had 1/50 to 1/100 the activity of SN-38 in cytotoxicity assays using two cell lines *in vitro*.

Excretion

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).

Effect of Age

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 $AUC_{0\text{-}24}$ 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 (2)]*.

Effect of Gender

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

Effect of Race

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

Effect of Hepatic Impairment

Irinotecan clearance is diminished in patients with hepatic impairment 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 (2.1), Warnings and Precautions (5.10) and Use in Specific Populations (8.7)]*.

Effect of Renal Impairment

The influence of renal impairment 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 *[see Use in Specific Populations (8.6)]*.

13 NONCLINICAL TOXICOLOGY

13.1 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. Irinotecan was clastogenic both *in vitro* (chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (micronucleus test in mice). Neither irinotecan nor its active metabolite SN-38 was mutagenic in the *in vitro* Ames assay.

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 and in dogs at 0.4 mg/kg. In separate studies in rodents, this dose produced an irinotecan C_{max} and AUC about 5 and 1 times, respectively, of the corresponding values in patients administered 125 mg/m^2 weekly. In dogs this dose produced an irinotecan C_{max} and AUC about one-half and 1/15th, respectively, of the corresponding values in patients administered 125 mg/m^2 weekly.

14 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 (2)]*. 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 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.

14.1 Metastatic Colorectal Cancer

First Line Therapy in Combination with 5-FU/LV: Studies 1 and 2

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 10.

Table 10. Combination Dosage Schedule: Study Results

	Study 1			Study 2	
	Irinotecan + Bolus 5-FU/LV weekly x 4 every 6 weeks	Bolus 5-FU/LV daily x 5 every 4 weeks	Irinotecan weekly x 4 every 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

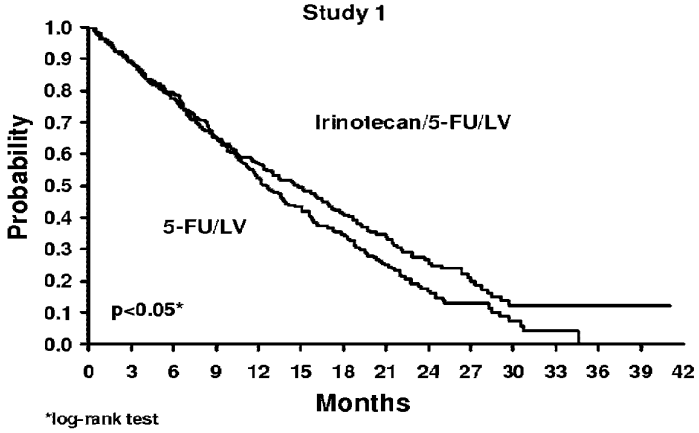
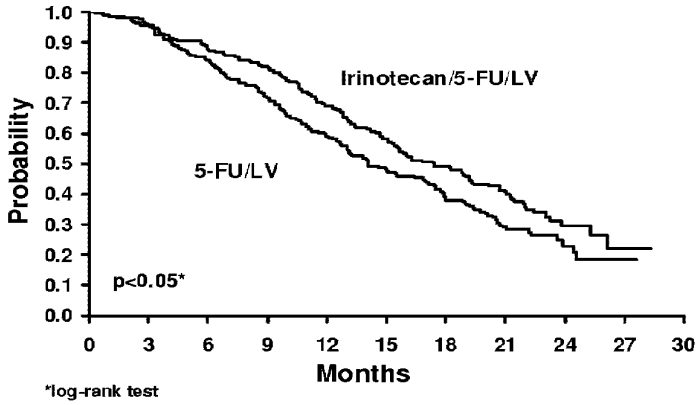


Figure 2. Survival
First-Line Irinotecan/5-FU/LV vs 5-FU/LV
Study 2



Second-Line Therapy After 5-FU-Based Treatment

4 Weekly Doses on a 6-Week Cycle: Studies 3, 4, and 5

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 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 high rates of grade 4 late diarrhea and febrile neutropenia). Study 3 enrolled 48 patients and was conducted by a single investigator at several regional hospitals. Study 4 was a multicenter

study conducted by the North Central Cancer Treatment Group. All 90 patients enrolled in Study 4 received a starting dose of 125 mg/m^2. Study 5 was a multicenter study that enrolled 166 patients from 30 institutions. The initial dose in Study 5 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 11.

Table 11. Weekly Dosage Schedule: Study Results

	Study			
	3	4	5	
Number of Patients	48	90	64	102
Starting Dose (mg/m^2/week 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

Table 11. Weekly Dosage Schedule: Study Results

[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 Study: Study 6

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 Studies: Studies 7 and 8

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 Study 7, second-line irinotecan therapy plus best supportive care was compared with best supportive care alone. In Study 8, 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 7 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 Study 8 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 7, median survival for patients treated with irinotecan was 9.2 months compared with 6.5 months for patients receiving best supportive care. In Study 8, 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 7 and p=0.017 for Study 8). 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 7, 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 7

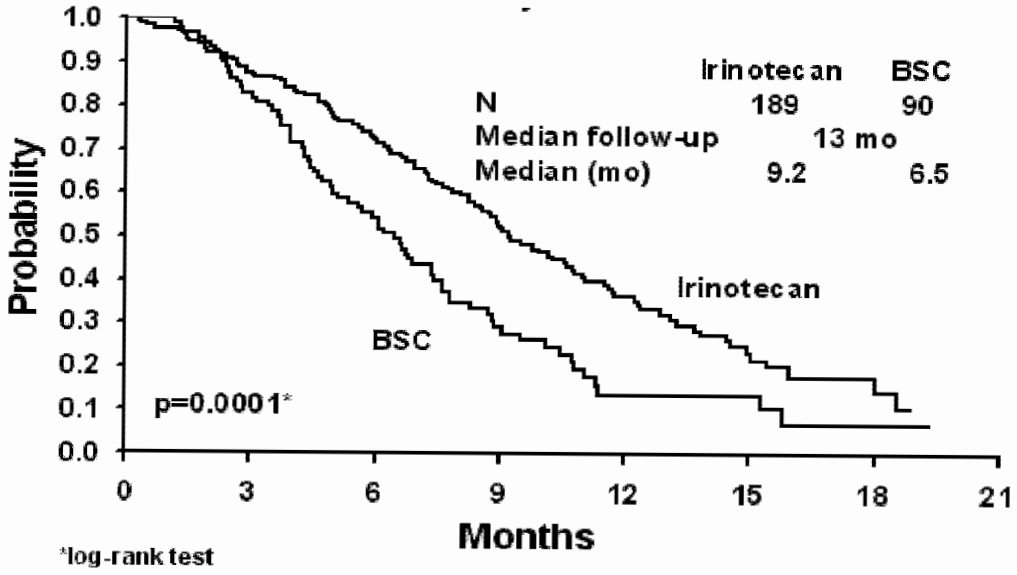


Figure 4. Survival
Second-Line Irinotecan vs Infusional 5-FU
Study 8

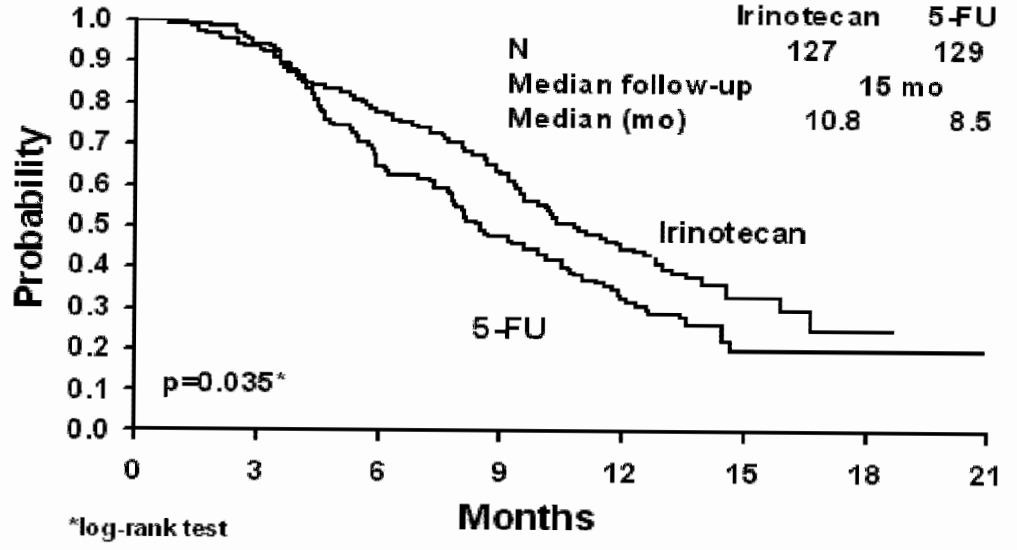


Table 12. Once-Every-3-Week Dosage Schedule: Study Results

	Study 7		Study 8	
	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
rvival				
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. The results as summarized in Table 13 are based on patients' worst post-baseline scores. In Study 7, 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 8, the multivariate analysis on all 15 subscales did not indicate a statistically significant difference between irinotecan and infusional 5-FU.

Table 13. EORTC QLQ-C30: Mean Worst Post-Baseline Score[a]

QLQ-C30 Subscale	Study 7			Study 8		
	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.

15 REFERENCES

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16 HOW SUPPLIED/STORAGE AND HANDLING

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

2 mL NDC 0009-7529-02
5 mL NDC 0009-7529-01

CAMPTOSAR Injection is available in single-dose amber colored polypropylene CYTOSAFE® vials in the following package sizes:

2 mL NDC 0009-7529-04
5 mL NDC 0009-7529-03
15 mL NDC 0009-7529-05

Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. Keep the vial in the carton until the time of use.

Inspect the vial for damage and visible signs of leaks before removing from the carton. If damaged, incinerate the unopened package.

17 PATIENT COUNSELING INFORMATION

- Patients and caregivers should be informed of gastrointestinal complications, such as nausea, vomiting, abdominal cramping, and diarrhea. Patients should have loperamide readily available to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of CAMPTOSAR). Begin loperamide at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normal. One dosage regimen for loperamide is 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. Patients should contact their physician 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; or inability to get diarrhea under control within 24 hours.

- Patients should be warned about the potential for dizziness or visual disturbances which may occur within 24 hours following the administration of CAMPTOSAR.

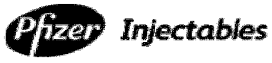
- Explain the significance of routine blood cell counts. Instruct patients to monitor their temperature frequently and immediately report any occurrence of fever or infection.

- CAMPTOSAR may cause fetal harm. Advise patients to avoid becoming pregnant while receiving this drug.

- Patients should be alerted to the possibility of alopecia.

- Contains sorbitol.

This product's label may have been updated. For current full prescribing information, please visit www.pfizer.com.



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Application Number:	15809815
International Application Number:	
Confirmation Number:	5137
Title of Invention:	Methods for Treating Metastatic Pancreatic Cancer Using Combination Therapies Comprising Liposomal Irinotecan and Oxaliplatin
First Named Inventor/Applicant Name:	Eliel Bayever
Customer Number:	153749
Filer:	Mary Rucker Henninger/Richard King
Filer Authorized By:	Mary Rucker Henninger
Attorney Docket Number:	263266-421428
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49	Other Reference-Patent/App/Search documents	EP2861210_Opp_D3_Zaniboni_2012.pdf	1151491 48300bab13a2fefc0bccf493d47096447e9365dc	no	5
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50	Non Patent Literature	EP2861210_Opp_D4_Neuzillet_2012.pdf	2004251 92dcff94a7c5428efad5a5dc667349573ccea7ca	no	9
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51	Non Patent Literature	EP2861210_Opp_D5_Yoo_2009.pdf	664989 1ca2654dbf1081717e8acbf7ecbebf8a1bb90a67	no	6
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52	Non Patent Literature	EP2861210_Opp_D6_Taieb_2007.pdf	1593467 7408565151d27acc5b5b94e53781463aae4e7c65	no	6
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53	Non Patent Literature	EP2861210_Opp_D7_Chen_2008_Abst.pdf	284054 c93dfcdccf2a90d570958f25137382b682940ce2	no	2
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54	Non Patent Literature	EP2861210_Opp_D8_Infante_2012.pdf	1480461 adcacc3852ee1d0f7e33fd8353080e441136b7aa	no	7
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55	Non Patent Literature	EP2861210_Opp_D9_Waterhouse_2011.pdf	3844861 6cd42a1c2dc74af1bff2fa1b148036a54219909c	no	10
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56	Non Patent Literature	EP2861210_Opp_D10_Camptosar_PI_2012.pdf	2026130 39234637e248a7c1e7e39f5e7834a319038b945d	no	39
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COMMENTARY

UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Dose Matters

Janelle M. Hoskins, Richard M. Goldberg, Pingping Qu, Joseph G. Ibrahim, Howard L. McLeod

The Food and Drug Administration and Pfizer changed the package insert for irinotecan to include a patient's UGT1A1*28 genotype as a risk factor for severe neutropenia on the basis of the findings of four pharmacogenetic studies, which found that irinotecan-treated patients who were homozygous for the UGT1A1*28 allele had a greater risk of hematologic toxic effects than patients who had one or two copies of the wild-type allele (UGT1A1*1). Findings of subsequent irinotecan pharmacogenetic studies have been inconsistent. In a meta-analysis, we reviewed data presented in nine studies that included a total of 10 sets of patients (for a total of 821 patients) and assessed the association of irinotecan dose with the risk of irinotecan-related hematologic toxicities (grade III–IV) for patients with a UGT1A1*28/*28 genotype. The risk of toxicity was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype at both medium (odds ratio [OR] = 3.22, 95% confidence interval [CI] = 1.52 to 6.81; $P = .008$) and high (OR = 27.8, 95% CI = 4.0 to 195; $P = .005$) doses of irinotecan. However, risk was similar at lower doses (OR = 1.80, 95% CI = 0.37 to 8.84; $P = .41$). Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range. The risk of experiencing irinotecan-induced hematologic toxicity for patients with a UGT1A1*28/*28 genotype thus appears to be a function of the dose of irinotecan administered.

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Irinotecan (Camptosar), a topoisomerase I poison, is approved for use in combination with 5-fluorouracil and leucovorin chemotherapy for first-line treatment of metastatic colorectal cancer and as a single agent in second-line salvage therapy of 5-fluorouracil refractory metastatic colorectal cancer disease. It is also commonly used to treat esophageal, non–small-cell lung, and breast cancers and other solid tumors in a second- or third-line setting. Irinotecan can be administered weekly, every 2 weeks, or every 3 weeks at doses ranging from 50 to 350 mg/m^2. The principal dose-limiting toxicities are delayed diarrhea and neutropenia; these toxicities are reversible, not cumulative, and related to irinotecan dose (1). Irinotecan is metabolized in vivo by carboxylesterases to the active metabolite SN-38, which is 100- to 1000-fold more potent than irinotecan as a topoisomerase I poison. SN-38 is eliminated predominantly by glucuronidation to SN-38 glucuronide. This glucuronidation reaction is mediated primarily by UDP-glucuronosyltransferase 1 family polypeptide A1, which is encoded by the UGT1A1 gene. Systemic exposure to SN-38 (as measured by area under the concentration–time curve) is related to the number of TA base repeats that a patient carries in the promoter region of each UGT1A1 allele (2–5). The wild-type allele (i.e., allele UGT1A1*1) has six TA repeats, and the variant allele (i.e., allele UGT1A1*28) has seven TA repeats. Patients who are homozygous for the UGT1A1*28 allele glucuronidate SN-38 less efficiently than patients who have one or two wild-type alleles; therefore, homozygous patients are exposed to higher plasma concentrations of SN-38 (3).

In November 2004, the US Food and Drug Administration (FDA) Advisory Committee on Pharmaceutical Sciences considered the findings of four pharmacogenetic trials that had assessed the association between UGT1A1*28 genotype and irinotecan-induced toxicities in a total of 30 patients who were homozygous for the UGT1A1*28 allele (4,6–8). In these studies, associations between the UGT1A1*28/*28 genotype and hematologic toxicity and/or diarrhea were observed. As a result of these findings, the FDA advised Pfizer Pharmaceuticals, the manufacturer of irinotecan, to amend the product information for the drug to include the association between the UGT1A1*28 genotype and hematologic toxicity and to recommend that patients with the UGT1A1*28/*28 genotype receive a lower starting dose of irinotecan. These changes took effect in July 2005. A diagnostic test for the UGT1A1*28 genotype (i.e., Invader UGT1A1 Molecular Assay; Third Wave Technologies, Inc, Madison, WI) for irinotecan dosing was approved in August 2005 by the FDA (9).

Subsequent results have begun to clarify the association between UGT1A1*28 and irinotecan-induced toxicities, particularly for dosing schedules that were not reviewed in the initial FDA committee meeting. Some studies (4,7) found that the UGT1A1*28/*28 genotype predicted grade III–IV neutropenia but not diarrhea, and other studies (6,10) found that the genotype predicted grade III–IV diarrhea but not hematologic toxicity. These results contrast with studies in which the UGT1A1*28

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genotype was not associated with neutropenia or diarrhea (11,12). Moreover, in most studies (4,6,7,10–12), many patients with a UGT1A1*28/*28 genotype did not experience severe toxicity, and in some of the studies (6,10,11), patients with the UGT1A1*1/*1 genotype had high-grade toxicity. Inconsistent relationships are common for genetic association studies and might be explained by false-positive associations or overestimation of the effect size in the initial studies. Whatever the cause, these findings pose the question: How should UGT1A1*28 genotyping be used to determine the optimum treatment regimen for a patient treated with irinotecan?

In a meta-analysis, we assessed the relationships between the incidence of irinotecan-induced hematologic toxicity (grade III–IV) and irinotecan dose among patients with the UGT1A1*28/*28 genotype. We identified nine studies (3,4,6,7,10–14) that assessed the relationship between UGT1A1*28 genotype and irinotecan-induced hematologic toxicity in a total of 821 patients. Two irinotecan-containing regimens were administered to patients in the N9741 study (14), and in our analyses, we analyzed the patients treated with each regimen as two separate samples. A summary of the 10 pharmacogenetic samples included in our analyses is presented in Table 1. Among the samples, patients received a variety of irinotecan-containing regimens, including commonly used higher doses (200–350 mg/m^2) administered every 21 days, an intermediate dose (180 mg/m^2) administered every 2 weeks, or lower doses (80–125 mg/m^2) administered weekly; irinotecan was given either alone or in combination with other anticancer agents. A UGT1A1*28/*28 genotype was associated with severe hematologic toxicity in three (3,4,14) of the 10 samples ($P<.05$, two-sided Fisher's exact test; see Table 1 for P values) and tended to be associated with toxicity in two of the samples (6,13) ($P<.1$). In the other five samples, the UGT1A1*28/*28 genotype was not associated with toxicity.

Heterogeneity among samples was tested by use of a chi-square test, and the presence of heterogeneity was not detected ($P = .25$). Publication bias was assessed by a funnel plot of the log odds ratio (OR) of individual samples against the standard error of the log odds ratios. The plot (Fig. 1) appeared to be symmetrical about the horizontal line (weighted average log OR = 1.35), with the diameter of the funnel decreasing with decreasing standard error (i.e., increasing sample size), indicating no evidence of publication bias. The observation suggests that studies demonstrating non–statistically significant associations between UGT1A1*28/*28 genotype and irinotecan-related hematologic toxicity were ascertained and included in the meta-analytic study.

To assess whether irinotecan dose modulates the association between UGT1A1*28 genotype and the risk of hematologic toxicity, we used a generalized linear mixed model (available in the SAS PROC GLIMMIX program, SAS Institute, Cary, NC) and considered dose as both a continuous and categorical variable. By using a unified regression model, we could account for the sample size of each genotype and of each sample. We first considered dose as a continuous variable and compared the rate of severe hematologic toxicity induced by irinotecan between patients with a UGT1A1*28/*28 genotype and patients with one or two wild-type alleles (UGT1A1*1/*1 or UGT1A1*1/*28 genotype). The results showed that the risk of hematologic toxicity between patients with a UGT1A1*28 and those with a UGT1A1*1/*1 or UGT1A1*1/*28 increased statistically significantly as irinotecan dose increased (slope = 0.012; $P = .028$). At a low dose level the risk was relatively low, but at a medium to high dose level the risk was higher. For example, at an irinotecan dose of 100 mg/m^2, the odds of hematologic toxicity for UGT1A1*28/*28 patients was 1.28 times higher than that for UGT1A1*1/*1 or UGT1A1*1/*28 patients (OR = 1.28, 95% confidence interval [CI] = 0.42 to 3.91; $P = .63$), and, at a dose of 250 mg/m^2, it was 8.07 times higher (OR = 8.07, 95% CI = 3.23 to 20.2; $P<.001$).

In a further analysis, we assessed the association between UGT1A1*28 genotype and hematologic toxicity and their interaction with irinotecan dose as a categorical variable. Irinotecan dose levels were pooled into the following three groups: low (<150 mg/m^2), medium (150–250 mg/m^2), and high (>250 mg/m^2) doses on the basis of the three most commonly used dosage regimens. At medium doses, the risk of toxicity was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (OR = 3.22, 95% CI = 1.52 to 6.81; $P = .008$); similarly, at high doses, the risk was higher among patients with a UGT1A1*28/*28 genotype than among

CONTEXT AND CAVEATS

Prior knowledge

In four previous studies, a UGT1A1*28 genotype among irinotecan-treated patients was associated with an increased risk of severe neutropenia.

Study design

A meta-analysis of nine studies that included 10 sets of patients (for a total of 821 patients) assessed the association between irinotecan dose and the risk of grade III and IV hematologic toxic effects by UGT1A1*1 or UGT1A1*28 genotype.

Contribution

The risk of hematologic toxic effects at high and medium irinotecan doses was higher among patients with a UGT1A1*28/*28 genotype than among those with a UGT1A1*1/*28 or UGT1A1*1/*1 genotype. However, at lower doses, risk was similar for patients with all genotypes. Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range.

Implications

At low doses of irinotecan, decisions about treating individual patients can be made according to standard clinical practice because genotype was not associated with risk. At higher doses, genotype-based decisions are advisable because of the association between the UGT1A1*28/*28 genotype and increased risk of irinotecan-induced toxic effects.

Limitations

There were many sources of heterogeneity among the studies analyzed. Some sources of heterogeneity could have influenced patient participation in a trial and, therefore, the dose of irinotecan that was received. Others could have been related to the dose of irinotecan administered by trials. These factors may also have directly modulated the association observed. Because of limited power or the unavailability of individual data, the relationship between these factors and the association could not be assessed.

Table 1. Summary of 10 samples included in our analyses that assessed the diagnostic value of the homozygous UGT1A1*28 genotype to predict irinotecan-induced grade III–IV hematologic toxicity†

Irinotecan dose, mg/m² (% patients)	Schedule	Concomitant chemotherapy	Trial type	Patient type	Toxicity (grade III–IV)	Grade‡ criteria	No. of patients	Overall incidence of toxicity§	No. of UGT1A1*28/*28 patients (frequency, %)	Toxicity incidence§		Two-sided Fisher's exact *P*	First author	Sample ref.
										UGT1A1 *28/*28	UGT1A1*1/*1 or *1/*28			
350	Every 3 wk	None	Phase I, prospective	Solid tumors, lymphoma	Neutropenia	NCI	61	18 (11/61)	6 (10)	83 (5/6)	11 (6/55)	.0004	Innocenti	(4)
300	Every 3 wk	None	Phase I, prospective	Solid tumors, lymphoma	Neutropenia	NCI	20	10 (2/20)	4 (20)	50 (2/4)	0 (0/16)	.03	Iyer	(3)
200	Every 3 wk	OXA	Prospective	Advanced colorectal	Neutropenia (IV only)	NCI	103	17 (17/103)	11 (11)	55 (6/11)	12 (11/92)	.002	McLeod	(14)
180	Biweekly	5FU	Prospective	Metastatic colorectal	Hematologic	NCI	250	15 (37/250)	22 (9)	18 (4/22)	14 (33/228)	.55	Toffoli	(12)
180	Biweekly	5FU	Prospective	Metastatic colorectal	Neutropenia	WHO	56	25 (14/56)	5 (9)	60 (3/5)	22 (11/51)	.09	Marcuello	(6)‖
180	Biweekly	None	No information	Advanced colorectal	Neutropenia	NCI	58	28 (16/58)	7 (12)	57 (4/7)	24 (12/51)	.08	Chiara	(13)
180	Biweekly	5FU	Retrospective	Advanced colorectal	Neutropenia	NCI	46	33 (15/47)	5 (11)	60 (3/5)	29 (12/41)	.31	Rouits	(7)‖
100	Weekly	5FU	Prospective	Advanced colorectal	Neutropenia (IV only)	NCI	109	10 (11/109)	11 (10)	18 (2/11)	9 (9/98)	.31	McLeod	(14)
80	Weekly	RAL	Prospective	Advanced colorectal	Neutropenia	NCI	56	7 (4/56)	7 (13)	14 (1/7)	6 (3/49)	.42	Massacesi	(10)
100 (22)	Weekly	CAP	Phase II, prospective	Metastatic colorectal	Neutropenia	NCI	64¶	5 (3/64)	6 (9)	0 (0/6)	5 (3/58)	1.00	Carlini	(11)
125 (78)	Weekly	CAP												

† ref. = reference; NCI = National Cancer Institute common toxicity criteria; OXA = oxaliplatin; 5FU = 5-fluorouracil; WHO = World Health Organization; RAL = raltitrexed; CAP = capecitabine.

‡ NCI and WHO systems grade an absolute neutrophil count of less than 1000×10^6 cells per L of blood as grade III–IV neutropenia (http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx, http://www.who.int/en/).

§ The toxicity incidence (overall or by genotype group) is reported as a percentage. Values in parentheses are number of patients overall or in each genotype group with hematologic toxicity/number of total patients in that group.

‖ More than one irinotecan-containing regimen was administered in these studies. Only results from patients who received irinotecan at 180 mg/m² biweekly with 5-fluorouracil were considered in these analyses.

¶ Patients with TA_5 and TA_9 alleles are included in these results.

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those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (OR = 27.8, 95% CI = 4.00 to 195; $P = .005$). In contrast, at low irinotecan doses, the risk of toxicity was not statistically significantly different between patients with a UGT1A1*28/*28 genotype and those with a wild-type allele (OR = 1.80, 95% CI = 0.37 to 8.84; $P = .41$). Results from the categorical-dose and the continuous-dose analyses were similar; i.e., a statistically significant association was found between genotype and toxicity at medium or high doses of irinotecan but not at low doses.

We also assessed whether irinotecan dose modulates the association between UGT1A1*28 genotype and irinotecan-induced diarrhea (grade III–IV). We identified nine studies (3,4,6,7,10,11, 13–15) that assessed the relationship between UGT1A1*28 genotype and toxicity. As noted above, the N9741 study (14) administered two irinotecan-containing regimens to patients, and we treated the patient who was administered the different regimens as two separate samples. In addition, only grade IV diarrhea data weres available for the study (14). Of the 10 samples, UGT1A1*28/*28 genotype was associated with severe diarrhea in only one sample (relative risk = 3.40, 95% CI = 1.76 to 6.59; $P = .02$, two-sided Fisher's exact test), indicating that UGT1A1*28 genotype was not associated with diarrhea (6). We next assessed the relationships between irinotecan dose and the incidence of irinotecan-induced diarrhea (grade III–IV) by genotype. The incidence of severe diarrhea in patients with a UGT1A1*28/*28 genotype was not related to irinotecan dose ($r^2 = .0$; $P = .8$; n = 10 samples) (data not shown); however, the rate of diarrhea among patients with one or two wild-type alleles was inversely associated with dose ($r^2 = .43$; $P = .04$; n = 10 samples) (data not shown). Thus, the risk of diarrhea among patients with a UGT1A1*28/*28 genotype was not associated with irinotecan dose, and so we did not examine this relationship further.

We observed that, at higher irinotecan doses (>150 mg/m^2), the risk of hematologic toxicity was strongly associated with the UGT1A1*28 polymorphism. In contrast, at lower doses (≤150 mg/m^2), the risk of hematologic toxicity among patients with a UGT1A1*28/*28 genotype was not statistically significantly different from that among patients with one or two wild-type alleles (i.e., UGT1A1*1/*28 or UGT1A1*1/*1, respectively). This observation is consistent with a classic gene–environment interaction, in which the association between genotype and outcome depends on the level of exposure to an environmental factor—in this case, the dose of irinotecan (16). To our knowledge, this is the first demonstration of a gene–environment interaction in the context of pharmacogenetics. In contrast, among patients with the UGT1A1*28/*28 genotype, irinotecan dose was not associated with diarrhea. Heterogeneity of irinotecan administration, diarrhea management with loperamide, coadministered chemotherapeutic agents among trials, and difficulty in scoring this toxicity might contribute to the incidence of this adverse event and explain some of the interstudy variation in the incidence of diarrhea among patients with a UGT1A1*28/*28 genotype. The utility of UGT1A1*28 genotype to predict irinotecan-induced diarrhea, therefore, remains unclear and requires further investigation.

We propose two potential strategies for irinotecan dosing to accommodate the modulatory effect of irinotecan dose on the risk of hematologic toxicities among patients with a UGT1A1*28/*28 genotype. The first is based on a prior selection of the most convenient and appropriate regimen for the individual patient. When regimens with a low dose of irinotecan (<150 mg/m^2) given weekly are being considered, decisions concerning the best irinotecan dose for individual patients could be made on the basis of standard clinical practice rather than genotype because genotype was not associated with an increased risk of toxicity. Low doses of irinotecan (100–125 mg/m^2) are in the commonly used therapeutic range (17). For patients receiving a more convenient high-dose regimen (>250 mg/m^2), however, genotype-based decisions are advisable because UGT1A1*28 genotype was associated with toxicity at higher doses of irinotecan (>150 mg/m^2). For patients with a UGT1A1*28/*28 genotype, a starting irinotecan dose reduction of one level is recommended in the package insert from the manufacturer (18), whereas, for patients with one or two wild-type alleles a standard irinotecan dose can be used. Patients with a UGT1A1*28/*28 genotype had a heightened risk of toxicity at intermediate doses (150–250 mg/m^2) that were given biweekly or every 3 weeks. However, the odds of toxicity at intermediate doses are likely to be within a range acceptable to many patients who do not have other risk factors for neutropenia (e.g., they are not elderly or have not had prior myelotoxic therapy). We suggest that patients and physicians should strongly consider UGT1A1*28 testing for patients with other predictors of irinotecan-induced neutropenia. Alternatively, all patients could initiate therapy at a dose reduction of one level, with doses being increased if toxicity is modest. This approach apparently does not adversely influence outcomes of patients treated with low doses of irinotecan, but clear survival data are not available for high-dose irinotecan regimens (19).

The second potential strategy is to select an irinotecan-containing regimen with a level of toxicity risk that is acceptable to the patient and physician by use of Fig. 2, A, and results of the random effects model for irinotecan as a categorical variable. For irinotecan doses of up to 150 mg/m^2, the absolute risk of severe neutropenia among patients with a UGT1A1*28/*28 genotype is similar to the overall risk for all patients (i.e., ~15%). The absolute

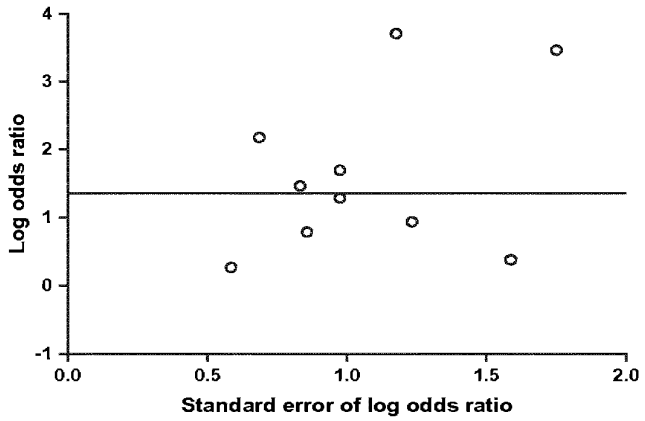

Fig. 1. Funnel plot of log odds ratio against standard error of log odds ratio for the UGT1A1*28/*28 genotype with irinotecan-related grade III–IV hematologic toxicity. Symmetry of the plot about the weighted average of the log odds ratios ($y = 1.3536$) indicates no evidence of publication bias.

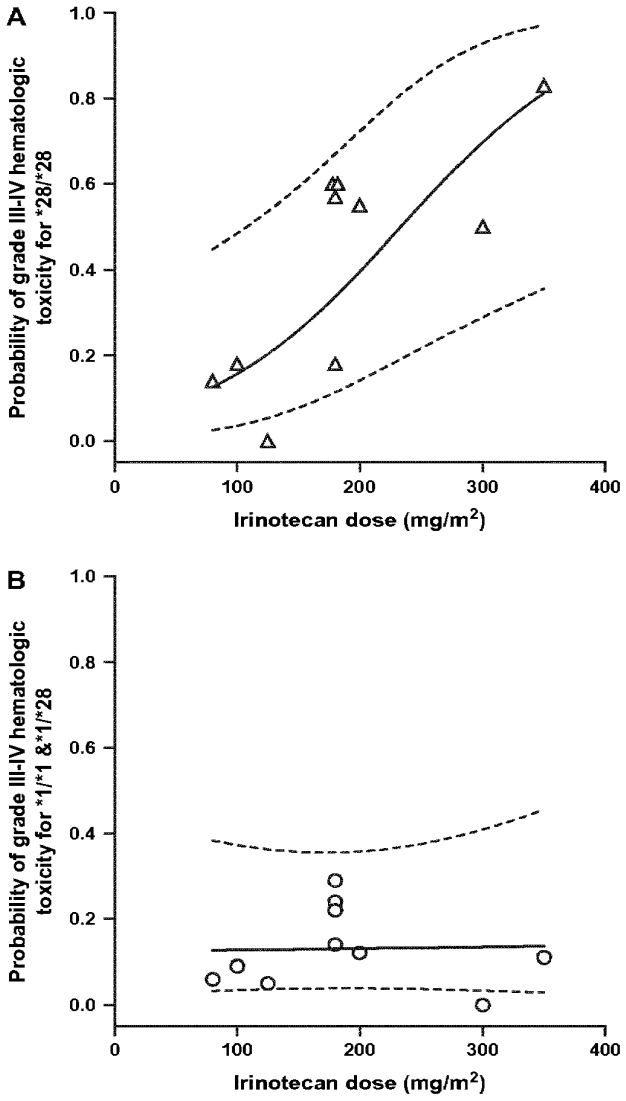

Fig. 2. Relationships between irinotecan dose and3 incidence of hematologic toxicity in patients with a UGT1A1*28/*28 genotype (**A**) and in those with a UGT1A1*1/*1 or UGT1A1*1/*28 genotype (**B**). Results from 10 samples (**triangles** in panel **A** and **circles** in panel **B**) were included in our analysis (3,4,6,7,10–14). **Solid lines** = predicted probabilities of experiencing hematologic toxicity under the generalized linear mixed model at any dose level in the original dose range; **dotted lines** = 95% confidence intervals. For trials in which more than one irinotecan regimen was administered, we considered only the results from patients who received irinotecan at 180 mg/m² biweekly with 5-fluorouracil (6,7). The two different irinotecan-containing regimens in the N9741 trial, which included only grade IV toxicity, were treated as two separate samples in our analyses (14).

risks of toxic effects in these patients increased to 25%–40% for intermediate doses (150–250 mg/m²) and to 50%–70% for higher doses (>250 mg/m²). If patients with a UGT1A1*28/*28 genotype and their physicians are willing to accept the risk of higher levels of toxicity, then the use of more convenient, less frequently administered regimens could be considered. This scenario offers a clear example for the use of patient preference or accepted risk in the selection of a treatment regimen. The greater refinement of the risk of toxicity within a genotype group is also an opportunity for the patient's threshold for risk to help dictate the drug schedule or even specific regimen.

This study has several limitations. We used a meta-analytic approach to combine information from independent trials that had addressed the question whether patients homozygous for the UGT1A1*28 allele have an elevated risk of hematologic toxicity to assess whether the interaction between genotype and toxicity was associated with the administered dose of irinotecan. There were many sources of heterogeneity among the studies, including patient characteristics (e.g., age, ethnicity, sex, performance status, and number of previous chemotherapies), patient eligibility criteria (e.g., type of tumor, stage of disease, and number of previous chemotherapies), treatment schedules (e.g., dose of irinotecan and time between courses and coadministered chemotherapies), and study design (e.g., phase I, phase II, prospective, and retrospective trial). Some sources of heterogeneity (including the stage of tumor, type of tumor, and line of chemotherapy) could have influenced patient participation in a trial and therefore the dose of irinotecan that was received, and other factors (including time between irinotecan doses and coadministered chemotherapies) could have been related to the dose of irinotecan administered by trials. These factors may have directly modulated the association between UGT1A1*28 genotype and irinotecan-related toxicity. Unfortunately, we were unable to assess whether these factors influenced the association between genotype and toxicity among the samples either because of limited power due to the small sample size or because the individual data were not available.

Confounding by genotype error, clinical phenotype, or other variables may also be sources of bias. UGT1A1*28 genotyping was conducted in different laboratories that used different methodologies; however, none of the samples in our study departed from the Hardy–Weinberg equilibrium ($P>.05$; chi-square test). Although this test, which assesses the relationship between the frequency of the UGT1A1*28 allele and UGT1A1*28 genotypes in a population, is not the most sensitive measure of assay reliability, it suggests that genotype error was not a large source of bias. Additionally, the genotyping assays for UGT1A1*28 are not especially prone to errors, suggesting that genotype error is an unlikely source of bias among the studies. In our analysis, grade III–IV neutropenia data (absolute neutrophil count nadir of $<1000 \times 10^6$ cells per L) were available for seven samples (3,4,6,7,10,11,13), whereas only grade IV neutropenia information (14) and grade III–IV hematologic toxicity data (12) could be extracted from the literature for other samples. We treated these clinical events as equivalent, which may have introduced bias into our analysis. Data were extracted from publications for some samples (6,7,10) and obtained via correspondence with authors for other samples (3,4,11–14). Possible data errors reported in publications and others introduced by extracting data from publications could be other sources of bias that were not addressed by our methodology.

A diagnostic test that identifies patients at high risk of dose-limiting toxicities to irinotecan would be clinically useful. Although initial studies (4,6–8) found UGT1A1*28 genotype to be strongly associated with risk of toxicity, results of subsequent studies (10–14) were inconsistent. In our meta-analysis, we found that

the irinotecan dose delivered modulated the association between UGT1A1*28 genotype and irinotecan-induced hematologic toxicity and that the interaction was clinically important only at higher irinotecan doses. At lower irinotecan doses, factors other than UGT1A1*28 genotype, either genetic or nongenetic, are likely to determine a patient's risk of hematologic toxicity, whereas at higher drug doses, UGT1A1*28 genotype appears to be an important determinant. We recommend that the product information for irinotecan be amended to describe the association between irinotecan dose and risk of hematologic toxicity among patients with a UGT1A1*28/*28 genotype. We also favor the development of consensus guidelines by national and regional bodies (e.g., the National Cancer Institute, American Society of Clinical Oncologists, European Society of Medical Oncology, or National Comprehensive Cancer Network) for optimal use of UGT1A1*28 genotype information to prescribe irinotecan doses. Finally, we caution that decisions that are based on only a few events may prove to be misleading. Determining the amount of evidence needed to justify the inclusion of black box warnings on product inserts to safeguard patients is a controversial issue that is worthy of further study.

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Nanovector-based therapies in advanced pancreatic cancer

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ABSTRACT Systemic therapy for advanced pancreatic cancer has been largely disappointing owing to the unfavorable pharmacokinetic profile and poor penetration of current chemotherapeutic agents ,as well as the fragile patient population with compromised tolerance to toxic chemotherapies. Nanovectors can provide passive drug delivery through abnormal tumor neo-vasculature microanatomy or active targeting via binding to receptors or macromolecules associated with the tumor. In such a manner, nanovector-based therapy may not only modulate the pharmacokinetics and therapeutic index of chemotherapeutic agents but also provide new treatment options in patients with advanced pancreatic cancer. In this article, we present the rationale and currently available clinical results of nanovector-based therapies to highlight the potential use of this class of agent in patients with advanced pancreatic cancer.

KEY WORDS nanovector; pancreatic cancer; liposome; PEP02; nab-paclitaxel; EndoTAG-1; nanoplatin; platinum; CPT-11

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Introduction

Pancreatic cancer is one of the most detrimental malignancies and the fourth most common cause of cancer-related death in the United Stated. There were 43,140 newly diagnosed cases and 36,800 deaths in 2010 (1). Early detection is uncommon with no more than 15–20% of the patients being amenable for curative intent surgery at the time of diagnosis. Gemcitabine either alone or in combination with erlotinib are the only approved treatments for patients with advanced pancreatic cancer, of whom the overall survival time is generally around 6 months (2-5). Recently, Conroy et al showed that a gemcitabine-free triplet chemotherapy, FOLFIRINOX regimen consisting of oxaliplatin, irinotecan and infusional 5-FU/leucovorin, could achieve significantly better tumor response rate, progression-free survival and overall survival than gemcitabine monotherapy in patients with metastatic pancreatic cancer in a randomization phase III trial (6,7). However, the application of either doublet of triplet combination chemotherapy in patients with advanced pancreatic cancer is often hindered by their toxicity and the performance status of the patients.

New treatment strategies are mandatory to improve the therapeutic outcomes of patients with advanced pancreatic cancer. Recently, two major potential new approaches are emerging that may have the chance to change our practice in treating advanced pancreatic cancer. The first one is molecular targeted agent targeting on dysregulated signaling pathway and the second is the use of nanovector drug delivery system to provide 'passive" or "active" targeting drug delivery thus to modulate the pharmacokinetics and therapeutic index of chemotherapeutic agents in pancreatic cancer (8).

This review will focus on the selective nanovector treatments in pancreatic cancer, especially those with available clinical data, including albumin-bound nanoparticles, liposome-encapsulation nanoparticle, cationic liposomal nanoparticle, polymeric micellar agents, and a non-replicating, retroviral vector delivered gene therapy construct.

Albumin-bound Nanoparticle Paclitaxel (Nab-

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paclitaxel)

Albumin is a particular vehicle for drug delivery in oncology because it is a natural carrier of hydrophobic molecules with reversible, noncovalent binding characteristics and able to enhance the delivery of drug into the extravascular space through a process of receptor-mediated endothelial transcytosis. Such process is initiated by the binding of albumin to an endothelium surface, 60-kDa glycoprotein (gp60) receptor (albondin), which will then bind with an intracellular protein (caveolin-1) to result in the invagination of the endothelium membrane to form transcytotic vesicles, the caveolae (9). The caveolae will subsequently move across the cytoplasm and release the albumin and its conjugated compound into the extracellular space (the peritumoral microenvironment) where the albumin will bind to SPARC (secreted protein acid and rich in cysteine), an extracellular matrix albumin-binding glycoprotein that is structurally and functionally closely related to gp60, and overexpressed in a variety of cancers, including breast cancer, gastric cancer and pancreatic cancer.

Nab-paclitaxel (Abraxane®) is a cremophor (CrEL)-free, albumin-bound, nanoparticle formulation of paclitaxel. Its CrEL-free formulation permits nab-paclitaxel to be administered within a shorter infusion period of time (30 minutes) and without the requirement of routine pre-medications for preventing the hypersensitivity reactions in association with the administration of cremophor solvent-based paclitaxel (10). In preclinical study, the transport of radiolabeled paclitaxel across the endothelial cell monolayer *in vitro*, and intratumor paclitaxel accumulation after equal doses of paclitaxel *in vivo* were both significantly enhanced by 4.2-folds ($P < 0.0001$) and 33% ($P < 0.0001$), respectively, for *nab*-paclitaxel as compared with CrEL-paclitaxel with an increase 4.2 folds. In addition, endothelial transcytosis was completely inhibited by inhibitor of gp60/caveolar transport, methyl ß-cyclodextrin (11). These observations supported that gp60-mediated transcytosis and SPARC-aided sequestration may be an important biological pathway to target tumor cells by novel albumin-bound therapeutics.

In a phase I trial, the maximum tolerated dose (MTD) of intravenous injection nab-paclitaxel monotherapy, every 3 weeks in 19 patients with standard therapy-failure solid tumors was 300 mg/m^2. No acute hypersensitivity reactions were observed. The most frequent toxicities were myelo-suppression, sensory neuropathy, nausea/vomiting, arthralgia and alopecia (12). The drug has subsequently approved for the treatment of metastatic breast cancer after failure of combination chemotherapy or relapse within 6 months of adjuvant chemotherapy. The commonly used dose/schedule was 260 mg/m^2, 30-min intravenous injection, every 3 weeks.

Because SPARC is frequently overexpressed and associated with poor clinical outcomes in pancreatic cancer, Von Hoff et al conducted a phase I/II study to evaluate the MTD of weekly nab-paclitaxel (100 – 150 mg/m^2/week) in combination with gemcitabine (1000 mg/m^2/week), and the therapeutic efficacies of the regimen. Both agents were given on day 1, 8, and 15 every 28 days (13). A total of 67 patients were treated. Despite MTD of nab-paclitaxel was determined as 125 mg/m^2/week, dose reduction was required in 30% (6/20), 18% (8/44) and 33% (1/3) of patients receiving 100 mg/m^2, 125 mg/m^2 and 150 mg/m^2, respectively. The most common grade 3-4 toxicity at the MTD dose were fatigue 23%, neutropenia 59% (grade 4 in 23%), thrombocytopenia 20% (grade 4 in 9%) and sensory neuropathy in 9%. Of the 58 patients whose CT image were revaluated with RECIST criteria by independent reviewer, the best tumor response was partial response in 40% and stable disease in 37%, with an overall disease control rate of 78%. The median progression-free and overall survival of the intent-to-treat (N=67) patients were 6.9 months and 10.3 months, respectively; while the survival parameters for the 44 patients receiving MTD dose were 7.9 months and not yet reached, respectively. Of 54 patients with available CA19.9 level, 42 (77.8%) patients had a more than 50% reduction of CA19.9 level after the treatment (14). The therapeutic efficacy of nab-paclitaxel in combination with vandetanib, a potent inhibitor of VEGF2, RET and EGFR, has also been evaluated in a phase I trial with expansion cohort of patients with pancreatic cancer (15). The MTD of vandetanib in combination with two different schedule of nab-paclitaxel, either 100 mg/m^2 weekly or 260 mg/m^2 every 3 weeks, was 300 mg daily. Of the 29 enrolled gemcitabine-refractory pancreatic cancer patients, the best tumor was partial response in 6 (20.7%) and stable disease in 10 (34.5%), and the median progression-free survival and overall survival were 5.3 (95% CI: 3.7 to 7.3) months and 8.2 (95% CI: 6.2 to 11.5) months, respectively. No statistical significant correlation between SNP (rs1059829 and rs3210714) of SPARC and clinical outcomes was observed.

Liposome-based Drugs

A liposome is often a spherical vesicle with a bilayer membrane whose size typically ranges from ~40 nanometers to several microns. Because the micro- or nanoparticles can form spontaneously and are generally easier to prepare compared to viral-mediated systems, this nontoxic phospholipid-based drug carrier has become a favorable drug delivery system for various purposes since the 1970s.

However, so-called conventional liposomes are easily bound with insoluble circulating plasma protein, i.e. opsonins and lipoproteins, and the complex will be subsequently eliminated from the circulation by reticuloendothelial cells system. Stealth liposome technology, with incorporationof high molecular weight polymers (i.e., polyethylene-glycol (PEG)) to the liposome surface, can effectively protect the liposome from circulating protein binding and subsequently phagocytosis by RER system, and thus improving its plasma clearance, prolonging the circulation time, and enhancing drug delivery efficacy.

Besides its characteristic slow-release pharmacokinetic property, liposome encapsulated drugs can potentially provide improved tumor localization via the "enhanced permeability and retention" (EPR) effect. Such agents can therefore, (i) lower drug elimination to increase systemic circulation time, (ii) lower maximum plasma concentration (C_{max}) to reduce drug side effects, (iii) enhance tumor tissue uptake and exposure to the anti-cancer drug; these principles can in turn yield an improved therapeutic index for cancer therapy.

Several liposomal formulated cancer drugs have been evaluated in various cancers, but only a limited number have been applied to pancreatic cancer.

Liposomal Doxorubicin

The first liposomal anti-cancer drug approved by the Food and Drug Administration (FDA) was pegylated liposomal doxorubicin (Caelyx®/Doxil®) in 1995 for Karposi's sarcoma (16-18). It has been subsequently approved for the treatment of multiple myeloma and recurrent epithelial ovarian cancer as well. It also has been evaluated for the treatment of pancreatic cancer in animal xenograft model and in clinical trials. In a preclinical study, Vagge et al showed that pegylated liposomal doxorubicin was significantly more effective in inhibiting the growth of human pancreatic cancer xenograft in nude mice as compared to free form doxorubicin (19). Using confocal laser scanning microscopy and microfluorimetry to quantitate the uptake of intravenously injected doxorubicin in tumor tissue, the authors found that the content of doxorubicin in tumor site of animal receiving liposomal formulated drug was 6 folds or higher compared to free doxorubicin. Based on the results, Halford et al conducted a phase II trial to evaluate the therapeutic efficacy of Caelyx® in 22 chemo-naïve patients with unresectable pancreatic carcinoma. The dose was escalated from 30 mg/m^2 (in the first two patients) to 50 mg/m^2 intravenous injection every 3 weeks (20). Of the 20 patients received the treatment, the most common grade 3 toxicity were stomatitis (20%) and nausea (10%), the best tumor response was stable diseases in 6 (30%), and the median overall survival was 3.2 months with one year survival rate of 10%. These finding excluded the use of Caelyx® monotherapy in the treatment of advanced pancreatic cancer.

The combination of Caelyx® with infusional 5-FU/leucovorin and mitomycin-C has been evaluated in a phase I trial in patients with upper gastrointestinal cancer. In that study, escalating dose of Caelyx® (15 – 35 mg/m^2) day 1 and 29 in combination with weekly 24-hour infusion of 5-FU and leucovorin (2,000 and 500 mg/m^2, respectively) for 6 weeks, and mitomycin-C 7 mg/m^2 day 8 and 36, every 8 weeks as one cycle. The most common grade 3-4 toxicities were nausea/vomiting (29%), diarrhea (18%) and leucopenia (12%). Of the 14 accruals with pre-treated pancreatic cancer, the best tumor response was partial response in one and minor response in 2, and the overall survival after the study treatment was 6.5 months (21).

Liposomal Platinum

Platinum is one of the most active and wildly used anti-cancer agents in the world, including in combination with gemcitabine to treat non-small cell lung cancer and pancreatic cancer. Although each single trial had failed to demonstrate the superiority of gemcitabine/platinum combination over gemcitabine single agent in the prolongation of the survival in patients with advanced pancreatic cancer, however, the survival benefit of gemcitabine/platinum doublets was demonstrated in a pooled, meta-analysis survival with a hazard ratio of 0.81, p = 0.031 (22).

It is also well known that the use of cisplatin is frequently limited by its nephrotoxicity, peripheral sensory neuropathy, ototoxicity and the aggravation of hematological toxicity while in combination with other cytotoxic agents. Therefore, several liposomal formulations of cisplatin have been developed aiming to reduce its toxicity profile and hopefully to enhance it activity. Based on previous experience of gemcitabine/cisplatin combination and the result of meta-analysis, several liposomal formulated cisplatin have been evaluated in patients with pancreatic cancer.

Lipoplatin is one of the pegylated liposome cisplatin, whose nanoparticulate liposomes are reverse-miscelles, composed of dipalmitoyl phosphatidyl glycerol (DPPG), soy phosphatidyl choline (SPC-3), cholesterol and methoxy- polyethylene glycol-distearoyl phosphatidylethanolamine (mPEG2000-DSPE). Lipoplatin exhibits the fundamental pharmacologic characteristics of pegylated liposomal agents, for example, protecting from the engulfment of reticuloendotheralial system

to prolong circulating time, and extravasating from the fenestrate between endothelial cells of tumor vasculature to preferentially localize in per-tumor interstitial tissue and uptake by tumor cells. The anionic, fusogenic nature of the DPPG lipids enables lipoplatin to cross cell membranes more easily than native cisplatin. In addition, with intraperitoneal injection of a "sheath" liposomes wrapped reporter β-galactosidase gene, which had same structure like lipoplatin, into human tumor bearing nude mice, Boulikas et al were able to demonstrate the preferential expression of the reporter gene in the tumor and the tumor neo-vasculature. The findings indicate the potential antiangiogenic activity of the lipoplatin (23).

In phase I trial of lipoplatin monotherapy, the drug was diluted in 5% glucose water and administered as 8 hour intravenous infusion every 14 days. The dose was escalated from 25 mg/m^2 to 125 mg/m^2. Even at the targeted dose of 125 mg/m^2, only grade 1-2 gastrointestinal and hematological toxicities were observed, but neither nephrotoxicity nor neuropathy. Higher doses, 200, 250 and 300 mg/m^2, were also tested in one each patient, respectively. The half-life of lipoplatin was estimated ranging from 60 – 117 hours. Of the 27 accruals (19 with pretreated, advanced pancreatic cancer) in this phase I trial, the objective tumor response rate and disease control rate were 11.1% and 63.0%, respectively. Based on the exciting results, the drug has been further tested in combination with gemcitabine in non-small cell lung cancer and pancreatic cancer patient cohorts (24).

In a phase I/II study, Stathopoulos GP et al evaluated the maximum tolerated dose of lipoplatin in combination with gemcitabine in patients with previously treated advanced pancreatic cancer (25). Lipoplatin was given as an 8-hour infusion followed by 60 minutes infusion of 1,000 mg/m^2 of gemcitabine at day 1 and 15 every 28 days. The dose of lipoplatin was stepwise escalated from 25 mg/m^2 to 125 mg/m^2. Of the 24 enrolled patients, two of four patients at 125 mg/m^2 experienced grade 3-4 neutropenia. Therefore, the MTD of lipoplatin in this combination was determined to be 100 mg/m^2. In this dose escalating study, there were two (8.3%) partial responders and 14 (58.3%) disease stabilizers, and the median overall survival was 4 month. Further randomized phase II/III trial against gemcitabine monotherapy is under evaluation.

Liposome-entrapped *cis*-bisneodecanoato-*trans*-R,R-1,2-diaminocyclohexane (DACH) platinum(II) (L-NDDP, Aroplatin™) is a lipophilic cisplatin analog that has been formulated in relatively large-size multi-lamellar liposomes measuring from 1 to 3 μm in diameter. L-NDDP has been demonstrated to be non–cross-resistant with cisplatin in cisplatin-resistant Lovo DDP 3.0 (human colon cancer cells) and L1210/PPD (human leukemia cells) both *in vitro* and *in vivo* models. In a phase I study, L-NDDP was given intravenously once every 4 weeks, ranging from 7.5 mg/m^2 to 390 mg/m^2 (26). The infusion rate was set at 4 mg NDDP per minute for all cases. In this particular study, intra-patient dose escalation was allowed. Grade 1-2 nausea/vomiting, diarrhea and fever were frequently observed in patients receiving 100 mg/m^2 or higher dose of L-NDDP. Six out of the 10 patients who had 390 mg/m^2 experienced grade 4 hematological toxicities manifesting as thrombocytopenia, granulocytopenia or both. The MTD of intravenous L-NDDP every 4 weeks was determined as 300 mg/m^2. In 2004, Aronex Pharmaceuticals had registered a phase I/II study of L-NDDP and gemcitabine combination in patients with advanced pancreatic cancer resistant to standard therapy in a public clinical trial registration website, the clinicaltrials.gov, with an indentifier of NCT00081549. Unfortunately, the latest trial information was updated in June 2005, and no further publication on this trial can be found.

Liposomal Irinotecan (Nanoliposomal CPT-11, PEP02, MM-398)

Irinotecan hydrochloride (CPT-11) is a water-soluble semi-synthetic derivative of camptothecin targeting topoisomerase I, and has been an approved agent for the treatment of metastatic colorectal cancer worldwide, and also for gastric cancer (Japan and Korea), non-small cell lung cancer, small cell lung cancer, cervical cancer, and non-Hodgkin's lymphoma in Japan. In pancreatic cancer, earlier trial showed that combination of gemcitabine and CPT-11 did not provide any survival benefit over gemcitabine monotherapy in patients with advanced pancreatic cancer, and thus CPT-11 has not been considered to be a clinically useful drug in this disease. However, in the recent PRODIGE 4/ACCORD 11 trial, Conroy et al demonstrated that a gemcitabine-free, CPT-11-containing regimen, FOLFIRINOX (CPT-11, oxaliplatin plus intermittent infusion of 5-FU/leucovorin), provided significantly better objective tumor response rate, progression-free survival and overall survival versus gemcitabine monotherapy in patients with metastatic pancreatic cancer. Notable and not unexpectedly, this triplet regimen is associated with significant hematologic toxicity including higher rates of grade-3/4 febrile neutropenia. The results of the PRODIGE/ACCORD 11 trial have revived interest in CPT-11-based therapy in advanced pancreatic cancer (6,7).

Although the original CPT-11 drug is now of interest in pancreatic cancer management, potentially superior versions incorporating drug delivery technologies offer a

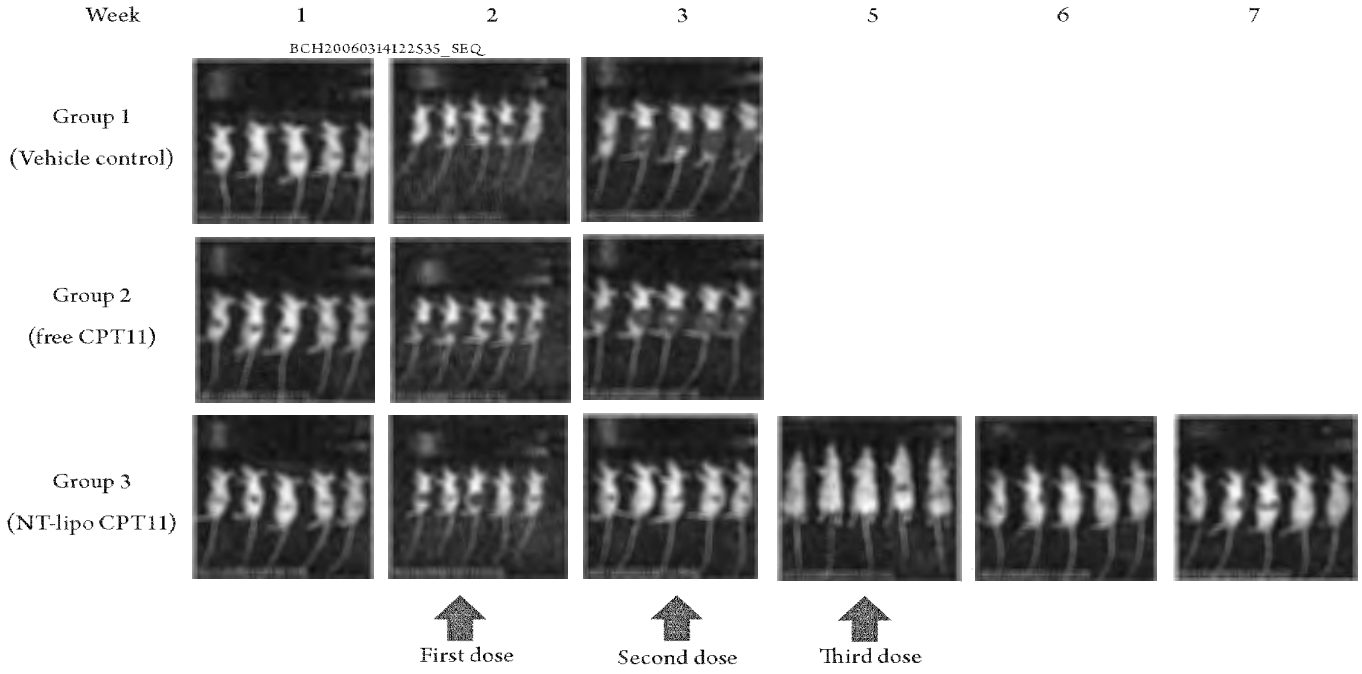
next generation approach. CPT-11 exhibits well-known pharmacologic liabilities and significant associated toxicities, which in turn make it an obvious candidate for drug delivery strategies The camptothecins exist in a pH-dependent equilibrium between an inactive carboxylate form (predominant at neutral-to-basic pH) and an active lactone form (predominant under acidic conditions); hence, intravenous injection of free CPT-11 results in rapid inactivation as well as clearance. Furthermore, CPT-11 is largely a prodrug which is converted into the much more potent metabolite SN-38. Hepatic activation and hepatobiliary excretion of SN-38 result in substantial risk of GI injury, especially in individuals having impaired SN-38 glucuronidation. These metabolic conversions contribute to notable heterogeneities in both efficacy and toxicity, and ultimately to a rather narrow therapeutic index. The concept of nanoparticle delivery of CPT-11 is thus very attractive based on potential advantages including: overcoming solubility limitations of the camptothecins; protecting drug in the active lactone configuration; chaperoning drug away from sites of toxicity such as the GI tract; prolonging circulation time and increasing tumor accumulation via the enhanced permeability and retention (EPR) effect; and providing sustained release and prolonged tumor exposure.

To realize the potential advantages of nanoparticle delivery, a novel liposome-based construct termed "nanoliposomal CPT-11 (nLs-CPT-11)" was developed, which encapsulates CPT-11 with unprecedented efficiency and stability (27). PK studies showed long circulation times for the carrier and undetectable drug release in plasma. Furthermore, nanoliposomal CPT-11 provides protection of drug in its active lactone form within the liposome aqueous interior, preventing its hydrolysis as well as premature conversion to the potent and toxigenic metabolite, SN-38. This contrasts markedly with free CPT-11, which is rapidly cleared from circulation, is subject to immediate hydrolysis of the lactone ring, and is also converted to SN-38 contributing to its dose-limiting GI toxicity.

In a series of preclinical studies, nanoliposomal CPT-11 demonstrated significantly superior efficacy when compared to free CPT-11 at the same or higher dose, including frequent cures in some models. The superiority of nanoliposomal CPT-11 over free CPT-11 has been observed in different tumor models including colorectal, gastric, breast, cervical, glioma, pancreatic and lung cancer models. In addition to superior efficacy, nanoliposomal CPT-11 has shown a more favorable pharmacologic profile and reduced toxicity in multiple preclinical models.

In order to evaluate this novel agent as a potential therapy for pancreatic cancer, a bioluminescence-based orthotopic xenograft model of pancreas cancer was developed (28). COLO357, a human pancreatic cell line, was passaged multiple times in vivo to generate the subline L3.6pl. This cell line was then modified by lentiviral transduction (L3.6pl-T) to express firefly luciferase. L3.6pl-T cells were implanted during open surgery directly into the pancreas of a nude mouse to form an orthotopic tumor xenograft. Therapeutic studies in this model compared nanoliposomal CPT-11 versus free drug at the equivalent dose, along with vehicle control (Figure 1). All treatments were administered intravenously by tail vein beginning at 7 days post-tumor implantation and continued weekly for a total of 3 planned treatments. At 20 mg/kg, free CPT-11 showed some tumor growth inhibition, but all mice required euthanization after 2 doses due to massive tumor progression. In contrast, nanoliposomal CPT-11 at the equivalent 20 mg/kg dose showed potent antitumor activity, including complete tumor inhibition during the entire post-treatment period. Systemic toxicity was not observed with any treatment. These studies indicated that nanoparticle- mediated delivery via nanoliposomal CPT-11 greatly enhances antitumor efficacy in the COLO357/L3.6pl-T orthotopic pancreatic xenograft model.

In the first-in-human phase I trial, patients with standard therapy-failure solid tumor were enrolled to determine the maximum tolerated dose, safety profile and pharmacokinetics of nanoliposomal CPT-11 (formerly PEP02, PharmaEngine, Inc., Taiwan, and now under the designation of MM-398, Merrimack Pharmaceuticals, Inc, USA). The drug was delivered intravenously for 90 minutes, once every 3 weeks, with starting dose of 60 mg/m^2. The maximum tolerated dose was 120 mg/m^2. Two patients achieved partial response including cervical cancer in one and pancreatic cancer in one (29). The observation was further extended in a phase I trial for nanoliposomal CPT-11in combination with weekly 24-hour infusion of high-dose 5-FU/LV (HDFL). In the two phase I trials, 7 pancreatic cancer patients who failed gemcitabine/HDFL +/- platinum had received PEP02 with or without HDFL. The best response was partial response in one, stable disease in 4 and progressive disease in 2, which indicated a potential activity of PEP02 in treating gemcitabine-refractory advanced pancreatic cancer. Based on these clinical observations and preclinical results, clinical testing of nanoliposomal CPT-11 was pursued in patients with gemcitabine-based chemotherapy failure advanced pancreatic cancer in an international phase II trial with the target of the primary end-point of 3-month overall survival rate ($OS_{3\text{-month}}$) = 65%. The results have been presented at the 2011 ASCO meeting (30). Of the 40 treated patients, more than three fourths had failed to first-line gemcitabine-based doublet or triplet chemotherapy. Mean cycle of treatment



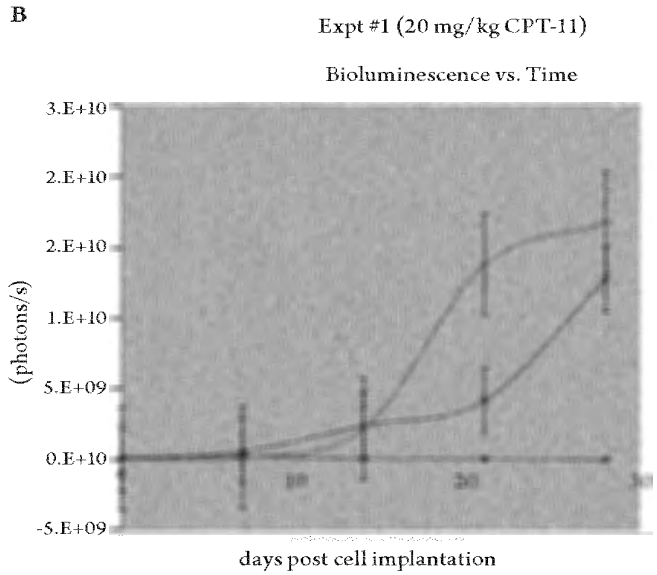


Figure 1 Nude mice were orthotopically implanted with COLO357/L3.6pI-T xenografts into the pancreas. Following ip administration of luciferin, animals were immediately imaged using a Xenogen IVIS 100 bioluminescence system, and subsequently imaged at weekly intervals. The signal was quantified by defining regions of interest (ROIs) and measuring photons/sec/str. Quantitative BLI values at post implantation day 7 were used to assign mice to treatment groups of five mice per group. Treatments included nanoliposomal CPT- 11 at 20 mg/kg, free CPT-11 at 20 mg/kg or vehicle control. All treatments were administered i.v. by tail vein injection beginning at 7 days post- tumor implantation and continued weekly for a total of 3 planned treatments. (A) Bioluminescence images of nude mice on weeks 1-7. (B) BLI values over time. Free CPT-11 treatment (diamonds) produced partial inhibition of tumor growth at initial time points, followed by rapid growth approaching that of the vehicle control group (+). Nanoliposomal CPT-11 treatment (circles) produced complete inhibition of tumor growth at all time points.

was 5.4 (range, 1 – 26) cycles. The most common G3/4 toxicities were: neutropenia (30%), leucopenia (22.5%), anemia (15%), diarrhea (7.5%), and fatigue (7.5%). Dose modification due to adverse events was required in 10 (25%) patients. The best tumor response rate was partial response in 7.5% and stable disease in 40% (overall disease control rate of 47.5%). The overall survival was 5.2 months with a 3-month and 6-month survival rate of 75% and 42.5%, respectively. The results highlight the feasibility and activity of nanoliposomal CPT-11 in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration.

Cationic Liposome Encapsulated Paclitaxel (EndoTAG™-I)

Tumor angiogenesis, the formation of neovasculature from pre-existed peri-tumor vessels, is a crucial process in supporting the development and growth of tumor mass, and the dissemination of tumor metastases. Tumor angiogenesis is mainly triggered by growth factors that are secreted by tumor cells per se and/or by miscellaneous types of cell within the microenvironment, for example, tumor associated macrophages or fibroblasts. Tumor vessels are often dilated and torturous, and characterized by large inter-endothelial cell gap (up to 100 – 600 nm *versus* < 6 nm in normal vessels), aberrant pericytes and basement membrane coverage, overexpression of specific surface receptor or antigen, and the presence of negative charged macro-molecules for example, anionic phospholipids and glycoprotein. Based on these characters, several strategies have been used to develop neo-vascular targeting liposomal drugs, which include conjugating with specific antibody again surface antigen or receptor and modified, non-functional receptor binding ligand, or incorporating positive (cationic) charged molecules in the surface of liposome. Of them, cationic liposome is a unique and interesting approach (31). In a preclinical study, Kalra and Campbell showed 5-FU and doxorubicin-loaded cationic liposome could preferentially bind with human endothelial (HMEC-1 and HUVEC) rather than pancreatic cancer cells. (HPAF-II and Capan-1)(32). Subsequently, Eichhorn et al showed that both cationic lipid complexed paclitaxel (EndoTAG™-1) and camptothecin (EndoTAG™-2) could preferentially bind at endothelial cells of neo-vasculature in solid tumor preclinical model (33-35). The selectively targeting of both agents on tumor microvasculature was confirmed by quantitative fluorescence microscopy. Further study suggested the anti-vascular effect of cationic liposome encapsulated paclitaxel (EndoTAG™-1) is schedule-dependent with metronomic schedule better than the maximum tolerated dose schedule. In addition, the combination of EndoTAG™-1 and gemcitabine could significantly inhibit the incidence of metastatsis in L3.6pl orthotopic pancreatic cancer mice model.

Based on these data, EndoTAG™-1, a cationic liposome (prepared from 1,2 dioleoyl-3-trimethyl- ammonium-propane (DOTAP) and 1,2 dioleoyl-sn-glycero-3-phosphocholine (DOPC)) encapsulated paclitaxel, has been used in combination with gemcitabine to treat chemo-naïve pancreatic cancer patients. The latest follow-up data of the four-arm randomized, phase II trial comparing weekly gemcitabine 1,000 mg/m^2 alone *versus* gemcitabine plus twice weekly EndoTAG™-1 at three different doses, 11, 22 and 44 mg/m^2) was presented in the 2009 ASCO Annual Meeting (36). Of the 200 chemo-naïve advanced pancreatic cancer patients who participated the study, 80% had metastatic diseases and 20% had locally advanced diseases. Disease-control rates in the gemcitabine monotherapy arm and the three gemcitabine plus EndoTAG-1 arms was 43% and ranging from 53% to 69%, respectively. The median progression-free survival time in corresponding group of patients were 2.7 months versus 4.1 to 4.6 months, respectively. The median overall survival time of patients receiving gemcitabine plus either high-dose (44 mg/m^2) or intermediate-dose of EndoTAG-1 were 9.4 months and 8.7 months, respectively, as compared with the 7.2 months in the gemcitabine monotherapy arm. The adjusted hazard ratio for overall survival for either arm was 0.72 (95% CI, 0.46 to 1.13) and 0.67 (95% CI, 0.43 to 1.07), respectively. The data is exciting but large-scale study to validate the data is mandatory.

Polymeric Micelles

Polymeric micelles-based anticancer drug, consisting of the incorporation of chemotherapeutic agent into polymeric micelles in size of 20–100 nm, was originally developed by Professor Kataoka(37). The polymeric micelle has two major components, a polyethylene glycol (PEG) constituted hydrophilic outer shell and a cytotoxic chemotherapeutic agent incorporated hydrophobic inner core. The main action mechanism of the polymeric micelles is similar to lipomosal agents and through the passive targeting based on the enhanced permeability of tumor neo-vasculature and the impeding clearance of macromolecules from lymphatic-deficient tumor interstitial tissue. Several cytotoxic chemotherapy-incorporating polymeric micellar nanoparticles have been in clinical trials, including paclitaxel-incorporating PEG-polyaspartate (NK105), cisplatin-incorporating PEG-polyglutamate/cisplatin complex (NC-6004) and SN-38-incorporating PEG-

ployglutamate/SN-38 (NK012). Of them, NC-6004 is currently evaluated in a phase Ib/II trial for patients with advanced pancreatic cancer, and will be discussed (38-41).

Cisplatin-incorporating Polymeric Micelles, NC-6004

In animal study, NC-6004 showed characteristic delayed total body clearance and higher area-under curve as compared with free cisplatin with a ratio of 1/19 and 65 folds, respectively (42). In addition, both histopathological and biochemical studies suggested NC-6004 significantly reduced cisplatin-associated nephrotoxicity. In phase I trial for patients with refractory advanced solid tumor, escalating dose of NC-6004 was administered intravenously every 3 weeks. Despite the implantation of pre-medication and post-therapy hydration, nephrotoxicity and allergic reaction were observed in patients receiving 120 mg/m^2 and further dose escalation was withheld. The MTD and the recommended dose were determined as 120 mg/m^2 and 90 mg/m^2, respectively. Pharmacokinetic study showed the maximum plasma concentration and area under curve of ultra-filterable platinum after 120 mg/m^2 of NC-6004 were 1/34 and 8.5 folds of those with free cisplatin (43). Seven out of 17 accruals achieved stable diseases, including two of two pancreatic cancer patients who had NC-6004 at dose level of 90 mg/m^2. Perhaps owing to earlier meta-analysis showed he combination of gemcitabine and platinum could significantly improved the overall survival of advanced pancreatic cancer patients as compared to gemcitabine monotherapy, NC-6004 is currently proceeded into a phase Ib/II trial to evaluate the maximum tolerated dose of NC-6004 in combination with gemcitabine and the therapeutic efficacy of the combination in patients with chemo-naïve advanced pancreatic cancer, clinicaltrials.gov identifier NCT00910741.

Rexin-G

Rexin-G is a highly engineered, nonreplicating retroviral vector displaying a von Willebrand factor–derived collagen-binding motif at its amphotropic envelope, and expressing a dominant negative cyclin G1 gene (44-46). This Willebrand factor-derived collagen-binding motif on the retrovector' s surface enables the nanoparticle drug to seek and be selectively delivered to primary and secondary tumor sites where angiogenesis and collagen matrix exposure characteristically occur. The encoded dominant negative cyclin G1 gene will thus to disrupt tumor cell cyclin G1 activity to lead to the destruction and/or growth inhibition of tumor.

There were two dose escalating phase I trials evaluating different dose/schedule of Rexin-G in patients with gemcitabine-failed advanced pancreatic cancer. The first trial evaluating 3 dose levels of Rexin-G administered intravenously, level I, 7.5 x 10^9 colony forming units (CFU) per day, days 1-7 and 15-21 every 28 days; level II, 1.1 x 10^{10} CFU per day, days 1-7 and 15-21 every 28 days; and level III, 3 x 10^{10} CFU per day, 5 days per week x 4 weeks/cycle with 6 weeks rest between two cycles. A total of 12 patients were enrolled, only one patient with dose-limiting toxicity manifesting as grade 3 transaminitis was observed at dose level II. However, the best tumor response was stable disease in one (8.3%) and the median time to tumor progression and overall survival of intent-to-treat population were 32 days and 3.5 months, respectively (47). In the second trial, the dose of Rexin-G was increased to 1 x 10^{11} CFU per day, twice or thrice per week for 4 weeks as one cycle (dose levels 0 and I), and 2 x 10^{11} CFU per day, thrice per week for 4 weeks as one cycle (dose levels II). A total of 13 patients were enrolled, 6 in dose level 0-I and 7 in dose level II. There was no DLT observed. On intent-to –treat analysis, the tumor control rate was 50% (3/6) and 85.7% (6/7 with one partial responder) of patients at dose level 0-I and II, respectively. The median overall survival in corresponding group of patients was 2.6 months and 9.3 months, respectively (48). Based on the results, the US FDA has granted Rexin-G fast-track designation as second-line treatment for pancreatic cancer in June 2009. Currently, a phase II/III pivotal two-arm randomized study aiming to validate the survival benefit of Rexin-G monotherapy *versus* physician's choice in gemcitabine-refractory pancreatic cancer is under discussion.

Conclusion

Systemic therapy for advanced pancreatic cancer has been largely disappointed owing to the unfavorable pharmacokinetic profile and poor penetration of current chemotherapeutic agents and the fragile patient population hard to tolerate toxic combination chemotherapy. Nanovector can provide passive or active targeting drug delivery to reduce the system exposure and enhance local drug retention in tumor tissue. In this review, we provide pre-clinical and clinical evidence to support the potential use of nanovector-based therapy in patients with advanced pancreatic cancer. Unfortunately, most of trials reported here are relatively small and without control group. Prospective, large-scale randomization trials are warranted to confirm their efficacy in this difficult tumor. In addition, the combination of the relatively low toxic nanoparticle drug with conventional cytotoxic agent and/or

Table 1 Nanovectors in pancreatic cancer treatment

Name	Compound	Nanocarrier	Size	Status
Abraxane™	Paclitaxel	Nanoparticle-albumin	130 nm	Phase I/II
Caelyx™	Doxorubicin	Liposome	100 nm	Phase I/II
Lipoplatin™	Cisplatin	Liposome	110nm	Phase I/II
Aroplatin™	Platunum	Liposome	1-3 μm	Phase I/II
MM-398	Irinotecan	Liposome	110±30 nm	Phase II
Endotag-1™	Paclitaxel	Liposome	180-200 nm	Phase II
Nanoplatin™	Cisplatin	Polymer Micelle	30 nm	Phase I/II
Rexin-G™	Cyclin G1 gene	Viral vector	110 nm	Phase I/II

recently emergent molecular targeted agent should also be investigated to improve the clinical outcomes of patients with advanced pancreatic cancer.

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A multinational phase II study of liposome irinotecan (PEP02) for patients with gemcitabine-refractory metastatic pancreatic cancer.

Meeting:
2011 Gastrointestinal Cancers Symposium

Category:
Cancers of the Pancreas Small Bowel and Hepatobiliary Tract

Subcategory:
Multidisciplinary Treatment

Session Type and Session Title:
General Poster Session B

Abstract Number:
237

Citation:
J Clin Oncol 29: 2011 (suppl 4; abstr 237)

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Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved pharmacokinetics and tumor biodistribution of both CPT-11 and its active metabolite-SN38 compared to the free form drug. PEP02 has showed encouraging safety and efficacy in various tumor types, including significant antitumor activity in a human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model. In previous phase I studies, PEP02 either alone or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC). This phase II study aims to evaluate PEP02 monotherapy as 2^{nd}-line treatment in pts with metastatic, GEM-refractory PC.
Methods: Pts were eligible if they had metastatic pancreatic adenocarcinoma, KPS $\geq$ 70, and progressed following one line of GEM-based therapy. Treatment consisted of PEP02 120 mg/m^2 administered as a 90-minute infusion every 3 weeks. A Simon's 2-stage design was used with 16 pts in the first stage and 39 pts in total; primary objective was 3-month survival rate ($OS_{3\text{-month}}$).
Results: Between March 2009 and August 2010, 37 pts were enrolled at 3 centers in the U.S. and Taiwan. Characteristics for the first 31 evaluable pts: 13 M/18 F; age 39-82 yrs; 19 Asian/12 Caucasian, KPS 100/90/80/70: 5/14/4/8. Mean number of treatment cycles is 5 (range, 1-22). Disease control rate (minor response + stable disease >2 cycles) is 52%. 8 of 24 pts (33%) with elevated baseline CA19-9 have had >50% biomarker decline. To date, 23/31 pts (74%) have survived > 3 months, with 4 pts still alive after 1 year. Reasons for study discontinuation: 74%

progressive disease, 9% drug-related toxicity, 17% other. Preliminary safety data is available for the first stage. Most common G3/4 adverse events included: fatigue (31%), neutropenia (25%), nausea/vomiting (19%), and diarrhea (13%). **Conclusions:** This study has already met its primary endpoint (predicted $OS_{3\text{-month}}$ >65%). PEP02 appears to have both activity and tolerable side effects for pts with metastatic, GEM-refractory PC, and represents a promising option for this pt population with few standard options.

View of NCT01494506 on 2013_01_25

ClinicalTrials Identifier: NCT01494506
Updated: 2013_01_25

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 400 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

CARPMAELS & RANSFORD

EUROPEAN PATENT 2 861 210

IPSEN BIOPHARM LTD.

PATENT PROPRIETOR'S RESPONSE TO THE NOTICE OF OPPOSITION

1 INTRODUCTION

1.1 European patent 2 861 210 ("the patent") owned by Ipsen Biopharm Ltd. ("the proprietor") has been opposed by Teva Pharmaceuticals Inc. ("the opponent"). This is the proprietor's response to the opposition.

1.2 The claimed invention is embodied by the medicine Onivyde®, which was approved by the FDA in the United States in October 2015. Onivyde® was also approved by the European Commission on 14th October 2016 (D17). Treatment with the Onivyde® regimen substantially prolongs the lives of patients suffering from an aggressive and difficult-to-treat form of cancer, namely pancreatic cancer.

2 REQUESTS

2.1 The proprietor's Main Request is for the patent to be maintained on the basis of the enclosed Main Request claims labelled "Main Request – August 2018".

2.2 Should the Opposition Division be considering anything other than the maintenance of the patent of the basis of the Main Request, oral proceedings are requested.

2.3 The proprietor requests permission to make further amendments to the Main Request, or to submit one or more auxiliary requests in future in response to points raised by the opponent and/or the Opposition Division.

2.4 Should the Opposition Division deem amendment to the description necessary, the proprietor requests that any such amendment be deferred until agreement has been reached on the claims.

2.5 For the avoidance of doubt, any unclaimed or deleted subject matter is not abandoned.

3 DOCUMENTS ENCLOSED

- D15a – "Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer" clinicaltrials.gov posting NCT01494506 as updated on 2011_12_16

  The document cited as "D15" by the opponent relates to a later version of D15a. The document cited by the opponent has been relabelled D15b.

- D17 – Commission Implementing Decision and Annexes (Summary of Product Characteristics for Onivyde®).

- D18 – "FDA approves new treatment for advanced pancreatic cancer" (2015), FDA News Releases

- D19 – "Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial", *Lancet*, 2016 Feb 6;387(10018):545-57
- D20 – MHRA Public Assessment Report for 5-FU (2006)
- D21 – EP 1 210 115 B1 with relevant parts highlighted
- Annex I – consolidated list of citations

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4 SUMMARY OF THE INVENTION

4.1 The invention relates to a new treatment for pancreatic cancer. Due to the location of the pancreas, pancreatic cancer is typically not diagnosed until a tumour has become large enough to produce systemic symptoms (paragraph 0002 of the patent). For at least this reason, patients usually have advanced, often metastatic, disease at the time of diagnosis, which had led to a dismal overall survival rate (*ibidem*).

4.2 The standard of care in first-line treatment for pancreatic cancer at the priority date was gemcitabine (as a monotherapy or in combination with erlotinib - see paragraphs 0005-0006). There was no standard of care - or even an approved treatment - for patients who had failed gemcitabine therapy, and clinical trials involving other agents had been disappointingly negative (paragraph 0006). There was thus a long-felt need for a treatment for patients whose pancreatic cancer had failed to respond to gemcitabine.

4.3 The claimed dosage regimen satisfies this long felt need, and its significant contribution to the art has been recognised by the wider scientific community (D18 and D19), not least because it provides the first approved second-line treatment for this previously-underserved group of patients. It was also awarded priority review status by the FDA in the United States. A priority review designation is awarded by the FDA for drugs that, if approved (as the claimed therapy was), would result in a significant improvement in safety or effectiveness in the treatment of a serious condition (D18).

4.4 The claimed dosage regimen was approved by the EMA and the FDA following a pivotal Phase III trial, referred to as NAPOLI-1, which when conducted was the largest ever Phase III study in metastatic pancreatic cancer of post-gemcitabine-based therapy. The results of this trial confirmed that the regimen of claim 1 of the patent is superior when compared to the control arm of the trial (5-flurouracil and leucovorin – hereafter "5-FU/LV" – see D19). These results confirmed the findings outlined in the patent specification, that is, that the presently claimed regimen is suitable for treating pancreatic cancer.

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5 MAIN REQUEST - AMENDMENTS (ART. 123(2) EPC)

5.1 The claims of the Main Request are based on the claims as granted, with an additional amendment which has been made in response to the attacks raised by the opponent.

5.2 In claim 1, step (c) has been amended to delete the text "or 400 mg/m^2 (l + d racemic form)":

"leucovorin is administered at a dose of 200 mg/m^2 (l form) ~~or 400 mg/m^2 (l+ d racemic form)~~"

5.3 In claim 1 as granted and the application as filed, "*200 mg/m^2 (l form)*" of leucovorin and "*400 mg/m^2 (l +d racemic form)*" of leucovorin are presented as alternatives (see, for example, page 3, final paragraph; page 5, fourth paragraph; page 14, final paragraph; and claims 3 and 12). Following the Guidelines (H-V, 3.3) the deletion of one of these two alternatives does not contravene Article 123(2) EPC.

5.4 The claims of the Main Request therefore do not add subject matter.

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6 MAIN REQUEST - PRIORITY

6.1 The opponent has stated that the patent's first priority claim is invalid. The proprietor disagrees. Nevertheless, in view of the amendments made in the enclosed Main Request, the proprietor's case is even stronger. In particular, it will be explained below that the first priority document (US61/659,211, hereafter "US'211") relates to the "same invention" as that claimed in the Main Request, meaning that the priority claim is valid.

The subject matter of claim 1 finds basis in US'211

6.2 Claim 1 of the Main Request relates to liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient. The method comprises:

"*co-administration 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 UGT1A 1 *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)".

This feature finds basis in claim 3 of US'211.

6.3 The final integer of claim 1:

"*and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU*"

finds basis in claim 4 of US'211.

6.4 The patient population specified in claim 1:

"*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"

finds basis on page 11 (final sentence) and page 12 (second sentence) of US'211.

6.5 Claim 1 of the Main Request specifies that the 200 mg/m^2 of leucovorin is the "l-form", whereas US'211 refers to "leucovorin" only, but this difference is not prejudicial to the validity of claim 1's priority claim to US'211.

6.6 The leucovorin molecule is optically active. This means that a given molecule of leucovorin can exist either as the l-form or as the d-form. The l-form and the d-form can be referred to as "optical isomers". At the filing date of US'211, leucovorin was commercially available as either the l-form (also referred to as "levoleucovorin" or "l-leucovorin") or as a 50:50 isomeric mixture of the l- and d-forms (also referred to as "l+d leucovorin" or "racemic leucovorin"). Of the two optical isomers, the l-form of leucovorin is pharmaceutically active, and the d-form is not (see paragraph 0042 of the patent and D1 at section "11 DESCRIPTION"). This is why the pure d-form of leucovorin was not approved at the priority date, nor has it been approved at the time of writing.

6.7 Based on this common general knowledge, the skilled person considering US'211 at the priority date would have considered the generic term "leucovorin" in US'211 to be a reference to either "l-leucovorin" or "racemic leucovorin" because these were the only two forms of "leucovorin" which were available. That is to say, the term "leucovorin" in US'211 when read by the skilled person constituted an implicit, direct and unambiguous, disclosure of "l-leucovorin or racemic leucovorin".

6.8 Claim 1 of the Main Request is limited to "l-leucovorin". Claim 1 therefore finds basis in US'211 because it merely specifies "l-leucovorin" as opposed to the "l-leucovorin or racemic leucovorin" disclosed in US'211. Claim 1 does not contain subject matter which is not disclosed in US'211. Claim 1 therefore relates to the "same invention" as that disclosed in US'211, meaning that claim 1 validly claims the priority date of 13th June 2012.

6.9 This approach is consistent with the Board of Appeal's case law on optically active compounds. For example, in T658/91 it was held that an example in a prior art document which disclosed a racemic compound anticipated a claim to a single optical isomer of the compound because it was known in the art that the racemic compound could exist either as a racemate or as each single optical isomer. Similar reasoning was given by the Board in T600/95. Following this reasoning, it is clear that the disclosure of "leucovorin" in US'211, which would be read as "l-leucovorin, or racemic leucovorin" because these reflected the only optical isomers of leucovorin which were known in the art to be therapeutically active at the priority date, supports the priority of the claim.

The dependent claims find basis in US'211

6.10 Claim 2 finds basis in claims 5, 7 and 8 of US'211.

6.11 Claim 3 finds basis in claims 6, 9 and 10 of US'211.

6.12 Claim 4 finds basis in claim 11 of US'211.

6.13 Claim 5 finds basis on page 12, fourth sentence of US'211.

Priority Conclusion

6.14 The claims of the Main Request are entitled to the earliest priority date of 13th June 2012. This means that documents D4, D8, D10 and D15b are not prior art because these documents were made available to the public after 13th June 2012.

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7 INVENTIVE STEP

7.1 The opponent's inventive step attacks can be summarised as follows:

i. Claim 1 lacks inventive step in view of D12/D13.

ii. Claim 1 lacks inventive step in view of D12/D13 in combination with D15b.

iii. Claim 1 lacks inventive step in view of D15b in combination with D12/D13.

7.2 As explained above in section 6, the Main Request validly claims its earliest priority date of 13th June 2012, meaning that document D15b is not prior art. Therefore it is only necessary to consider attack (i) in this section. However should the Opposition Division unexpectedly take the view that the Main Request does not validly claim its earliest priority date, it will also be shown that attacks (ii) and (iii) do not prejudice the maintenance of the patent on the basis of the Main Request.

7.3 In the table in paragraph 2 of the opposition statement, the opponent states that document D12 is a reference to Tsai et al. and that D13 refers to Ko et al. The opponent reverses this notation in paragraph 15, where D12 is said to be a reference to Ko et al. and D13 is said to be Tsai et al. In this section, the proprietor will refer to Ko et al. as D12, and Tsai et al. as D13. In addition, the document referred to as "D15" in the opposition statement will be referred to as "D15b" hereafter. To assist the Opposition Division, Annex I is enclosed which lists all of the citations filed by the opponent and by the proprietor, and which gives each document a unique D-number.

Claim 1 is inventive in view of D12/D13

7.4 In paragraph 15, the opponent has stated that either of D12 or D13 could be considered as the closest prior art.

7.5 D12 states in the "Background" section that PEP02 (a liposomal irinotecan formulation) had been administered both in combination with 5-FU/LV (5-fluorouracil and leucovorin) and as a monotherapy in previous Phase I trials, and states that these studies had demonstrated "disease control" in patients with gemcitabine-refractory advanced pancreatic cancer. When discussing the PEP02/5-FU/LV combination regimen, details of the regimen are not disclosed.

7.6 Contrary to the opponent's statement, D12 does not state that "the combination … demonstrated prolonged disease control in 5 out of 7 (71%) patients with gemcitabine refractory pancreatic cancer" (see paragraph 15 of the opposition statement). In making this statement the opponent is reporting on the disclosure of D12 in a misleading manner. The passage of D12 from which the opponent is reporting is reproduced below:

> "*In previous phase I studies, PEP02 either **alone** **[i.e. as a monotherapy]** or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC).*" [emphasis added]

7.7 The opponent's discussion of D12 omits the fact that the "Background" section discusses liposomal irinotecan **monotherapy** together with the combination regimen (indeed, the study which D12 reports on relates to such a **monotherapy** only). More so, the opponent's discussion misrepresents the Phase I data given in D12, by implying that the "prolonged disease control in 5 out of 7 (71%) patients" is solely a reference to the results obtained for patients given the combination regimen. In actual fact, the "5 out of 7" patients is a reference to the results obtained for both patients given the monotherapy and for patients given the combination regimen. That is, when the patient populations for the monotherapy study and the combination regimen study were combined, there were seven patients in total with gemcitabine-refractory advanced pancreatic cancer, and five of these patients were said to exhibit "prolonged disease control". However, based on D12 alone it is not possible to determine how many of these five patients were given the combination regimen, or indeed if any of the five patients were given the combination regimen. Therefore the opponent has mischaracterised the disclosure of D12.

7.8 After discussing a PEP02/5-FU/LV combination regimen in the "Background" section, the remainder of D12 reports data from a multinational Phase II trial of PEP02 monotherapy in the second-line treatment of patients with metastatic, gemcitabine-resistant pancreatic cancer. In the trial, subjects were administered 120 mg/m^2 PEP02 as a monotherapy every three weeks. D12 concludes by stating that PEP02 monotherapy "appears to have both activity and tolerable side-effects" and that it represents a "promising option" for patients with metastatic gemcitabine-refractory pancreatic cancer.

7.9 The skilled person would have noted from D12 that only liposomal irinotecan monotherapy was taken forward in a Phase II trial, where it appeared to have activity and tolerable side effects. The combination regimen of liposomal irinotecan and 5-FU/LV that had also been tested in Phase I was not taken forward to Phase II. The skilled person would have concluded that the previously-tested liposomal irinotecan and 5-FU/LV combination regimen was deliberately not taken forward to Phase II trials because it was in some way inadequate. The skilled person would have been aware that, unlike the monotherapy Phase I trial in which one of the pancreatic patients was taught to have shown a promising, partial response (which is referred to as the "best response"), none of the pancreatic cancer patients in the Phase I trial of the combination regimen was taught to have shown a partial response (see D13 and 7.26 below). Furthermore, as no safety data for the combination regimen were given in D12, it would have been reasonable for the skilled person to conclude that the combination regimen showed unacceptable levels of adverse events without sufficient signs of activity. In other words, that there were insufficient signs of activity at the maximum tolerated dose.

7.10 It is therefore clear from D12 that the most promising starting point for further development would have been the liposomal irinotecan monotherapy regimen disclosed in D12 because this is the only regimen that had reported data for activity and safety in Phase II trials. No such activity had been shown for the combination regimen in Phase II, or indeed in any phase of clinical trials. Moreover, no safety data had been shown for the combination regimen in any

phase. Thus the skilled person would not have considered the combination regimen of D12 to be a promising starting point, and certainly not the most promising starting point.

7.11 D13 cites D12 (see reference (30) of D13) and concludes that the Phase II trial reported in D12 highlights the feasibility and activity of the liposomal irinotecan monotherapy regimen in the treatment of metastatic gemcitabine-refractory pancreatic cancer. While D13 adds little information about a Phase I combination regimen, it states that therapy involved "weekly 24-hour infusion of high-dose 5-FU/LV (HDFL)" (underlining added to emphasise a further difference versus the claimed regimen; the latter requires a cycle of two weeks). Again, D13 (page 189, bottom-right hand paragraph) indicates that the most promising starting point for development would have been the liposomal irinotecan monotherapy regimen administered every three weeks, not the combination regimen studied in the Phase I trial.

7.12 As D12 and D13 report on the same Phase II clinical trial of liposomal irinotecan monotherapy, they will be considered together in this section and will be referred to as "D12/D13".

Distinguishing features

7.13 Claim 1 differs from D12/D13 at least because it relates to a dosage regimen for treating pancreatic cancer in which:

liposomal irinotecan, 5-FU and LV are co-administered in at least one cycle, wherein the cycle is a period of two weeks;

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);

wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

7.14 In other words, the claimed regimen differs from the Phase II trial discussed in D12/D13 at least because:

- Claim 1 requires the co-administration of liposomal irinotecan, 5-FU and LV. The Phase II trial discussed in D12/D13 is a liposomal irinotecan monotherapy;
- Claim 1 requires the three drugs to be administered in at least one two-week cycle. D12/D13 mentions irinotecan administration "every 3 weeks". Moreover, D13 teaches that 5-FU and LV administration in the Phase I combination regimen was "weekly";
- Claim 1 requires liposomal irinotecan to be administered at a dose of 80 mg/m^2 on day 1 of each cycle to patients not homozygous for the UGT1A 1 *28 allele, and for liposomal irinotecan to be administered at a dose of 60 mg/m^2 on day 1 of each cycle for patients homozygous for the UGT1A1 *28 allele and at a dose of 60 mg/m^2 or 80 mg/m^2 on day 1 of each subsequent cycle. The Phase II monotherapy trial reported in D12/D13 administered 120 mg/m^2 to all subjects;

- Claim 1 requires 5-FU to be administered at a dose of 2400 mg/m^2. D12/13 does not specify the dose of 5-FU used in the Phase I combination regimen trial, while the Phase II monotherapy trial reported in D12/D13 does not use 5-FU at all;
- Claim 1 requires leucovorin (l-form) to be administered at a dose of 200 mg/m^2. D12/13 does not specify the dose of leucovorin used in the Phase I combination regimen trial, while the Phase II monotherapy trial reported in D12/D13 does not use leucovorin at all;
- Claim 1 requires the liposomal irinotecan to be administered prior to the leucovorin, and the leucovorin to be administered prior to the 5-FU. The Phase II trial reported in D12/D13 uses a monotherapy regimen, so there is no order of administration. Furthermore, D12/13 does not specify the order of drug administration for the combination regimen Phase I trial.

Technical effects

7.15 As stated above, liposomal irinotecan for use as recited in claim 1 was approved following a pivotal Phase III trial, referred to as NAPOLI-1. The protocol for this trial is explained in detail in Example 7 of the patent. The results of this trial show that the claimed invention is associated with a number of beneficial technical effects.

7.16 In the NAPOLI-1 trial, and as explained in section B of Example 7, patients were separated into three arms. Patients in Arm A were administered 120 mg/m^2 of liposomal irinotecan (referred to as "MM-398" in Example 7) over 90 minutes every three weeks. Arm B was referred to as the control arm, in which patients were treated with 5-FU/LV. Patients in Arm C were administered 80 mg/m^2 of liposomal irinotecan over 90 minutes every two weeks in combination with 5-FU/LV according to claim 1. The results from each of Arms A and C were individually compared with those from Arm B.

The claimed dosage regimen shows improved efficacy

7.17 Several efficacy endpoints were considered in the NAPOLI-1 trial. The primary endpoint (i.e. the endpoint which was designed to ultimately show whether the therapy is efficacious) was overall survival (OS). OS is defined in paragraph 0164 of the patent as the time from the date of patient randomisation to the date of death or the date the patient was last known alive. The results of the trial showed a clinically relevant and statistically significant superiority in overall survival in patients receiving the liposomal irinotecan and 5-FU/LV combination regimen according to claim 1 (the median OS was 6.1 months) relative to patients receiving the 5-FU/LV control arm (the median OS was 4.2 months). By contrast, patients in the liposomal irinotecan monotherapy arm did not show superiority in overall survival relative to the 5-FU/LV control (4.9 months versus 4.2 months). These data alone demonstrate the advantageous efficacy of the claimed combination regimen, and were specifically cited by the FDA in its press release issued following the approval of Onivyde® (see D18).

7.18 In addition to the above, the claimed combination regimen also demonstrated its efficacy when the secondary endpoints were considered.

7.18.1 When progression free survival (PFS – defined in paragraph 0169 as "the number of months from the date of randomization to the date of death or progression, whichever occurred

earlier") data are considered, patients treated with the combination regimen according to claim 1 achieved a median PFS of 3.1 months – approximately twice that of the patients in the 5-FU/LV control arm who exhibited a median PFS of 1.5 months. By contrast, patients treated with liposomal irinotecan monotherapy showed a median PFS of 2.7 months compared to 1.6 months seen in the control arm.

7.18.2 Patients in the liposomal irinotecan and 5-FU/LV combination therapy arm achieved a median time to treatment failure (TTF – defined in paragraph 0171 as "the time from randomisation to either disease progression, death or study discontinuation due to toxicity") of 2.3 months compared to a median TTF of 1.4 months for patients in the 5-FU/LV control arm. By contrast, patients in the liposomal irinotecan monotherapy arm had a TTF of 1.7 months which was similar to the 5-FU/LV control (1.4 months).

7.18.3 Patients in the combination therapy arm showed an improved objective response rate (ORR – discussed in paragraphs 0172 and 0173) over the 5-FU/LV control arm (16% vs. 1%, respectively). Patients in the monotherapy arm showed an ORR of 6% relative to the 1% seen in the control arm.

7.18.4 Patients in the combination arm according to claim 1 showed lower levels of the pancreatic cancer tumour marker CA19-9 (discussed in paragraph 0174) than patients in the 5-FU/LV control arm. Of the patients with elevated baseline CA19-9 who were treated in the study, 29% of patients treated with the liposomal irinotecan and 5-FU/LV combination regimen of claim 1 showed reductions of ≥50% from baseline levels, compared to 9% of patients in the 5-FU/LV control arm. Out of the patients with elevated baseline CA19-9 levels in the monotherapy arm, 24% of these patients showed reductions of ≥50% versus 11% seen in the control arm.

7.18.5 In the patient reported outcome analysis (discussed in paragraph 0175), there were no substantial differences in patient quality of life between the three arms, indicating that the increased efficacy of the claimed combination regimen surprisingly does not have a detrimental effect on patients' quality of life.

7.19 By way of a summary, the efficacy data endpoints from the NAPOLI-1 trial are reproduced in the table below. These data demonstrate that the claimed combination therapy regimen (right-hand column) is associated with therapeutic advantages without having a detrimental effect on patients' quality of life. The data reproduced below are also discussed in D19 (copy enclosed).

	Arm A (monotherapy)	Arm C (combination)
Median Overall survival / months (Arm B value)	4.9 (4.2)	6.1 (4.2)
Median progression-free survival / months (Arm B value)	2.7 (1.6)	3.1 (1.5)
Median time to treatment failure / months (Arm B value)	1.7 (1.4)	2.3 (1.4)
Overall response rate / % (Arm B value)	6.0 (1)	16 (1)
Patients showing ≥50% reduction in CA19-9 levels / % (Arm B value)	24 (11)	29 (9)

The efficacy of the claimed dosage regimen was not accompanied by an increase in treatment emergent adverse events (TEAEs)

7.20 Given the above improved overall survival data, the claimed combination regimen was also surprisingly associated with a lower frequency of serious treatment emergent adverse events (TEAEs – discussed in paragraph 0176) than the liposomal irinotecan monotherapy regimen. In the NAPOLI-1 trial, 61% of patients in the liposomal irinotecan monotherapy arm experienced a serious TEAE, compared with the combination therapy arm, where the percentage of patients experiencing a serious TEAE was similar to that of patients in the 5-FU/LV control arm (48% for the combination therapy and 45% for the control). This demonstrates that the efficacy of the claimed combination regimen did not come at the cost of additional TEAEs.

7.20.1 For example, patients treated with the combination regimen according to claim 1 also had less frequent severe diarrhoea than patients in the liposomal irinotecan monotherapy arm (13% of patients vs 21% of patients, respectively). As mentioned in paragraphs 0119 and 0120, irinotecan was known to induce both early and late forms of diarrhoea, the latter of which can be life-threatening. Thus, any regimen which is both therapeutically efficacious whilst causing less frequent diarrhoea would be greatly advantageous. The reduced frequency of severe diarrhoea in the combination arm coupled with the combination regimen's therapeutic efficacy would have come as a surprise to the skilled person, not least because diarrhoea is also a "frequently reported" side effect of 5-FU administration (D20).

7.20.2 Further, patients treated in the liposomal irinotecan/5-FU/LV combination arm showed a lower frequency of alopecia compared with patients in the liposomal monotherapy arm (14% vs. 22%, respectively).

7.21 For ease of reference, the safety data discussed above are summarised in the below table. These data are also discussed in D19.

	Arm A (monotherapy)	Arm B (control)	Arm C (combination)
Frequency of serious TEAEs (%)	61.2	44.8	47.9
Frequency of patients experiencing severe diarrhoea (%)	21	5	13
Frequency of patients experiencing alopecia (%)	22	5	14

7.22 In summary, the technical effect achieved by claim 1 is an improved therapy for gemcitabine-resistant pancreatic cancer.

Objective technical problem

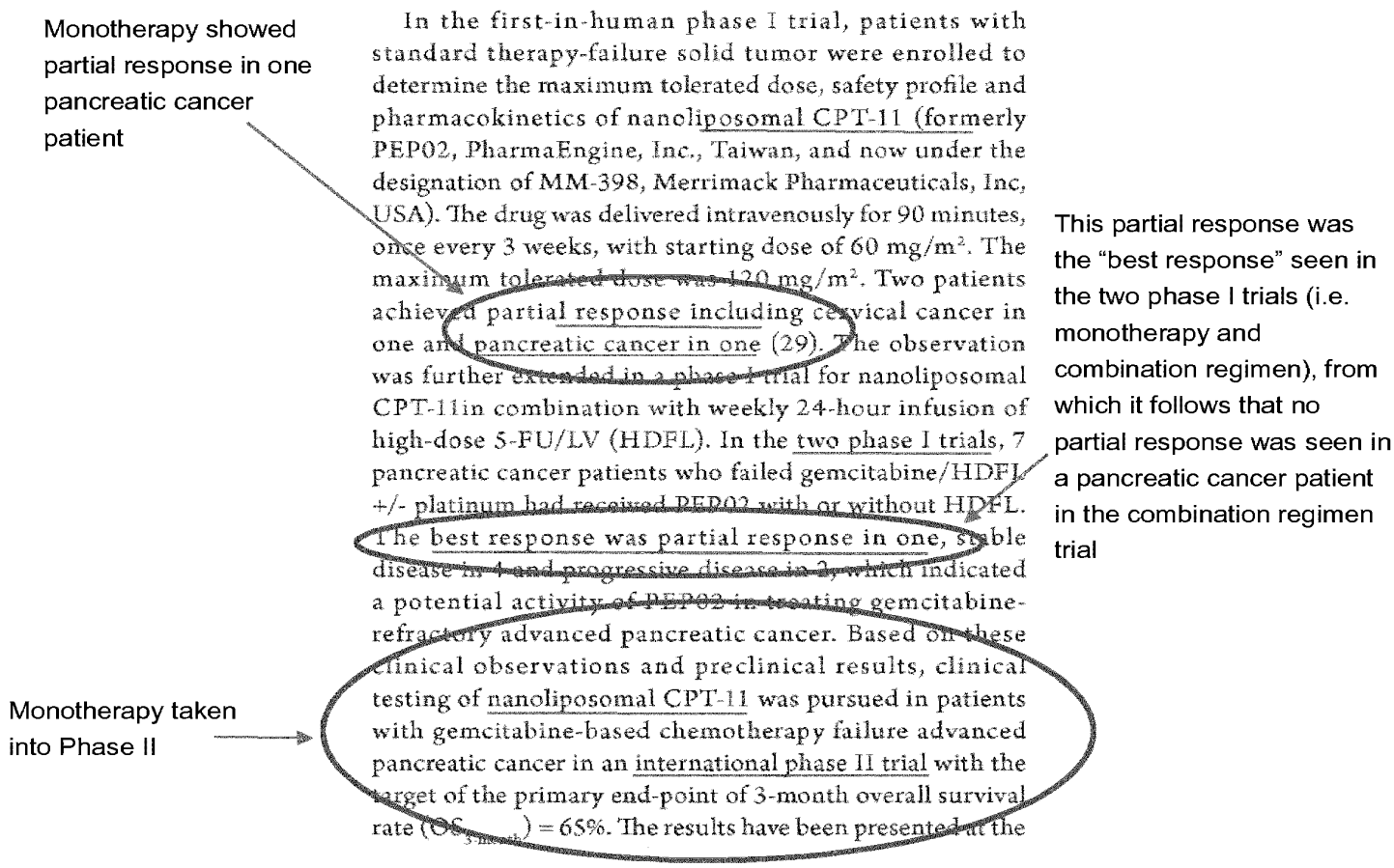
7.23 The objective technical problem is the provision of an improved therapy for gemcitabine-resistant pancreatic cancer. As explained above, this problem is solved by claim 1.

The claimed solution is not obvious

7.24 When faced with D12/D13 and the objective technical problem, there would have been nothing that would have motivated the skilled person to solve it in the manner claimed. D12/D13 direct the skilled person to a dosage regimen which is totally different from the one presently claimed (a monotherapy which, inter alia, uses different doses and a different administration frequency from those used in the claimed combination regimen), and the opponent's arguments are based on a hindsight interpretation of D12/D13.

7.25 D12/D13 do mention that liposomal irinotecan, both alone and in combination with 5-FU/LV, had been studied in Phase I trials. The dosage regimen used in the Phase I combination trial is not fully disclosed. In the specific context of the Phase I trial, no explicit comparisons between the monotherapy and the combination regimen are made, and at no point is the combination regimen said to be in any way preferable over the monotherapy. In fact, due to its simplicity and the desire to avoid administering additional drugs unnecessarily, the skilled person would, other things being equal, have wished to take forward the monotherapy rather than the more complex combination regimen. Reasons why the skilled person would not have developed a combination regimen are elaborated on below, e.g. in paragraph 7.29 below.

7.26 After certain Phase I data are briefly mentioned in D12/D13, the discussion moves on to the Phase II monotherapy trial, and the combination regimen is not mentioned again. In clinical research, a Phase II trial is carried out after a successful Phase I trial, and aims to establish whether a drug or dosage regimen is sufficiently efficacious to be moved forward to a larger scale Phase III trial, the results of which may lead to an application for authorisation to market the drug or dosage regimen. In the present case, the monotherapy regimen was taken forward to Phase II, consistent with the partial response observed in a patient with refractory pancreatic cancer in the Phase I monotherapy trial but not the Phase I combination regimen trial; see D13, page 189, right hand column; emphasis added:



In fact, D12/D13 do not present any efficacy data about the Phase I combination regimen.

7.27 The results of the Phase II trial of the monotherapy regimen are discussed in D12/D13, which concludes that liposomal irinotecan monotherapy "appears to have both activity and tolerable side effects" for patients with metastatic, gemcitabine-refractory pancreatic cancer (D12), and that "[t]he results highlight the feasibility and activity of nanoliposomal CPT-11 [i.e., liposomal irinotecan monotherapy] in previously heavily treated patients with gemcitabine-refractory advanced pancreatic cancer, which deserves further exploration." (D13 – page 191, fifth complete sentence).

7.28 The skilled person seeking to provide an improved therapy for gemcitabine-resistant pancreatic cancer is thus taught by D12/D13 that a liposomal irinotecan monotherapy should be developed. A move to a combination regimen, which was not taken forward after Phase I, would have been viewed as a backward step, obvious only with the benefit of hindsight. Thus, D12/D13 teaches the skilled person away from the claimed combination dosage regimen, and claim 1 involves an inventive step for at least this reason.

7.29 Developing the liposomal irinotecan monotherapy regimen in an attempt to solve the objective technical problem would have been consistent with the skilled person's common general knowledge. As mentioned above, the skilled person would have been aware that irinotecan is known to cause, amongst other things, both the early and late forms of diarrhoea, the latter of which can be life threatening. 5-FU was also known to cause diarrhoea (D20). Thus, the skilled person seeking an improved therapy would have been drawn to liposomal irinotecan monotherapy, as s/he would have had a strong motivation to avoid the combination regimen

which combines two drugs which are both known to be associated with a potentially lethal adverse event (diarrhoea). This is particularly pertinent given that the monotherapy regimen, which did not require the use of multiple drugs, had shown safety and efficacy in both Phase I and Phase II trials, and was known to be the subject of a Phase III trial at the priority date (see paragraph 0007 of the patent, which refers to this trial, whose protocol is given in D15a).

7.30 The skilled person would also have been attracted to develop the liposomal irinotecan monotherapy because it is a simpler regimen, which requires an infrequent (every three weeks) and relatively brief 90 minute administration of one drug only. In addition, the omission of 5-FU from the regimen would have meant that an infusion pump would not need to be used in the regimen.

7.31 Furthermore, D12/D13 teach the skilled person that the liposomal irinotecan should be administered once every three weeks at a dose of 120 mg/m^2 (see the "Methods" section of D12, for example). There is nothing in D12/D13 which would have motivated the skilled person to administer liposomal irinotecan (as a monotherapy or in combination with other drugs) once every two weeks at a dose of 80 mg/m^2, as required by claim 1, with an expectation of providing an improvement.

The opponent's arguments are based on hindsight

7.32 The opponent's arguments suggest that the skilled person faced with D12/D13 would have developed the claimed combination regimen of liposomal irinotecan and 5-FU/LV. The opponent is suggesting that the skilled person would have totally ignored the only positive Phase II data in D12/D13, which was for the monotherapy regimen, and instead would have arbitrarily chosen to focus his/her attention on a poorly-specified combination regimen that had been tested only in Phase I, and for which no data are given. No justification is given as to why the skilled person would have focussed on a poorly-specified combination regimen tested only in Phase I which was not taken forward to Phase II. It is therefore clear that the opponent's arguments are a result of ex post facto analysis which is forbidden under the problem-solution approach.

7.33 The opponent's ex post facto analysis can also be seen in its formulation of the objective technical problem as "to provide a suitable dosage regimen for the combination regimen described in either D12 or D13". This problem has been formulated to contain a pointer to the solution (the use of a combination regimen). It is established case law of the Boards of Appeal that formulating the problem in this way is impermissible (Case Law of the Boards of Appeal, 8th Ed., I.D.4.3.1).

7.34 If the problem-solution approach is to be applied correctly, avoiding ex post facto analysis, it is necessary to consider whether the skilled person would have carried out the invention in the hope of solving the underlying problem, not whether s/he merely could have done so. Because the present invention relates to a commercially successful pharmaceutical treatment regimen which is now known to have made a valuable contribution to the art, and because liposomal irinotecan was known (but not approved as a medicament) at the priority date, there is a particular risk of ex post facto analysis in this case. Thus, it must be borne in mind at all times whether the skilled person would have taken a particular course of action when seeking to solve the above-mentioned problem (see Case Law of the Boards of Appeal, I.D.5). The opponent's arguments incorrectly focus on what the skilled person could have done, rather than what s/he would have done in the hope of solving the objective technical problem.

The case law cited by the opponent is not relevant to the present case

7.35 In T1409/06, which is relied on by the opponent, it was stated by the Board that the use of a "1 mg to 3 mg unit dose" of an active substance was an obvious solution to the problem of "optimising the effect" of the active substance. In T1409/06, there was no evidence for any additional non-obvious effects associated with the 1 mg to 3 mg dose range.

7.36 The claimed subject matter is not concerned with merely optimising the effect of a single active substance. Rather, it is concerned with providing a new treatment option for patients with gemcitabine-resistant pancreatic cancer. For example, it involved taking the apparently retrograde step of moving to a combination regimen, even though the only prior art teaching of a poorly-specified combination regimen was not taken beyond Phase I.

7.37 Moreover, there are several non-obvious effects associated with the claimed dosage regimen, which are discussed above. These beneficial effects led to the claimed regimen's significant contribution to the art being recognised by the wider scientific community (see D18– see paragraph 7.38 immediately below). This further justifies the inventive merit of the invention (T915/00).

The claimed subject matter satisfies a long-felt need

7.38 As alluded to above, in the prior art, pancreatic cancer patients who had failed gemcitabine-based first line therapy had no established options for further treatment. Whilst incidences of pancreatic cancer had markedly increased in the past several decades, efforts to evaluate other agents for use in second-line therapy had been disappointingly negative (see the patent, paragraphs 0002 and 0006). The state of the art had thus been inactive for a long period in spite of the fact that there was an urgent need in this period for a second-line therapy for pancreatic cancer. The claimed invention provides the first (and, thus far, only) approved treatment for patients who have progressed following gemcitabine-based therapy, and therefore the invention satisfies a long-felt need (Case Law of the Boards of Appeal, I.D.10.4).

Conclusion

7.39 When faced with D12/D13 and the objective technical problem mentioned above, there was nothing that would have motivated the skilled person to solve it in the manner required by claim 1, not least because the prior art taught that liposomal irinotecan monotherapy should be developed. Any conclusion to the contrary can be arrived at only using hindsight, which is not permitted. Claim 1 therefore involves an inventive step.

Claim 1 is inventive in view of D12/D13 in combination with D15b

7.40 It has been demonstrated above that the patent validly claims its earliest priority date of 13th June 2012, meaning that D15b is not prior art. Should the Opposition Division unexpectedly take the view that the patent is not entitled to its earliest priority date, it remains the case that the claims involve an inventive step.

7.41 In particular, if D12/D13 is taken as the closest prior art, the skilled person would still have had no motivation to solve the objective technical problem in the manner of claim 1.

7.42 D15b gives some information about a clinical trial, and its official title states that it aims to compare liposomal irinotecan monotherapy and liposomal irinotecan in combination with 5-

FU/LV with a regimen which uses 5-FU/LV (without liposomal irinotecan). The subjects of the trial are patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy. No safety or efficacy data are given in D15b, and no treatment is disclosed. D15b also fails to specify the order in which the three drugs are administered in the combination regimen arm.

7.43 Consideration of D15b in view of D12/D13 would not have changed the fact that the skilled person would have been motivated to use liposomal irinotecan monotherapy to solve the objective technical problem. The skilled person would have learned from D12/D13 that liposomal irinotecan had been administered to gemcitabine-resistance patients both alone and in combination with 5-FU/LV in Phase I trials. As mentioned above, no explicit comparison is made between the two regimens in D12/13, and the combination regimen is not said to be preferred over the monotherapy. In these Phase I trials, a refractory pancreatic cancer patient showed a partial response in the monotherapy trial, but not in the combination regimen trial (see both D7 and D13; the latter discussed above, in particular at paragraph 7.26). However, no Phase I safety data were given in D12/D13, and there is no efficacy data about the combination regimen. As stated previously, the skilled person would have then seen that only liposomal irinotecan monotherapy was taken forward into a Phase II trial where it appeared to have activity and tolerable side effects. The skilled person would have thus concluded that the liposomal irinotecan and 5-FU/LV combination was deliberately not taken through to Phase II trials because it was in some way inadequate. As no safety data for the combination regimen were given in D12/D13, it would have been reasonable for the skilled person to conclude that the combination regimen showed unacceptable levels of adverse events without sufficient signs of activity, not least because both irinotecan and 5-FU were known to cause diarrhoea, which could be "severe" in the case of irinotecan (see D20 and paragraphs 0119 and 0120 of the patent).

7.44 On turning from D12/D13 to D15b, the conclusion given in D13 that the liposomal irinotecan monotherapy tested in Phase II is "feasible" and deserving of further exploration would have been at the forefront of the skilled person's mind. In view of this, the skilled person would have been drawn to Arm A of D15b, which administers the same liposomal irinotecan monotherapy regimen, as this would have reinforced the conclusion reasonably drawn from D12/D13. The skilled person would have been aware that Arm A was the only experimental arm in the trial described in D15b when it was initiated (see D15a). The presence of Arm C is not explained, in contrast to Arm A (monotherapy) whose presence is consistent with the technical teaching of the prior art.

7.45 Indeed, the skilled person would have read with conservative circumspection the disclosure that a combination regimen was undergoing a Phase III trial (Arm C). Following the teaching of D12/D13 and the skilled person's common general knowledge, s/he would not have reasonably expected a combination regimen to provide a safe and effective second-line treatment for pancreatic cancer. No efficacy or safety data at all were disclosed in D12/D13 for the combination regimen studied in Phase I, whereas the pancreatic cancer patient's partial response was associated with the monotherapy Phase I trial (see the discussion in paragraph 7.26 above). And, unlike the monotherapy regimen, the combination regimen disclosed in D12/D13 had not been progressed to a Phase II trial. That is to say, the skilled person would have been dissuaded from the combination regimen mentioned in D15b by the teaching of the other prior art documents.

7.46 It is therefore clear that the skilled person would not have developed the combination regimen (Arm C) mentioned in D15b with a reasonable expectation of success, which must not be confused with a mere "hope to succeed" (T296/93). This is particularly true in the present case. As acknowledged in paragraph 0006, whilst a variety of targeted agents have been evaluated, only one active substance (gemcitabine, with or without erlotinib) had been approved for first-line use in pancreatic cancer. The results of other clinical trials had been "disappointingly negative". The skilled person would thus have been acutely aware that the field of pancreatic cancer treatments was unpredictable and less likely to be successful than other fields. The skilled person would have had a very low expectation of success in this field (T694/92). This would apply to the combination regimen especially, as the combination regimen tested in Phase I had not been disclosed as showing any signs of activity at all, let alone a partial response, and it had not even been tested in a Phase II trial. This unpredictability would have persuaded the skilled person to take a conservative approach and develop only the monotherapy regimen, which appeared to have activity and tolerable side effects in a Phase II trial.

7.47 As stated above, when D12/D13 is considered together with D15b without hindsight knowledge, it becomes clear that the skilled person would have chosen to develop liposomal irinotecan monotherapy when seeking to solve the objective technical problem. The fact that the skilled person could have pursued a combination regimen is not relevant.

Claim 1 is inventive in view of D15b

7.48 In paragraph 22 of the opposition statement, the opponent suggests that D15b could be the closest prior art. To the extent that D15b is prior art at all (which, as stated above, the proprietor does not concede), D15b is not an appropriate choice for the closest prior art.

7.49 As the Opposition Division will be aware, the closest prior art should be a document which relates to the same purpose or effect as the invention. The closest prior art should not be a disclosure which merely shows superficial structural similarities with the claim at issue (T506/95). In the case of a medical use claim, where the achievement of a particular therapeutic effect is a functional feature of the claim, a document relating to the "same purpose or effect" should be a document that discloses a treatment which achieves this therapeutic effect. Such an approach is logical because a skilled person wishing to treat a particular condition would naturally start with a known way of treating the condition.

7.50 The disclosure of D15b is silent as to the effects of combination therapy in humans, and no actual treatment of patients is disclosed. Whilst the regimen used in Arm C of D15b bears some superficial structural similarities with the regimen of claim 1, this cannot justify the selection of D15b as closest prior art. D12/D13, by contrast, do disclose the apparent activity of liposomal irinotecan monotherapy in the patient population referred to in claim 1.

7.51 Following the Board's reasoning in T2154/14 (reasons 38 and 39), it follows that D12/D13 constitute a more promising springboard than D15b. This means that the Phase II monotherapy data given in D12/D13 constitute the closest prior art, and that D15b is not the closest prior art.

7.52 As D15b is not the closest prior art, there is no need to consider it further in this section. Nevertheless, should the Opposition Division unexpectedly consider D15b to be the closest

prior art, claim 1 still involves an inventive step. As mentioned above, D15b briefly discusses a Phase III clinical trial. No treatment is disclosed in D15b.

7.53 Claim 1 differs from D15b because claim 1 requires that liposomal irinotecan be administered in the claimed dosage regimen to treat pancreatic cancer in the patient population specified in the claim. Claim 1 is a medical use claim, and thus achieving the therapeutic effect is a functional feature of the claim (T609/02 and many others). Whilst D15b gives some basic information about a clinical trial, it does not provide a detailed protocol and is missing information about the regimen necessary to conduct the trial. Importantly, it does not disclose the treatment of any patients.

7.54 Claim 1 also differs from D15b because claim 1 requires liposomal irinotecan to be administered prior to LV, and for the LV to be administered prior to 5-FU. The order in which the drugs are administered is not specified in D15b. Claim 1 also requires that patients homozygous for UGT1A1*28 allele receive a lower starting dose of liposomal irinotecan compared to patients not homozygous for the UGT1A1*28 allele. D15b makes no mention of the UGT1A1*28 allele, and merely states that liposomal irinotecan is administered in an amount of 80 mg/m^2.

7.55 The technical effects of the claimed dosage regimen are discussed above in sections 7.15 - 7.22. Solely for the sake of brevity they will not be repeated here, although for the avoidance of doubt, the points made at 7.15 - 7.22 are equally valid if D15b is taken as the closest prior art.

7.56 The objective technical problem is therefore the provision of an improved therapy for gemcitabine-resistant pancreatic cancer. As explained above, this problem is solved by claim 1.

7.57 Faced with D15b and this objective technical problem, the skilled person would not have been motivated to solve it in the manner presently claimed. It has been acknowledged by the Boards of Appeal that the disclosure that a particular treatment is undergoing clinical trials is merely "speculative" (T715/03) of the treatment having any therapeutic efficacy and safety. Therefore, it is clear that the skilled person would not have looked to the combination regimen mentioned in D15b and used it in patients with a reasonable expectation of success. Rather, the use of the combination regimen would, at best, represent a mere "hope to succeed" (T296/93). As acknowledged in paragraph 0006, whilst a variety of targeted agents have been evaluated, only one active substance (erlotinib, when administered with gemcitabine) had been approved for first-line use in pancreatic cancer, and there was no approved treatment for patients who have progressed following gemcitabine-based therapy. Other clinical trials had been "disappointingly negative" (paragraph 0006). The skilled person would thus have been acutely aware that the field of pancreatic cancer treatments was unpredictable and less likely to be successful than other cancers.

7.58 The unpredictability of the field of pancreatic cancer treatments would have led the skilled person to adopt a conservative approach. The skilled person would thus have been motivated to develop only the monotherapy arm mentioned in D15b (Arm A) because the skilled person would not have expected to obtain the necessary levels of efficacy in the combination arm to mitigate his/her safety concerns and to warrant exposing patients to additional drugs. This motivation would have been strengthened by the skilled person's knowledge that 5-FU, like

irinotecan, was known to cause diarrhoea (D20). Thus, the conservative skilled person would have been dissuaded from the combination regimen in favour of the monotherapy, particularly seeing as the monotherapy, unlike the combination regimen, had been tested successfully in Phase II.

7.59 Such a conclusion would have been strengthened by a consultation of D12/D13. D12/D13 report on a successful Phase II clinical trial of liposomal irinotecan monotherapy, which "deserves further exploration" (D13). The skilled person would have noted the lack of Phase I and II data for the liposomal irinotecan and 5-FU/LV combination regimen, and would have come to the conclusion that the combination regimen was not taken through to Phase II trials because it was in some way inadequate.

7.60 As stated above, when D15b is considered, optionally in combination with D12/D13, without the benefit of hindsight knowledge, it becomes clear that the skilled person would have chosen liposomal irinotecan monotherapy when seeking to solve the objective technical problem. The fact that the skilled person could have pursued a combination regimen is not relevant. Whilst it has been established now (i.e. after the relevant date) that the claimed combination regimen is both safe and efficacious, such a conclusion would not reasonably have been expected by the skilled person at the relevant date. It is for at least this reason that claim 1 involves an inventive step over D15b in combination of D12/D13.

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8 SUFFICIENCY OF DISCLOSURE

8.1 The opponent argues that the patent is insufficient as it is allegedly devoid of any evidence that the claimed dosage regimen is suitable for treating pancreatic cancer. However, the opponent has applied the wrong legal standard in its arguments. In particular, the opponent's arguments arise from an incorrect application of the case law relating to sufficiency of medical use inventions.

8.2 When the law on sufficiency of medical use claims is applied correctly, it becomes clear that the requirements of Article 83 EPC are met.

The claimed dosage regimen is sufficiently disclosed

8.3 The basic requirement for sufficiency set out in Article 83 EPC is 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", i.e. the skilled person must be able follow the teaching in the patent and make the invention work. There is no question as to whether or not the claimed dosage regimen works because its efficacy has been confirmed in a Phase III clinical trial and the claimed regimen has been authorised in, for example, the United States, the EU (see section 4.2 of the Annex of D17) and Switzerland.

8.4 As the Opposition Division will be aware, the Board's decision in T609/02 laid out the requirements for the sufficiency of medical use claims. The Board stated that where a therapeutic application is claimed in the form of the use of a substance or composition for the manufacture of a medicament[1] for a defined therapeutic application, attaining the claimed

[1] This wording appears in the Board's decision because the claim at issue in T609/02 was in Swiss form. For the avoidance of doubt, the reasoning given in T609/02 applies equally to EPC-2000 medical use claims (see, for example, T895/13).

therapeutic effect is a functional technical feature of the claim. As a consequence, under Article 83 EPC, unless this is already known to the skilled person at the priority date, the application must disclose "the suitability of the product to be manufactured for the claimed therapeutic application" (see reason 9 of T609/02, emphasis added). In the present case, the "claimed therapeutic application" is the treatment of pancreatic cancer in the patient population defined in claim 1.

8.5 The "suitability" of the product for the claimed therapeutic application was set as a low hurdle by the Board in T609/02. It was established that the patent system does not require "absolute proof" that the product is approved for a particular therapeutic indication, or even in vivo data, before it may be claimed for use in treating said therapeutic indication (see reason 9 of T609/02). It is therefore enough, for example, "that the skilled person is made aware of the structure of the active ingredient proposed for the pharmaceutical composition as well as, in technical terms, of a definite link between the ingredient and the mechanism allegedly involved in the disease state" such that a "cause/effect relationship" is "made plausible" (see reason 10 of T609/02, emphasis added). Data in the application is not required to make a therapeutic application plausible (see, for example, T1616/09). As will be explained below, it was plausible at the effective filing date that administration of liposomal irinotecan in the claimed dosage regimen was suitable for the treatment of pancreatic cancer in the patient population specified in claim 1.

8.6 If it can be established that the technical teaching was made plausible by the application as filed, post-published evidence may be relied on when assessing sufficiency of disclosure to back-up the findings in the application.

8.7 As mentioned above, in the present case there is no doubt that post-published evidence (such as D17) shows that liposomal irinotecan can effectively treat pancreatic cancer as required by claim 1. As explained below, the application as filed discloses the suitability of the product for this therapeutic application, and so the requirements of Article 83 EPC are met.

The claimed therapeutic application was plausible at the relevant date

8.8 The opponent has pointed out that the results of the study described in Example 7 do not appear in the application as filed, and alleges that the claimed therapeutic application is not plausible on this basis. As already mentioned, T609/02 and the decisions citing it make it clear that "absolute proof" of the therapeutic application is not required for plausibility to be acknowledged, and that the Boards have acknowledged plausibility when no data are present in the application as filed (see T1616/09 cited above). Thus the absence of Phase III data in the application does not mean that plausibility is absent.

8.9 It is clear from the disclosure of the patent and the application as filed that liposomal irinotecan administered in combination with 5-FU/LV is suitable for the claimed indication. Combination therapies including non-liposomal irinotecan, 5-FU and leucovorin had been used previously to treat some pancreatic cancers (see paragraph 0003 of the patent, second sentence). Example 6 of the patent shows that the three-way combination of liposomal irinotecan, 5-FU and leucovorin showed "promising efficacy and safety data" (paragraph 0083 of the patent) when administered to 15 patients with solid tumours, five of whom had

pancreatic cancer. Example 6 thus discloses treatment of pancreatic cancer using a combination regimen of liposomal irinotecan, 5-FU and leucovorin.

8.10 For example, Table 2 on page 13 of the patent indicates that 3 pancreatic cancer patients (see the reference to "pancreatic cancer patients" in the title) received 80 mg/m^2: Example 6 discloses at line 44 that "Among the 6 patients who received the MTD dose of 80 mg/m^2, there were 1 PR [partial response, the best outcome], 4 SD [stable disease, which is indicative of activity] and 1 PD [progressive disease, which is not indicative of activity]". As explained in the first sentence of this paragraph, 3 of those 6 patients had pancreatic cancer. Even if it is assumed that 1 of those 3 patients showed progressive disease (i.e. a negative assumption is made about the efficacy of the combination regimen), it follows that 2 out of the 3 patients (67%) exhibited stable disease. In other words, example 6 teaches that activity was seen in at least 67% of the pancreatic cancer patients administered the combination regimen at the MTD dose of 80 mg/m^2.

8.11 Furthermore, Example 6 discloses that the maximum tolerated dose of liposomal irinotecan, when administered once every three weeks in combination with 5-FU and LV, was 80 mg/m^2. This compares with the MTD of 120 mg/m^2 for single-agent liposomal irinotecan. Moreover, Figure 6 discloses that $AUC_{0-\infty}$ (SN-38) after administration of liposomal irinotecan at MTD (80 mg/m^2 q3w) in combination with 5FU/LV was roughly half of the $AUC_{0-\infty}$ (SN-38) achieved after administration of single-agent liposomal irinotecan at MTD (120 mg/m^2 q3w). These results were reviewed by the inventors and further analysed in PK models developed by the inventors, which further strengthened the inventors' recognition at the priority date that the combination of liposomal irinotecan, 5-FU and LV, when administered using the regimen recited in claim 1 was suitable for treating pancreatic cancer.

8.12 It is therefore clear from the teaching of the patent that liposomal irinotecan with 5-FU/LV used in the claimed dosage regimen is suitable for therapeutic indication given in claim 1. There is certainly no evidence to the contrary.

The case law cited by the opponent has been wrongly applied to the present facts

8.13 The alleged legal basis for the opponent's insufficiency attack is Board 3.3.04's decision in T1592/12. In raising this attack, the opponent has failed to apply properly the Board's decision in T1592/12 to the facts of the present case. Instead, the opponent has cherry-picked a short passage from the decision and generalised it. When the Board's decision in T1592/12 is properly applied to the facts of the present case it becomes even clearer that the requirements of Article 83 are met by claim 1.

8.14 To allow the Board's teaching in T1592/12 to be applied correctly to the present case, it is necessary to consider the factual background which led the Board to dismiss the patentee's appeal and revoke the patent. To assist the Opposition Division, a copy of the patent at issue in T1592/12 (EP1210155; D21) is enclosed with the relevant parts highlighted.

8.15 Claim 1 at issue in T1592/12 claimed a dosage regimen for the administration of an antibody commonly known by its trade name "Herceptin®":

> *"Use of the anti-ErbB2 antibody huMab 4D5-8 in the manufacture of a medicament for use in a method for treating a human patient diagnosed with a breast cancer*

characterized by overexpression of ErbB2, said method comprising the steps of administering to the patient an initial dose of 8 mg/kg of the anti-ErbB2 antibody; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is 6 mg/kg, wherein the doses are separated in time from each other by three weeks."

8.16 Herceptin® was known in the art to treat breast cancer when administered in an initial dose of 4 mg/kg followed by weekly doses of 2 mg/kg. The only difference between the claims and the prior art was that the claims required Herceptin® to be administered once every three weeks with different dosage amounts – i.e. the invention's contribution to the art was the different administration frequency and the different initial/subsequent doses. In view of this the Board, citing T609/02, stated that it is the suitability of the **new dosage regimen** to treat breast cancer which needed to be disclosed for the patent to be sufficient (see Reasons 20 cited by the opponent). The Board then considered the relevant common general knowledge, and reviewed the teaching of the patent in detail. Following this the Board took the view that the suitability of the new dosage regimen for treating breast cancer was not disclosed, and thus the patent was found to be insufficient. However, the patent in question was very different from the present one, as explained below.

8.17 The specific disclosure (or lack thereof) in the description of the patent was the key factor in T1592/12. The Board cited paragraphs 0016 and 0214 of the patent which stated that, contrary to what was stated in claim 1, the first subsequent dose is administered "most preferably 1 week or less" (emphasis added) after the initial dose. Further, paragraph 0028 stated that the initial dose and the first subsequent dose may be separated in time by anything from "at least about two weeks" to "about two months". A plethora of permutations of possible dosage amounts and administration frequencies were disclosed in paragraphs 0017 to 0024. Examples 1 and 2 of the patent related to weekly administration, not administration once every three weeks (Reasons 24-27).

8.18 The Board concluded that the most preferred dosing frequency disclosed was weekly, meaning that the skilled person would have had serious doubts that three-weekly administration would have been suitable to treat breast cancer (Reasons 28). The patent was thus held to be insufficient.

8.19 The Board also considered a further submission from the patentee relating to Example 6 of the patent. This very brief example was prophetic and no data were included in it. Whilst the Board did comment on the lack of data in this example (Reasons 35), at no point did the Board make any statement to the effect that data are required to establish plausibility. As explained above, the reason for denying plausibility was the fact that the patent taught weekly administration as being preferred, meaning that the skilled person would have had serious doubts as to the suitability of three-weekly administration for treating breast cancer.

8.20 Like T1592/12, the patent at issue in these proceedings relates to a dosage regimen. Like Example 6 in T1592/12, Example 7 of the description does not contain the numerical clinical trial data. But this is where the factual similarity between the two cases begins and ends. Example 6 of the opposed patent does contain data (see paragraphs 8.9 to 8.9 above). The patent discloses that the dosage regimen of claim 1 is suitable for treating pancreatic cancer in the patient population defined in claim 1.

8.21 The first paragraph of the "Summary" section of the application as filed (page 3) states that the invention relates to dosage regimens for the administration of liposomal irinotecan. Two dosage regimens are mentioned – liposomal irinotecan "alone [i.e. monotherapy] or in combination with 5-fluorouracil and leucovorin". The following two paragraphs give more information on liposomal irinotecan monotherapy and combination therapy, respectively. It is noteworthy that the paragraph describing the combination therapy mentions the same dosages and administration frequencies which appear in claim 1. These dosages and administration frequencies which appear in claim 1 of the patent are repeated in the fourth paragraph of page 5 in the context of a liposomal irinotecan formulation which is to be used in a combination regimen. They are repeated again in the final paragraph of page 14, and yet again in claim 3 of the application as filed. The dosages and administration frequencies are also discussed at length in Example 7.

8.22 It is clear then that the preferred combination therapy disclosed in the application as filed is that which employs the dosages and administration frequencies recited in claim 1 of the patent. This is in stark contrast to the situation in T1592/12, where a great many different dosages and administration frequencies were disclosed, and where the application as filed stated a preferred administration frequency that differed from the one that was claimed. It follows that, unlike the case in T1592/12, the skilled person would have no doubts that the claimed dosage regimen was suitable for the claimed therapeutic indication. As established in paragraphs 8.9 to 8.9 above, the application also discloses that liposomal irinotecan was suitable for this indication when administered with 5-FU/LV. No evidence has been provided which suggests otherwise. It follows that the plausibility hurdle set by T609/02 has been met by the claimed subject matter. The requirements of Article 83 EPC are therefore met.

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

EUROPEAN
COMMISSION

Bruxelles, 14.10.2016
C(2016) 6778 (final)

COMMISSION IMPLEMENTING DECISION

of 14.10.2016

granting marketing authorisation under Regulation (EC) No 726/2004 of the European Parliament and of the Council for "Onivyde - irinotecan", an orphan medicinal product for human use

(Text with EEA relevance)

(ONLY THE GERMAN TEXT IS AUTHENTIC)

COMMISSION IMPLEMENTING DECISION

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(Text with EEA relevance)

(ONLY THE GERMAN TEXT IS AUTHENTIC)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency[1], and in particular Article 10(2) thereof,

Having regard to Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products, and in particular Article 5(12) thereof,

Having regard to the application submitted by Baxalta Innovations GmbH, on 28 May 2015, under Article 4(1) of Regulation (EC) No 726/2004,

Having regard to the opinions of the European Medicines Agency, formulated on 21 July 2016 by the Committee for Medicinal Products for Human Use and on 8 September 2016 by the Committee for Orphan Medicinal Products,

Whereas:

(1) Commission Decision C(2011)9419(final), adopted in accordance with Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products[2] designated "Nanoliposomal irinotecan" as an orphan medicinal product.

(2) The orphan medicinal product "Onivyde - irinotecan" complies with the requirements set out in Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use[3] .

(3) It is therefore appropriate to authorise its placing on the market.

(4) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Medicinal Products for Human Use,

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1 OJ L 136, 30.4.2004, p. 1.
2 OJ L 18, 22.1.2000, p. 1.
3 OJ L 311, 28.11.2001, p. 67.

HAS ADOPTED THIS DECISION:

Article 1

The marketing authorisation provided for in Article 3 of Regulation (EC) No 726/2004 is granted for the orphan medicinal product "Onivyde - irinotecan", the characteristics of which are summarised in Annex I to this Decision. "Onivyde - irinotecan" shall be registered in the Community register of medicinal products under number EU/1/16/1130.

Article 2

The marketing authorisation concerning the medicinal product referred to in Article 1 shall be subject to compliance with the conditions set out in Annex II and, in particular, with those relating to manufacture and importation, control and issue.

Article 3

The labelling and package leaflet concerning the orphan medicinal product referred to in Article 1 shall conform to Annex III.

Article 4

The period of validity of the authorisation shall be five years from the date of notification of this Decision.

Article 5

This Decision is addressed to Baxalta Innovations GmbH, Industriestrasse 67, A-1221 Wien, Österreich.

Done at Brussels, 14.10.2016

For the Commission

Xavier PRATS MONNÉ
Director-General

ANNEX I

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

ONIVYDE 5 mg/ml concentrate for solution for infusion

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.

One ml of concentrate contains the equivalent of 5 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 4.3 mg irinotecan.

Excipient with known effect

One ml of concentrate contains 0.144 mmol (3.31 mg) sodium.
For the full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

Concentrate for solution for infusion.
White to slightly yellow opaque isotonic liposomal dispersion.
The concentrate has a pH of 7.2 and an osmolality of 295 mOsm/kg.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Treatment of metastatic adenocarcinoma of the pancreas, in combination with 5-fluorouracil (5-FU) and leucovorin (LV), in adult patients who have progressed following gemcitabine based therapy.

4.2 Posology and method of administration

ONIVYDE (liposomal irinotecan) must only be prescribed and administered to patients by healthcare professionals experienced in the use of anti-cancer therapies.

ONIVYDE (liposomal irinotecan) is not equivalent to non-liposomal irinotecan formulations and should not be interchanged.

Posology

ONIVYDE, leucovorin and 5-fluorouracil should be administered sequentially. The recommended dose and regimen of ONIVYDE is 80 mg/m^2 intravenously over 90 minutes, followed by LV 400 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,400 mg/m^2 intravenously over 46 hours, administered every 2 weeks. ONIVYDE should not be administered as a single agent.

A reduced starting dose of ONIVYDE (liposomal irinotecan) of 60 mg/ m^2 should be considered for patients known to be homozygous for the UGT1A1*28 allele (see sections 4.8 and 5.1). A dose increase of ONIVYDE to 80 mg/m^2 should be considered if tolerated in subsequent cycles.

Pre-medication
It is recommended that patients receive pre-medication with standard doses of dexamethasone (or an equivalent corticosteroid) together with a 5-HT_3 antagonist (or other antiemetic) at least 30 minutes prior to ONIVYDE infusion.

Dosage adjustments
All dose modifications should be based on the worst preceding toxicity. LV dose does not require adjustment. For Grade 1 and 2 toxicities there are no dose modifications recommended. Dose adjustments, as summarised in Table 1 and Table 2, are recommended to manage Grade 3 or 4 toxicities related to ONIVYDE.

For patients who start treatment with 60 mg/m^2 ONIVYDE and do not dose escalate to 80 mg/m^2, the recommended first dose reduction is to 50 mg/m^2 and the second dose reduction is to 40 mg/m^2. Patients who require further dose reduction should discontinue treatment.

Patients who are known to be homozygous for UGT1A1*28 and without drug related toxicities during the first cycle of therapy (reduced dose of 60 mg/m^2) may have the dose of ONIVYDE increased to a total dose of 80 mg/m^2 in subsequent cycles based on individual patient tolerance.

Table 1: Recommended dose modifications for ONIVYDE+5-FU/LV for Grade 3-4 toxicities for patients not homozygous for UGT1A1*28

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
Haematological toxicities		
Neutropenia	A new cycle of therapy should not begin until the absolute neutrophil count is ≥ 1500/mm^3	
Grade 3 or Grade 4 (< 1000/mm^3) **or Neutropenic fever**	***First occurrence***	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2).
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2).
	Third occurrence	Discontinue treatment
Thrombocytopenia **Leukopenia**	A new cycle of therapy should not begin until the platelet count is ≥ 100,000/mm^3 Dose modifications for leukopenia and thrombocytopenia are based on NCI CTCAE toxicity grading and are the same as recommended for neutropenia above.	
Nonhaematological toxicities[2]		
Diarrhoea	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	
Grade 2	A new cycle of therapy should not begin until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency).	

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients not homozygous for UGT1A1*28)**	
Grade 3 or 4	*First occurrence*	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Nausea/vomiting	A new cycle of therapy should not begin until nausea/vomiting resolves to ≤ Grade 1 or baseline	
Grade 3 or 4 (despite antiemetic therapy)	*First occurrence*	Optimise antiemetic therapy Reduce ONIVYDE dose to 60 mg/m^2
	Second occurrence	Optimise antiemetic therapy Reduce ONIVYDE dose to 50 mg/m^2
	Third occurrence	Discontinue treatment
Hepatic, renal, respiratory or other[2] toxicities *Grade 3 or 4*	A new cycle of therapy should not begin until the adverse reaction resolves to ≤ Grade 1	
	First occurrence	Reduce ONIVYDE dose to 60 mg/m^2 Reduce 5-FU dose by 25% (1800 mg/m^2)
	Second occurrence	Reduce ONIVYDE dose to 50 mg/m^2 Reduce 5-FU dose by an additional 25% (1350 mg/m^2)
	Third occurrence	Discontinue treatment
Anaphylactic reaction	*First occurrence*	Discontinue treatment

[1] NCI CTCAE v 4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

[2] Excludes asthenia and anorexia; Asthenia and Grade 3 anorexia do not require dose adjustment.

Table 2: Recommended dose modifications for ONIVYDE +5-FU/LV for Grade 3-4 toxicities in patients homozygous for UGT1A1*28

Toxicity grade (value) by NCI CTCAE v 4.0[1]	**ONIVYDE/5-FU adjustment (for patients homozygous for UGT1A1*28 without previous increase to 80 mg/m^2)**	
Adverse reactions[2] *Grade 3 or 4*	A new cycle of therapy should not begin until adverse event resolves to ≤ Grade 1	
	First occurrence	Reduce ONIVYDE dose to 50 mg/m^2 5-FU dose modification as in Table 1
	Second occurrence	Reduce ONIVYDE dose to 40 mg/m^2 5-FU dose modification as in Table 1
	Third occurrence	Discontinue treatment

[1] NCI CTCAE v 4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

[2] Excludes asthenia and anorexia; asthenia and Grade 3 anorexia do not require dose adjustment.

Special populations

Hepatic impairment
No dedicated hepatic impairment study has been conducted with ONIVYDE. The use of ONIVYDE should be avoided in patients with bilirubin > 2.0 mg/dl, or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 times upper limit of normal (ULN) or > 5 times ULN if liver metastasis is present (see section 4.4).

Renal impairment
No dedicated renal impairment study has been conducted with ONIVYDE. No dose adjustment is recommended in patients with mild to moderate renal impairment (see sections 4.4 and 5.2). ONIVYDE is not recommended for use in patients with severe renal impairment (CLcr < 30 ml/min).

Elderly
Forty-one percent (41%) of patients treated with ONIVYDE across the clinical program were ≥ 65 years. No dose adjustment is recommended.

Paediatric population
The safety and efficacy of ONIVYDE in children and adolescents aged ≤ 18 years have not yet been established. No data are available.

Method of administration

ONIVYDE is for intravenous use. The concentrate must be diluted prior to administration and given as single intravenous infusion over 90 minutes. For more details see section 6.6.

Precautions to be taken before handling or administering the medicinal product
ONIVYDE is a cytotoxic medicinal product. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. Pregnant staff should not handle ONIVYDE.

4.3 Contraindications

History of severe hypersensitivity to irinotecan or to any of the excipients listed in section 6.1.

Breast-feeding (see section 4.6).

4.4 Special warnings and precautions for use

General

ONIVYDE is a liposomal formulation of irinotecan with different pharmacokinetic properties compared to non-liposomal irinotecan. The dose concentration and strength are different in comparison to non-liposomal irinotecans.

ONIVYDE is not equivalent to other non-liposomal irinotecan formulations and should not be interchanged.

In the limited number of patients with prior exposure to non-liposomal irinotecan, no benefit of ONIVYDE has been demonstrated.

Myelosuppression/neutropenia

Complete blood cell count monitoring is recommended during ONIVYDE treatment. Patients should be aware of the risk of neutropenia and the significance of fever. The median time to nadir for ≥ Grade 3 neutropenia is 23 (range 8-104) days post first dose of treatment with ONIVYDE. Febrile neutropenia (body temperature > 38°C and neutrophil count≤ 1,000 cells/mm³) should be urgently treated in the hospital with broad-spectrum intravenous antibiotics. ONIVYDE should be

withheld if neutropenic fever occurs or the absolute neutrophil count drops below 1500/mm^3. Sepsis with neutropenic fever and consequent septic shock with fatal outcome has been observed in patients with metastatic pancreatic adenocarcinoma treated with ONIVYDE.
In patients who experienced severe haematological events, a dose reduction or treatment discontinuation is recommended (see section 4.2). Patients with severe bone marrow failure should not be treated with ONIVYDE.
History of prior abdominal radiation increases the risk of severe neutropenia and febrile neutropenia following ONIVYDE treatment. Close monitoring of blood counts is recommended, and the use of myeloid growth factors should be considered for patients with a history of abdominal radiation. Caution should be exercised in patients receiving concurrent administration of ONIVYDE with irradiation.

Patients with deficient glucuronidation of bilirubin, such as those with Gilbert's syndrome, may be at greater risk of myelosuppression when receiving therapy with ONIVYDE.

Compared to Caucasian patients, Asian patients have an increased risk of severe and febrile neutropenia following treatment with ONIVYDE+5-FU/LV (see sections 4.8 and 5.2).

Immunosuppressive effects and vaccines

Administration of live or live-attenuated vaccines in patients immunocompromised by chemotherapeutic medicinal products including ONIVYDE may result in serious or fatal infections; therefore vaccination with a live vaccine should be avoided. Killed or inactivated vaccines may be administered; however, the response to such vaccines may be diminished.

Interactions with strong CYP3A4 inducers

ONIVYDE should not be administered with strong CYP3A4-enzyme inducers such as anticonvulsants (phenytoin, phenobarbital or carbamazepine), rifampin, rifabutin and St. John's wort unless there are no therapeutic alternatives. The appropriate starting dose for patients taking these anticonvulsants or other strong inducers has not been defined. Consideration should be given to substituting with non-enzyme inducing therapies at least 2 weeks prior to initiation of ONIVYDE therapy (see section 4.5).

Interactions with strong CYP3A4 inhibitors or strong UGT1A1 inhibitors

ONIVYDE should not be administered with strong CYP3A4-enzyme inhibitors (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole). Strong CYP3A4 inhibitors should be discontinued at least 1 week prior to starting ONIVYDE therapy.
ONIVYDE should not be administered with strong UGT1A inhibitors (e.g. atazanavir, gemfibrozil, indinavir) unless there are no therapeutic alternatives.

Diarrhoea

Diarrhoea can occur early (onset in $\leq$ 24 hours after starting ONIVYDE) or late (> 24 hours) (see section 4.8).
In patients experiencing early diarrhea, therapeutic and prophylactic atropine should be considered unless contraindicated. Patients should be made aware of the risk of delayed diarrhoea which can be debilitating and, on rare occasions, life threatening since persistent loose or watery stools can result in dehydration, electrolyte imbalance, colitis, gastrointestinal (GI) ulceration, infection or sepsis.
As soon as the first liquid stool occurs, the patient should start drinking large volumes of beverages containing electrolytes. Patients should have loperamide (or equivalent) readily available to begin treatment for late diarrhoea. Loperamide should be initiated at first occurrence of poorly formed or loose stools or at the earliest onset of bowel movements more frequent than normal. Loperamide should be given until patient is without diarrhoea for at least 12 hours.

If diarrhoea persists while patient is on loperamide for more than 24 hours, adding oral antibiotic support (e.g. fluoroquinolone for 7 days) should be considered. Loperamide should not be used for more than 48 consecutive hours due to risk of paralytic ileus. If diarrhoea persists for more than 48 hours, stop loperamide, monitor and replace fluid electrolytes and continue antibiotic support until resolution for accompanying symptoms.
ONIVYDE treatment should be delayed until diarrhoea resolves to ≤ Grade 1 (2-3 stools/day more than pre-treatment frequency). ONIVYDE must not be administered to patients with bowel obstruction, and chronic inflammatory bowel disease, until it is resolved.
Following Grade 3 or 4 diarrhoea, the subsequent dose of ONIVYDE should be reduced, (see section 4.2).

Cholinergic reactions

Early onset diarrhoea may be accompanied by cholinergic symptoms such as rhinitis, increased salivation, flushing, diaphoresis, bradycardia, miosis and hyperperistalsis. In case of cholinergic symptoms atropine should be administered.

Acute infusion and related reactions

Infusion reactions primarily consisting of rash, urticaria, periorbital oedema or pruritus were reported in patients receiving ONIVYDE treatment. New events (all grade 1 or grade 2) occurred generally early during ONIVYDE treatment, with only 2 out of 10 patients noted with events after the fifth dose. Hypersensitivity reactions, including acute infusion reaction may occur. ONIVYDE should be discontinued in case of severe hypersensitivitiy reactions.

Prior Whipple procedure

Patients with a history of a Whipple procedure have a higher risk of serious infections following ONIVYDE in combination with 5-FU and leucovorin (see section 4.8). Patients should be monitored for signs of infections.

Pulmonary toxicity

Interstitial Lung Disease (ILD)-like events leading to fatalities have occurred in patients receiving non-liposomal irinotecan. No cases of ILD-like events have been reported with ONIVYDE therapy in clinical studies. Risk factors include pre-existing lung disease, use of pneumotoxic medicinal products, colony stimulating factors or having previously received radiation therapy. Patients with risk factors should be closely monitored for respiratory symptoms before and during ONIVYDE therapy. A reticulo-nodular pattern on chest X-ray was observed in a small percentage of patients enrolled in a clinical study with irinotecan. New or progressive dyspnoea, cough, and fever should prompt interruption of ONIVYDE treatment, pending diagnostic evaluation. ONIVYDE should be discontinued in patients with a confirmed diagnosis of ILD.

Hepatic impairment

Patients with hyperbilirubinaemia had higher concentrations for total SN-38 (see section 5.2) and therefore the risk of neutropenia is increased. Regular monitoring of complete blood counts should be conducted in patients with total bilirubin of 1.0-2.0 mg/dl. Caution should be exercised in patients with hepatic impairment (bilirubin > 2 times upper limit of normal [ULN]; transaminases > 5 times ULN). Caution is required when ONIVYDE is given in combination with other hepatotoxic medicinal products, especially in patients with pre-existing hepatic impairment.

Renal impairment

The use of ONIVYDE in patients with significant renal impairment has not been established (see section 5.2).

Underweight patients (body mass index < 18.5 kg/m^2)

In the clinical study evaluating ONIVYDE+5-FU/LV, 5 of 8 underweight patients experienced a Grade 3 or 4 adverse reactions, mostly myelosuppression, while 7 of the 8 patients required dose modification such as dose delay, dose reduction or dose discontinuation. Caution should be exercised when using ONIVYDE in patients with body mass index <18.5 kg/m^2.

Excipients

Each ml of ONIVYDE contains 0.144 mmol (3.31 mg) sodium. This needs to be taken into consideration by patients on a controlled sodium diet.

4.5 Interaction with other medicinal products and other forms of interaction

Information about drug interactions with ONIVYDE is referenced from the published scientific literature for nonliposomal irinotecan.

Interaction affecting the use of ONIVYDE

Strong CYP3A4 inducers
Patients receiving concomitant non-liposomal irinotecan and CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital or carbamazepine have substantially reduced exposure to irinotecan (AUC reduction by 12% with St John's wort, 57%-79% with phenytoin, phenobarbital, or carbamazepine) and SN-38 (AUC reduction by 42% with St John's wort, 36%-92% with phenytoin phenobarbital, or carbamazepine). Therefore, co-administration of ONIVYDE with inducers of CYP3A4 may reduce systemic exposure of ONIVYDE.

Strong CYP3A4 inhibitors and UGT1A1 inhibitors
Patients receiving concomitant non-liposomal irinotecan and ketoconazole, a CYP3A4 and UGT1A1 inhibitor, have increased SN-38 exposure by 109%. Therefore, co-administration of ONIVYDE with other inhibitors of CYP3A4 (e.g. grapefruit juice, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole) may increase systemic exposure of ONIVYDE. Based on the drug interaction of non-liposomal irinotecan and ketoconazole, co-administration of ONIVYDE with other inhibitors of UGT1A1 (e.g. atazanavir, gemfibrozil, indinavir) may also increase systemic exposure of ONIVYDE.

Co-administration of ONIVYDE+5-FU/LV does not alter the pharmacokinetics of ONIVYDE based on the population pharmacokinetic analysis.

No interaction of ONIVYDE (liposomal irinotecan) with other medicinal products is known.

4.6 Fertility, pregnancy and lactation

Women of childbearing potential / contraception in males and females

Women of childbearing potential should use effective contraception during ONIVYDE treatment and 1 month thereafter. Males should use condoms during ONIVYDE treatment and 4 months thereafter.

Pregnancy

There are no adequate data on the use of ONIVYDE in pregnant women. ONIVYDE can cause harm to the foetus when administered to the pregnant woman, as the main ingredient irinotecan has been shown to be embryotoxic and teratogenic in animals (see section 5.3). Therefore, based on results from animal studies and the mechanism of action of irinotecan, ONIVYDE should not be used during pregnancy unless clearly necessary. If ONIVYDE is used during pregnancy or if the patient becomes

pregnant while receiving therapy, the patient should be informed about the potential hazard to the foetus.

Breast-feeding

It is unknown whether ONIVYDE or its metabolites are excreted into human milk. Because of the potential for serious adverse reactions of ONIVYDE in breast-feeding infants, ONIVYDE is contraindicated during breast-feeding (see section 4.3). Patients should not breast-feed until one month after the last dose.

Fertility

There are no data on the impact of ONIVYDE on human fertility. Non-liposomal irinotecan was shown to cause atrophy of male and female reproductive organs after multiple daily irinotecan doses in animals (see section 5.3).

4.7 Effects on ability to drive and use machines

ONIVYDE has moderate influence on the ability to drive and use machines. During treatment patients should observe caution when driving or using machines.

4.8 Undesirable effects

Summary of the safety profile

The following adverse reactions, considered to be possibly or probably related to the administration of ONIVYDE, were reported in 264 patients with metastatic adenocarcinoma of the pancreas, 147 of whom received ONIVYDE monotherapy (120 mg/m^2) and 117 received ONIVYDE (80 mg/m^2) in combination with 5-FU/LV.
The most common adverse reactions (incidence ≥ 20%) of ONIVYDE+5FU/LV were: diarrhoea, nausea, vomiting, decreased appetite, neutropenia, fatigue, asthenia, anaemia, stomatitis and pyrexia.
The most common serious adverse reactions (≥ 2%) of ONIVYDE therapy were diarrhoea, vomiting, febrile neutropenia, nausea, pyrexia, sepsis, dehydration, septic shock, pneumonia, acute renal failure, and thrombocytopenia.
The rates of adverse reactions leading to permanent treatment discontinuation were 11% for the ONIVYDE+5-FU/LV arm and 12% for the monotherapy arm.
The most frequently reported adverse reactions leading to discontinuation were infection and diarrhoea for ONIVYDE+5-FU/LV arm, and vomiting and diarrhoea for the monotherapy arm.

Tabulated list of adverse reactions

The adverse reactions that may occur during treatment with ONIVYDE are summarised below and are presented by system organ class and frequency category (Table 3). Within each system organ class and frequency category, adverse reactions are presented in order of decreasing seriousness. Frequencies categories used for adverse reactions are: very common (≥ 1/10); common (≥ 1/100 to <1/10); uncommon (≥ 1/1,000 to <1/100) and rare (≥ 1/10,000 to < 1/1,000)**.

Table 3: Adverse reactions reported with ONIVYDE therapy in the NAPOLI-1 clinical study

MedDRA* system organ class	Adverse reaction frequency**
Infections and infestations	*Common:* Septic shock, Sepsis, Pneumonia, Febrile neutropenia, Gastroenteritis, Oral candidiasis *Uncommon:* Biliary sepsis

MedDRA* system organ class	Adverse reaction frequency**
Blood and lymphatic system disorders	*Very common:* Neutropenia, Leukopenia, Anaemia, Thrombocytopenia *Common:* Lymphopenia
Immune system disorders	*Uncommon: Hypersensitivity*
Metabolism and nutrition disorders	*Very common:* Hypokalaemia, Hypomagnesaemia, Dehydration, Decreased appetite *Common:* Hypoglycaemia, Hyponatraemia, Hypophosphataemia
Psychiatric disorders	*Common:* Insomnia
Nervous system disorders	*Very common:* Dizziness *Common:* Cholinergic syndrome, Dysgeusia
Cardiac disorders	*Common:* Hypotension
Vascular disorders	*Common*: Pulmonary embolism, Embolism, Deep vein thrombosis *Uncommon:* Thrombosis
Respiratory, thoracic and mediastinal disorders	*Common*: Dyspnoea, Dysphonia *Uncommon:* Hypoxia
Gastrointestinal disorders	*Very common:* Diarrhoea, Vomiting, Nausea, Abdominal pain, Stomatitis *Common:* Colitis, Haemorrhoids *Uncommon:* Oesophagitis, Proctitis
Hepatobiliary disorders	*Common:* Hypoalbuminaemia
Skin and subcutaneous tissue disorders	*Very common:* Alopecia *Uncommon:* Rash maculo-papular, Nail discolouration
Renal and urinary disorders	*Common:* Acute renal failure
General disorders and administration site conditions	*Very common:* Pyrexia, Peripheral oedema, Mucosal inflammation, Fatigue, Asthenia *Common:* Infusion related reaction, Oedema
Investigations	*Very common*: Weight decrease *Common*: Increased bilirubin, Increased alanine aminotransferase, Increased aspartate aminotransferase, Increased international normalized ratio

* MedDRA version 14.1

** Rare occurrence cannot be estimated from the NAPOLI-1study due to the small sample size

Description of selected adverse reactions

The following adverse reactions were observed in the NAPOLI-1 clinical study:

Myelosuppression

Myelosuppression (neutropenia/leukopenia, thrombocytopenia and, anaemia) was more common in the ONIVYDE+5-FU/LV arm compared to the 5-FU/LV control arm.

Neutropenia/leukopenia
Neutropenia/leukopenia was the most notable important haematological toxicity. Grade 3 or higher neutropenia occurred more frequently in patients treated with ONIVYDE+5-FU/LV (27.4%) compared to patients treated with 5-FU/LV (1.5%). Neutropenic fever/sepsis appeared more frequently in the ONIVYDE+5-FU/LV combination arm [in 4 patients (3.4%)] compared to 5-FU/LV control arm [in 1 patient (0.7%)].

Thrombocytopenia
Grade 3 or higher thrombocytopenia occurred in 2.6% of patients treated with ONIVYDE+5-FU/LV and 0% in patients treated with 5-FU/LV.

Anaemia
Grade 3 or higher anaemia occurred in 10.3% of patients treated with ONIVYDE+5-FU/LV and in 6.7% of patients treated with 5-FU/LV.

Acute renal failure
Renal impairment and acute renal failure have been identified, usually in patients who become volume depleted from nausea/vomiting and/or diarrhoea. Acute renal failure was reported in 6 of 117 patients (5.1%) in the ONIVYDE+5-FU/LV arm, 10 of 147 (6.8%) in the ONIVYDE monotherapy arm and 6 of 134 patients (4.5%) in the 5-FU/LV arm.

Diarrhoea and related adverse reactions
Diarrhoea is a very common adverse reaction leading to colitis, ileus, gastroenteritis, fatigue, dehydration, weight loss, renal toxicities, hyponatraemia, and hypokalaemia. Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhoea. In the clinical study Grade 3 or Grade 4 diarrhoea occurred in 15 out of 117 patients (12.8%) receiving ONIVYDE+5-FU/LV. For patients experiencing late diarrhoea, the median time to late diarrhoea onset was 8 days from the previous dose of ONIVYDE. Early onset diarrhoea, typically appearing ≤24 hours after dose administration, can occur and is usually transient. Early onset diarrhoea may also be accompanied by cholinergic symptoms that can include rhinitis, increased salivation, flushing, diaphoresis, bradycardia, miosis and hyperperistalsis that can induce abdominal cramping. In the clinical study, early diarrhoea onset occurred in 35 patients (29.9%) and cholinergic events occurred in 4 patients (3.4%) receiving ONIVYDE+5-FU/LV.
Withhold ONIVYDE for Grade 2-4 diarrhoea and initiate treatment for diarrhoea. Following recovery to Grade 1 diarrhoea, resume ONIVYDE at a reduced dose (see section 4.2).

Infusion reaction
Acute infusion reactions were reported in 8 of 117 patients (6.8%) in the ONIVYDE+5-FU/LV arm, 3 of 147 patients (2.0%) in the ONIVYDE monotherapy arm, and 8 of 134 patients (6.0%) in the 5-FU/LV arm.

Other special populations

Elderly
Overall, no major clinical differences in safety or efficacy were reported between patients ≥ 65 years and patients < 65 years, although a higher frequency of discontinuation (14.8% vs 7.9%) was noted in the former group treated with ONIVYDE+5-FU/LV in the NAPOLI-1 study and in some cases the adverse reactions did not resolve. Grade 3 or higher and serious treatment emergent adverse reactions were more frequent in patients < 65 years (84.1% and 50.8%) compared to patients ≥ 65 years (68.5 % and 44.4%). Conversely, patients > 75 years (n=12) experienced more frequent serious adverse reactions, dose delay, dose reduction and discontinuation compared to patients ≤ 75 years (n=105) when treated with ONIVYDE+5-FU/LV in the pancreatic adenocarcinoma study.

Asian population
Compared to Caucasians, Asian patients were observed with a lower incidence of diarrhoea [14 (19.2%) out of 73 Caucasians had a ≥ Grade 3 diarrhoea, and 1 out of 33 (3.3%) Asians had a

≥ Grade 3 diarrhoea], but a higher incidence and higher severity of neutropenia. In patients receiving ONIVYDE+5-FU/LV, the incidence of ≥ Grade 3 neutropenia was higher among Asian patients [18 of 33 (55%)] compared to White patients [13 of 73 (18%)]. Neutropenic fever/neutropenic sepsis was reported in 6% of Asian patients compared to 1% of White patients. This is consistent with the population pharmacokinetic analysis that showed a lower exposure to irinotecan and a higher exposure to its active metabolite SN-38 in Asians than in Caucasians.

Patients with hepatic impairment
In clinical studies of non-liposomal irinotecan administered on a 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 Grade 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dl.

Patients with prior Whipple procedure
In the clinical study evaluating ONIVYDE+5-FU/LV, patients with a prior Whipple procedure had a higher risk of serious infections following treatment with ONIVYDE+5-FU/LV [9 of 29 (30%)] compared to 11 of 88 (12.5%) patients with no prior Whipple procedure.

Patients with UGT1A1 allele
Individuals who are 7/7 homozygous for the UGT1A1*28 allele are at increased risk for neutropenia from non-liposomal irinotecan. In the clinical study evaluating ONIVYDE+5-FU/LV, the frequency of ≥ Grade 3 neutropenia in these patients [2 of 7 (28.6%)] was similar to the frequency in patients not homozygous for the UGT1A1*28 allele who received a starting dose of ONIVYDE of 80 mg/m^2 [30 of 110 (27.3%)] (see section 5.1).

Underweight patients (body mass index < 18.5 kg/m^2)
In the clinical study evaluating ONIVYDE+5-FU/LV, 5 of 8 underweight patients experienced a grade 3 or 4 adverse reaction, mostly myelosuppression, while 7 of the 8 patients required dose modification such as dose delay, dose reduction or dose discontinuation (see section 4.4).

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the national reporting system listed in Appendix V.

4.9 Overdose

In clinical trials, ONIVYDE was administered at doses up to 240 mg/m^2 to patients with various cancers. The adverse reactions in these patients were similar to those reported with the recommended dosage and regimen.

There have been reports of overdosage with non-liposomal irinotecan at doses up to approximately twice the recommended therapeutic dose of irinotecan, which may be fatal. The most significant adverse reactions reported were severe neutropenia and severe diarrrhoea.

There is no known antidote for overdose of ONIVYDE. Maximum supportive care should be instituted to prevent dehydration due to diarrhoea and to treat any infectious complications.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: antineoplastic agents, other antineoplastic agents, ATC code: L01XX19

Mechanism of action

The active substance in ONIVYDE is irinotecan (topoisomerase I inhibitor) encapsulated in a lipid bilayer vesicle or liposome.

Irinotecan is a derivative of camptothecin. Camptothecins act as specific inhibitors of the enzyme DNA topoisomerase I. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase I-DNA complex and induce single-strand DNA lesions which block the DNA replication fork and are responsible for the cytotoxicity. Irinotecan is metabolized by carboxylesterase to SN-38. SN-38 is approximately 1000 times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumour cell lines.

Pharmacodynamic effects

In animal models, ONIVYDE has been shown to extend plasma levels of irinotecan and prolong the exposure to the active metabolite SN-38 at the site of the tumour.

Clinical efficacy and safety

The safety and efficacy of ONIVYDE were investigated in a multinational, randomized, open label, controlled clinical trial (NAPOLI–1) that tested two treatment regimens for patients with metastatic pancreatic adenocarcinoma who had documented disease progression after gemcitabine or gemcitabine-containing therapy. The trial was designed to assess the clinical efficacy and safety of ONIVYDE monotherapy or ONIVYDE+5-FU/LV compared to an active control arm of 5-FU/LV.

Patients randomized to ONIVYDE+5-FU/LV received ONIVYDE at 80 mg/m^2 as an intravenous infusion over 90 minutes, followed by LV 400 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,400 mg/m^2 intravenously over 46 hours, administered every 2 weeks. Patients homozygous for the UGT1A1*28 allele were given a lower initial dose of ONIVYDE (see section 4.2). Patients randomised to 5-FU/LV received leucovorin 200 mg/m^2 intravenously over 30 minutes, followed by 5-FU 2,000 mg/m^2 intravenously over 24 hours, administered on Days 1, 8, 15 and 22 of a 6 week cycle. Patients randomised to ONIVYDE monotherapy received 120 mg/m^2 as an intravenous infusion over 90 minutes every 3 weeks.

Key eligibility criteria for patients with metastatic adenocarcinoma of the pancreas in the NAPOLI-1 clinical study were Karnofsky Performance Status (KPS) $\geq$ 70, normal bilirubin level, transaminase levels $\leq$ 2.5 times the ULN or $\leq$ 5 times the ULN for patients with liver metastases and albumin $\geq$ 3.0 g/dl.

A total of 417 patients were randomised to the ONIVYDE+5-FU/LV arm (N=117), ONIVYDE monotherapy arm (N=151) and 5-FU/LV arm (N=149). Patient demographic and entry disease characteristics were well balanced between trial arms.

In the intent to treat (all randomised) population, the median age was 63 years (range 31-87 years), 57 % were males, and 61% were White and 33% were Asian. Mean baseline albumin level was 3.6 g/dl, and baseline KPS was 90-100 in 55% of patients. Disease characteristics included 68% of patients with liver metastases and 31% with lung metastases; 12% of patients had no prior lines of metastatic therapy, 56 % of patients had 1 prior line of metastatic therapy, 32% of patients had 2 or more prior lines of metastatic therapy.

Patients received treatment until disease progression or unacceptable toxicity. The primary outcome measure was Overall Survival (OS). Additional outcome measures included Progression Free Survival (PFS) and Objective Response Rate (ORR). Results are shown in Table 4. Overall survival is illustrated in Figure 1.

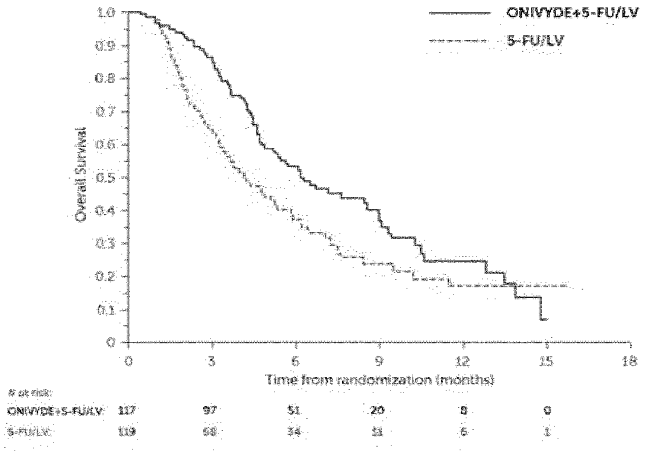
Table 4: Efficacy results from NAPOLI-1 clinical study

	ONIVYDE+5-FU/LV (N= 117)	**5-FU/LV (N= 119)**
Overall Survival[1]		
Number of deaths, n (%)	**75 (64)**	**80 (67)**
Median OS (months)	6.1	4.2
(95% CI)	(4.8, 8.9)	(3.3, 5.3)
Hazard Ratio (95% CI)[3]	0.67 (0.49-0.92)	
p-value[4]	0.0122	
Progression-Free Survival[1,2]		
Death or progression, n (%)	83 (71)	92 (77)
Median PFS (months)	3.1	1.5
(95% CI)	(2.7, 4.2)	(1.4, 1.8)
Hazard Ratio (95% CI)[3]	0.56 (0.41-0.75)	
p-value[4]	0.0001	
Objective Response Rate[2]		
N	19	1
ORR (%)	16.2	0.8
95% CI of Rate[5]	9.6, 22.9	0.0, 2.5
Rate Difference (95% CI)[5]	15.4 (8.5, 22.3)	
p-value[6]	< 0.0001	

1 Median is the Kaplan-Meier estimate of the median survival time
2 Per RECIST guidelines, v 1.1.
3 Cox model analysis
4 Unstratified log-rank test
5 Based on Normal approximation
6 Fisher's exact test

Abbreviations: 5-FU/LV=5-fluorouracil/leucovorin; CI=confidence interval

Figure 1: Overall survival



In the limited number of patients with prior exposure to non-liposomal irinotecan, no benefit of ONIVYDE has been demonstrated.

Paediatric population

The European Medicines Agency has waived the obligation to submit the results of studies with ONIVYDE in all subsets of the paediatric population in treatment of adenocarcinoma of the pancreas (see section 4.2 for information on paediatric use).

5.2 Pharmacokinetic properties

Absorption

Liposome encapsulation of irinotecan extends circulation and limits distribution relative to those of the non-liposomal irinotecan.

The plasma pharmacokinetics of total irinotecan and total SN-38 were evaluated in patients with cancer who received ONIVYDE, as a single agent or as part of combination chemotherapy, at doses between 60 and 180 mg/m2. The pharmacokinetic parameters of total irinotecan and SN-38 analytes, following the administration of ONIVYDE 80 mg/m2 are presented in Table 5.

Table 5: Summary of mean (±standard deviation) total irinotecan and total SN-38

Analyte	PK parameters	Unit	ONIVYDE geomean (95% CI)[a] 80 mg/m^2(n=353)[b]	Non-liposomal irinotecan mean (SD) 125 mg/m^2 (n=99)[c]
Total irinotecan	AUC	h ng/ml	919228 (845653-999204)	10529 (3786)
	C_{max}	ng/ml	28353 (27761-28958)	1492 (452)
	Clearance (CL)	l/h/m2	0.087 (0.080-0.094)	13.0 (5.6)
	Volume (V)	l/m2	2.6 (2.6-2.7)	138 (60.9)
	$t_{1/2\ effective}$	h	20.8 (19.4-22.3)	6.07 (1.19)

Analyte	PK parameters	Unit	ONIVYDE geomean (95% CI)[a] 80 mg/m^2(n=353)[b]	Non-liposomal irinotecan mean (SD) 125 mg/m^2 (n=99)[c]
Total SN-38	AUC	h ng/ml	341 (326-358)	267 (115)
	C_{max}	ng/ml	3.0 (2.9-3.1)	27.8 (11.6)
	$t_{1/2\ effective}$	h	40.9 (39.8-42.0)	11.7 (4.29)

SD= standard deviation
AUC= area under the plasma concentration curve (extrapolated to infinity for ONIVYDE and AUC24h for non-liposomal irinotecan)
C_{max}= maximum plasma concentration
$t_{1/2\ effective}$= effective half-lives
[a]Values are estimated from population PK analysis
[b]N=353 refers to all the subjects included in the population PK analysis
[c]Values are obtained from published data [Schaaf LJ et al. *Clin Cancer Res.* 2006 Jun 15;12:3782-91]

Distribution

Direct measurement of liposomal irinotecan shows that 95% of irinotecan remains liposome-encapsulated during circulation. Non-liposomal irinotecan displays a large volume of distribution (138 l/m^2). The volume of distribution of ONIVYDE 80 mg/m^2 was 2.6 l/m^2, which suggests that ONIVYDE is largely confined to vascular fluid.
The plasma protein binding of ONIVYDE is negligible (< 0.44% of total irinotecan in ONIVYDE). The plasma protein binding of non-liposomal irinotecan is moderate (30% to 68%), and SN-38 is highly bound to human plasma proteins (approximately 95%).

Biotransformation

Irinotecan released from liposome encapsulation follows a similar metabolic pathway reported with non-liposomal irinotecan.
The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes. *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. In the population pharmacokinetic analysis in patients with ONIVYDE using the results of a subset with UGT1A1*28 genotypic testing, in which the analysis adjusted for the lower dose administered to patients homozygous for the UGT1A1*28 allele, patients homozygous (N=14) and non-homozygous (N=244) for this allele had total SN-38 average steady-state concentrations of 1.06 and 0.95 ng/ml, respectively.

Elimination

The disposition of ONIVYDE and non-liposomal irinotecan has not been fully elucidated in humans. The urinary excretion of non-liposomal irinotecan is 11% to 20%; SN-38 <1%; and SN-38 glucuronide is 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).

Renal impairment

No dedicated pharmacokinetic study has been conducted in patients with renal impairment. In a population pharmacokinetic analysis, mild-to-moderate renal impairment had no effect on the exposure of total SN-38 after adjusting for BSA. The analysis included 68 patients with moderate

(CLcr 30-59 ml/min), 147 patients with mild (CLcr 60-89 ml/min) renal impairment, and 135 patients with normal renal function (CLcr > 90 ml/min). There was insufficient data in patients with severe renal impairment (CLcr < 30 ml/min) to assess its effect on pharmacokinetics (see sections 4.2 and 4.4).

Hepatic impairment

No dedicated pharmacokinetic study has been conducted in patients with hepatic impairment. In a population pharmacokinetic analysis, patients with baseline total bilirubin concentrations of 1-2 mg/dl (n=19) had average steady state concentrations for total SN-38 that were increased by 37% (0.98 [95%CI: 0.94-1.02] and 1.29 [95%CI: 1.11-1.5] ng/ml, respectively) compared to patients with baseline bilirubin concentrations of < 1 mg/dl (n=329); however, there was no effect of elevated ALT/AST concentrations on total SN-38 concentrations. No data are available in patients with total bilirubin more than 2 times the ULN.

Other special populations

Age and gender
The population pharmacokinetic analysis in patients aged 28 to 87 years, of whom 11% were ≥75 years suggests that age had no clinically meaningful effect on the exposure to irinotecan and SN-38.
The population pharmacokinetic analysis in 196 male and 157 female patients suggests that gender had no clinically meaningful effect on the exposure to irinotecan and SN-38 after adjusting for body surface area (BSA).

Ethnicity
The population pharmacokinetic analysis suggest that Asians have 56% lower total irinotecan average steady state concentration (3.93 [95%CI: 3.68-4.2] and 1.74 [95%CI: 1.58-1.93] mg/l, respectively) and 8% higher total SN-38 average steady state concentration (0.97 [95%CI: 0.92-1.03] and 1.05 [95%CI: 0.98-1.11] ng/ml, respectively) than Caucasians.

Pharmacokinetic/pharmacodynamic relationship

In a pooled analysis from 353 patients, higher plasma SN-38 C_{max} was associated with increased likelihood of experiencing neutropenia, and higher plasma total irinotecan C_{max} was associated with increased likelihood of experiencing diarrhoea.

In the clinical trial demonstrating effectiveness of ONIVYDE, higher plasma exposures of total irinotecan and SN-38 for patients in the ONIVYDE+5-FU/LV treatment arm were associated with longer OS and PFS as well as with higher ORR (objective response rate).

5.3 Preclinical safety data

In single and repeated dose toxicity studies in mice, rats and dogs, the target organs of toxicity were the gastrointestinal tract and the hematologic system. The severity of effects was dose-related and reversible. The no-observed-adverse-effect level (NOAEL) in rats and dogs following 90 min intravenous infusion of ONIVYDE once every 3 weeks for 18 weeks was at least 180 mg/m^2.
In safety pharmacology studies in dogs, ONIVYDE had no effect on cardiovascular, hemodynamic, electrocardiographic, or respiratory parameters at doses up to 21 mg/kg (420 mg/m^2). No findings indicative of CNS related toxicity were observed in the repeated dose toxicity studies in rats.

Genotoxic and carcinogenic potential

No genotoxicity studies have been performed with ONIVYDE. Non-liposomal irinotecan and SN-38 were genotoxic *in vitro* in the chromosomal aberration test on CHO-cells as well as in the *in vivo* micronucleus test in mice. However, in other studies with irinotecan they have been shown to be devoid of any mutagenic potential in the Ames test.

No carcinogenicity studies have been performed with ONIVYDE. For non-liposomal irinotecan, in rats treated once a week during 13 weeks at the maximum dose of 150 mg/m², no treatment related tumours were reported 91 weeks after the end of treatment. 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. Due to its mechanism of action, irinotecan is considered a potential carcinogen.

Reproduction toxicity

No reproductive and developmental toxicity studies have been performed with ONIVYDE.
Non-liposomal irinotecan was teratogenic in rats and rabbits at doses below the human therapeutic dose. In rats, pups born from treated animals and having external abnormalities showed a decrease in fertility. This was not seen in morphologically normal pups. In pregnant rats there was a decrease in placental weight and in the offspring a decrease in foetal viability and increase in behavioural abnormalities.
Non-liposomal irinotecan caused atrophy of male reproductive organs both in rats and dogs after multiple daily doses of 20 mg/kg and 0.4 mg/kg, respectively.These effects were reversible upon cessation of treatment.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Liposome forming lipids
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)
Cholesterol
N-(carbonyl-methoxypolyethylene glycol-2000)-1, 2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE)

Other excipients
Sucrose octasulphate
2- [4- (2-Hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES buffer)
Sodium chloride
Water for injections

6.2 Incompatibilities

ONIVYDE must not be mixed with other medicinal products except those mentioned in section 6.6.

6.3 Shelf life

Unopened vial
30 months.

After dilution
Chemical and physical stability for the diluted solution for infusion has been demonstrated at 15-25°C for up to 6 hours or in the refrigerator (2°C-8°C) for no more than 24 hours.
From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user.

6.4 Special precautions for storage

Store in a refrigerator (2°C-8°C).
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

For storage conditions after dilution of the medicinal product, see section 6.3.

6.5 Nature and contents of container

Type I glass vial with a grey chlorobutyl stopper and an aluminium seal with a flip-off cap, containing 10 ml of concentrate.
Each pack contains one vial.

6.6 Special precautions for disposal and other handling

ONIVYDE is a cytotoxic medicinal product, and caution should be exercised in handling it. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. If the solution contacts the skin, the skin should be washed immediately and thoroughly with soap and water. If the solution contacts mucous membranes, they should be flushed thoroughly with water. Pregnant staff should not handle ONIVYDE considering the cytotoxic nature of the medicinal product.

Preparation of the solution and administration

ONIVYDE is supplied as a sterile liposomal dispersion at a concentration of 5 mg/ml and must be diluted prior to administration. Dilute with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection to prepare a solution of the appropriate dose of ONIVYDE diluted to a final volume of 500 ml. Mix the diluted solution by gentle inversion. The diluted solution is clear to slightly white to slightly opalescent and free from visible particles.

ONIVYDE should be administered before LV followed by 5-FU. ONIVYDE must not be administered as a bolus injection or an undiluted solution.

Aseptic techniques must be followed during the preparation of the infusion. ONIVYDE is for single use only.

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 sodium chloride 9 mg/ml (0.9%) solution for injection and/or sterile water and applications of ice are recommended.

For storage conditions after dilution of the medicinal product, see section 6.3.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

Baxalta Innovations GmbH
Industriestrasse 67
1221 Vienna
Austria

8. MARKETING AUTHORISATION NUMBER(S)

EU/1/16/1130/001

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

10. DATE OF REVISION OF THE TEXT

Detailed information on this medicinal product is available on the website of the European Medicines Agency http://www.ema.europa.eu.

ANNEX II

A. MANUFACTURER(S) RESPONSIBLE FOR BATCH RELEASE

B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE

C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

A. MANUFACTURER(S) RESPONSIBLE FOR BATCH RELEASE

Name and address of the manufacturer(s) responsible for batch release
Baxter AG
Industriestrasse 67, 1221 Vienna, Austria

B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION

- **Periodic safety update reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

ANNEX III

LABELLING AND PACKAGE LEAFLET

A. LABELLING

PARTICULARS TO APPEAR ON THE OUTER PACKAGING

OUTER CARTON

1. NAME OF THE MEDICINAL PRODUCT

onivyde 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate

2. STATEMENT OF ACTIVE SUBSTANCE

One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as irinotecan sucrosofate salt in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.

3. LIST OF EXCIPIENTS

Excipients:
DSPC
Cholesterol
MPEG-2000-DSPE
Sucrose octasulphate
HEPES buffer
Sodium chloride
Water for injections
See leaflet for further information.

4. PHARMACEUTICAL FORM AND CONTENTS

Concentrate for solution for infusion.
50 mg/10 ml
1 vial

5. METHOD AND ROUTE OF ADMINISTRATION

For single use only.
Read the package leaflet before use.
Intravenous use after dilution.

6. SPECIAL WARNING THAT THE MEDICINAL PRODUCT MUST BE STORED OUT OF THE SIGHT AND REACH OF CHILDREN

Keep out of the sight and reach of children.

7. OTHER SPECIAL WARNING(S), IF NECESSARY

onivyde (liposomal irinotecan) is not equivalent to non-liposomal formulations. Do not interchange.

8. EXPIRY DATE

EXP:

9. SPECIAL STORAGE CONDITIONS

Store in a refrigerator.
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Cytotoxic: handle with caution and special disposal.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

Baxalta Innovations GmbH
Industriestrasse 67
A-1221 Vienna
Austria

12. MARKETING AUTHORISATION NUMBER(S)

EU/1/16/1130/001

13. BATCH NUMBER

Lot:

14. GENERAL CLASSIFICATION FOR SUPPLY

15. INSTRUCTIONS ON USE

16. INFORMATION IN BRAILLE

Justification for not including Braille accepted.

17. UNIQUE IDENTIFIER – 2D BARCODE

2D barcode carrying the unique identifier included.

18. UNIQUE IDENTIFIER – HUMAN READABLE DATA

PC:
SN:
NN:

MINIMUM PARTICULARS TO APPEAR ON SMALL IMMEDIATE PACKAGING UNITS

VIAL LABEL

1. NAME OF THE MEDICINAL PRODUCT AND ROUTE(S) OF ADMINISTRATION

onivyde 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate
IV use after dilution

2. METHOD OF ADMINISTRATION

3. EXPIRY DATE

EXP:

4. BATCH NUMBER

Lot:

5. CONTENTS BY WEIGHT, BY VOLUME OR BY UNIT

50 mg/10 ml

6. OTHER

B. PACKAGE LEAFLET

Package leaflet: Information for the user

ONIVYDE 5 mg/ml concentrate for solution for infusion
Pegylated liposomal irinotecan hydrochloride trihydrate

Read all of this leaflet carefully before you start using this medicine because it contains important information for you.

- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or nurse.
- If you get any side effects, talk to your doctor or nurse. This includes any possible side effects not listed in this leaflet. See section 4.

What is in this leaflet

1. What ONIVYDE is and what it is used for

What ONIVYDE is and how it works

ONIVYDE is a cancer medicine that contains the active substance irinotecan. This active substance is held in tiny lipid (fatty) particles called liposomes.

Irinotecan belongs to a group of cancer medicines called 'topoisomerase inhibitors'. It blocks an enzyme called topoisomerase I, which is involved in the division of cell DNA. This prevents the cancer cells from multiplying and growing, and they eventually die.

The liposomes are expected to accumulate within the tumour and release the medicine slowly over time, thereby allowing it to act for longer.

What ONIVYDE is used for

ONIVYDE is used to treat adult patients with metastatic pancreatic cancer (cancer of the pancreas that has already spread elsewhere in the body) whose previously cancer treatment included a medicine called gemcitabine. ONIVYDE is used in combination with other cancer medicines, called 5-fluorouracil and leucovorin.

If you have any questions about how ONIVYDE works or why this medicine has been prescribed for you, ask your doctor.

2. What you need to know before you use ONIVYDE

Follow carefully all instructions given to you by your doctor. They may differ from the general information contained in this leaflet.

Do not use ONIVYDE:

- if you have a history of a severe allergy to irinotecan, or any of the other ingredients of this medicine (listed in section 6).
- if you are breastfeeding

Warnings and precautions

Talk to your doctor or nurse before you are given ONIVYDE
- if you have ever had any liver problems or jaundice
- if you have ever had lung disease or have previously received medicines (colony stimulating factors) to increase your blood count or radiation therapy
- if you are taking other medicines (see section "Other medicines and ONIVYDE")
- if you are planning to have a vaccination as many vaccinations must not be given during chemotherapy
- if you are on a controlled sodium diet as this medicine contains sodium.

Talk to your doctor or nurse immediately during treatment with ONIVYDE
- if you feel sudden shortness of breath, flushing, headache, skin rash or hives (itchy rash with swollen red bumps on the skin that appear suddenly), itching, swelling around the eyes, tightness in the chest or throat during or shortly after your infusion
- if you experience fever, chills or other symptoms of infection
- if you get diarrhoea with frequent liquid stools and cannot control this after 12 to 24 hours of treatment (see below)
- if you get breathlessness or cough.

What to do in case of diarrhoea

As soon as the first liquid stool occurs, start drinking large volumes ofrehydration fluids (e.g. water, soda water, fizzy drinks, soup) to avoid losing too much liquid and salts from your body. Contact your doctor immediately to give you a suitable treatment. Your doctor may give you a medicine which contains loperamide to begin treatment at home but it must not be used for longer than 48 consecutive hours. If loose stools persist, contact your doctor.

Blood tests and medical examinations

Before you start treatment with ONIVYDE, your doctor will perform blood tests (or other medical examinations) to determine the best starting dose for you. You will need to have further (blood or other) tests during treatment so that your doctor can monitor your blood cells and assess how you are responding to the treatment. Your doctor may need to adjust the dose or stop treatment.

Children and adolescents

ONIVYDE is not recommended for use in adolescents and children below the age of 18 years.

Other medicines and ONIVYDE

Tell your doctor if you are taking, have recently taken or might take any other medicines.
It is especially important that you tell your doctor if you have been given irinotecan in any form earlier.
ONIVYDE must not be used instead of other medicines containing irinotecan because it behaves differently when it is contained in the liposomes than when it is given in its free form.

It is also especially important that you tell your doctor if you are also taking the following medicines, since they reduce the availability of irinotecan in your body:
- phenytoin, phenobarbital or carbamazepin (medicines used to treat convulsions and falls)
- rifampicin and rifabutin (medicines used to treat tuberculosis)

- St. John´s wort (a plant based medicine used to treat depression and low mood)
- as ONIVYDE should not be given to you together with this medicines.

It is especially important that you tell your doctor if you are also taking the following medicines, since they increase the availability of irinotecan in your body:
- ketoconazole, itraconazole or voriconazole (medicines used to treat fungal infections)
- clarithromycin (an antibiotic medicine used to treat bacterial infections)
- indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, atazanavir (medicines against HIV infection)
- telaprevir (a medicine used to treat a liver disease called hepatitis C)
- nefazodone (a medicine used to treat depression, low mood)
- gemfibrozil (medicine used to treat high fat levels in the blood)

ONIVYDE with food and drink

Avoid eating grapefruits and drinking grapefruit juice while you are receiving ONIVYDE as it may increase the availability of the active substance of ONIVYDE in your body.

Pregnancy and breast-feeding

You should not be given ONIVYDE if you are pregnant as it may harm the baby. Tell your doctor if you are or think you may be pregnant. Ask your doctor for advice if you are planning to have a baby. If you are given ONIVYDE you should not breast-feed until one month after the last dose.

During your ONIVYDE treatment and one month after you should choose an effective birth control method which suits you, to prevent pregnancy in this period of time. Males should use condoms during ONIVYDE treatment and 4 months thereafter.

Tell your doctor if you are breast-feeding. You must not be given ONIVYDE if you are breast-feeding as this may be harmful to your baby.

Driving and using machines

ONIVYDE may influence your ability to drive and use machines (as you may be sleepy, dizzy and exhausted with the use of ONIVYDE). You should avoid driving, using machines or performing other tasks that need full attention if you feel sleepy, dizzy and exhausted.

ONIVYDE contains sodium

One millilitre of this medicine contains 0.144 mmol (3.31 mg) sodium—keep this in mind if you are on a controlled sodium diet.

3. How ONIVYDE is used

ONIVYDE must only be given by healthcare professionals trained in giving anticancer medicines
Carefully follow all instructions given to you by your doctor or nurse.

Your doctor will decide upon the doses you will receive.
ONIVYDE is given as a drip (infusion) into a vein, which should take at least 90 minutes and should be given as a single dose.
After you have been given ONIVYDE you will be given two other medicines, leucovorin and 5-fluorouracil.
The treatment will be repeated every two weeks.

In certain cases, lower doses or longer dosing intervals may be required.

You may receive pre-medication against nausea and vomiting. If you have experienced sweating, abdominal cramping and salivation together with early frequent and liquid stools in previous treatments with ONIVYDE, you may receive additional medicines before ONIVYDE to prevent or reduce this in the following treament cycles.

If you have any further questions on the use of this medicine, ask your doctor or nurse.

4. Possible side effects

Like all medicines, this medicine can cause side effects, although not everybody gets them. It is important that you are aware of what these side effects may be.

Your doctor may also prescribe other medicines to help control your side effects.

Tell your doctor or nurse about any of the following serious side effects straight away:

- if you experience sudden shortness of breath, flushing, nausea, headache, skin rash or hives (itchy rash with swollen red bumps on the skin that appear suddenly), itching, swelling around the eyes, tightness in the chest or throat during the infusion or shortly after it (as the infusion may need to be stopped and you may need to be treated or observed for the side effects)
- if you get fever, chills and signs of an infection (as this might require immediate treatment)
- if you have severe persistent diarrhoea (liquid and frequent stools)—see section 2

The following side effects may occur:

Very common (may affect more than 1 in 10 people)
- Low levels of white blood cells (neutropenia and leukopenia), Low level of red blood cells (anaemia)
- Low level of blood platelets (thrombocytopenia)
- Diarrhoea (loose or watery and frequent stools)
- Nausea and vomiting
- Pain in the stomach or in the gut area
- Sore mouth
- Loss of weight
- Loss of appetite
- Loss of body fluid (dehydration)
- Low level of salts (electrolytes) in the body (e.g. of potassium, magnesium)
- Unusual hair loss
- Tiredness
- Dizziness
- Swelling and fluid retention in the soft tissues (peripheral oedema)
- Soreness and swelling of the digestive tract lining (mucosal inflammation)
- Fever
- Generalised weakness

Common (may affect up to 1 in 10 people)
- Chills
- Infections, for example fungal infections in the mouth (oral candidiasis), fever with low counts of white blood cells (febrile neutropenia), infections related to the administration of the product into a vein
- Inflammation of the stomach and the guts (gastroenteritis)
- Systemic body inflammation, caused by infection (sepsis)
- Potentially life-threatening complication of whole body inflammation (septic shock)
- Infection of the lungs (pneumonia)
- Low level of white blood cells subtype, called lymphocytes with important function for the immune system (lymphopenia)

- Decrease in some salts (electrolytes) in the body (e.g. phosphate, sodium)
- Low blood sugar (hypoglycaemia)
- Sleeplessness
- Bad taste in the mouth
- A syndrome called cholinergic syndrome with sweating, salivation and abdominal cramping
- Low blood pressure (hypotension)
- Formation of a blood clot in a deep vein (deep vein thrombosis) or blockage of the main artery of the lung or one of its branches (pulmonary embolism), or blockage due to a blood clot elsewhere in the blood stream (embolism)
- Voice impairment, hoarse or excessively breathy voice
- Shortness of breath
- Inflammation in the gut
- Piles(haemorrhoids)
- Increases in liver enzymes (alanine aminotransferase or aspartate aminotransferase) in laboratory blood tests
- Increase in bilirubin levels (an orange-yellow pigment, waste product of the normal breakdown of the red blood cells) in other laboratory measurements related to liver function
- Increase in other laboratory measurements (increased international normalized ratio) related to the blood clotting system function
- Abnormally low blood levels of albumin (major protein in the body)
- Sudden problems with kidney function which may lead to rapid deterioration or loss of the kidney function
- Abnormal reaction to the infusion causing symptoms like shortness of breath, flushing, headache, tightness in the chest or throat
- Abnormal fluid retention in the body causing swelling in the affected tissues (oedema)

Uncommon (may affect up to 1 in 100 people)
- Systemic body inflammation, caused by infection of the gall bladder and bile ducts (biliary sepsis)
- Allergic reaction to ONIVYDE (the active substance or the excipients)
- Diminished availability of oxygen to the body tissues
- Inflammation of the oesophagus (food pipe)
- Formation or presence of a blood clot within a blood vessel – vein or artery (thrombosis)
- Inflammation of the lining of the rectum (the end of the large intestine)
- Type of rash, characterised by appearance of a flat, red area on the skin covered with bumps (maculo-papular rash)
- Change in the colour of the nail plates

Reporting of side effects

If you get any side effects, talk to your doctor or nurse. This includes any possible side effects not listed in this leaflet. You can also report side effects directly via the national reporting system listed in Appendix V. By reporting side effects you can help provide more information on the safety of this medicine.

5. How to store ONIVYDE

Keep this medicine out of the sight and reach of children.

Do not use this medicine after the expiry date which is stated on the carton and vial after "EXP". The expiry date refers to the last day of that month.

Store in a refrigerator (2°C - 8°C).
Do not freeze.
Keep the vial in the outer carton in order to protect from light.

Once the concentrate has been diluted for infusion with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection, the solution should be used as soon as possible, but may be stored at ambient temperature (15°C to 25°C) for up to 6 hours. The diluted solution for infusion can be stored in the refrigerator (2°C - 8°C) for no more than 24 hours prior to use. It must be protected from light, and it must not be frozen.

Do not throw away this medicine via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

6. Contents of the pack and other information

What ONIVYDE contains

- The active substance is irinotecan hydrochloride trihydrate. One 10 ml vial of concentrate contains the equivalent of 50 mg irinotecan hydrochloride trihydrate (as the sucrosofate salt irinotecan sucrose octasulphate, in a pegylated liposomal formulation) which corresponds to 43 mg irinotecan.
- The other ingredients are: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); cholesterol, N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoly-sn-glycero-3-phosphoethanolamine (MPEG-2000-DSPE); Sucrose octasulphate; 2- [4- (2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES buffer); sodium chloride and water for injections. ONIVYDE contains sodium, if you are on a controlled sodium diet, see section 2.

What ONIVYDE looks like and contents of the pack

ONIVYDE is supplied as a white to slightly yellow opaque isotonic liposomal dispersion in a glass vial.

Each pack contains one vial with 10 ml of concentrate.

Marketing Authorisation Holder
Baxalta Innovations GmbH
Industriestrasse 67
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Tel.: +44(0)1256 894 959
E-mail: medinfoEMEA@shire.com

Manufacturer
Baxter AG
Industriestrasse 67
1221 Vienna
Austria

This leaflet was last revised in

Detailed information on this medicine is available on the European Medicines Agency web site: http://www.ema.europa.eu.

The following information is intended for healthcare professionals only:

How to prepare and administer ONIVYDE

- ONIVYDE is supplied as a sterile liposomal dispersion at a concentration of 5 mg/ml and must be diluted prior to administration. Dilute with 5% glucose solution for injection or sodium chloride 9 mg/ml (0.9%) solution for injection to prepare a solution of the appropriate dose of ONIVYDE diluted to a final volume of 500 ml. Mix diluted solution by gentle inversion.
- ONIVYDE should be administered before leucovorin followed by 5-fluorouracil. ONIVYDE must not be administered as a bolus injection or an undiluted solution.
- Aseptic techniques must be followed during the preparation of the infusion. ONIVYDE is for single use only.
- From a microbiological point of view, the product should be used as soon as possible after dilution. The diluted solution for infusion can be stored at ambient temperature (15°C to 25°C)for up to 6 hours or in the refrigerator (2°C - 8°C) for no more than 24 hours prior to use. It must be protected from light, and it must not be frozen.
- 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 sodium chloride 9 mg/ml (0.9%) solution for injection and/or sterile water and applications of ice are recommended.

How to handle and dispose of ONIVYDE

- ONIVYDE is a cytotoxic medicinal product and caution should be exercised in handling it. The use of gloves, goggles and protective clothing when handling or administering ONIVYDE is recommended. If the solution contacts the skin, the skin should be washed immediately and thoroughly with soap and water. If the solution contacts mucous membranes, they should be flushed thoroughly with water. Pregnant staff should not handle ONIVYDE considering the cytotoxic nature of the medicinal product.
- Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

FDA News Release

FDA approves new treatment for advanced pancreatic cancer

For Immediate Release

October 22, 2015

Release

The U.S. Food and Drug Administration today approved Onivyde (irinotecan liposome injection), in combination with fluorouracil and leucovorin, to treat patients with advanced (metastatic) pancreatic cancer who have been previously treated with gemcitabine-based chemotherapy.

According to the National Cancer Institute, there will be 48,960 new cases of pancreatic cancer diagnosed in the U.S. in 2015, and nearly the same number of deaths caused by the disease (40,560). Pancreatic cancer can be difficult to diagnose early and treatment options are limited, especially when the disease has spread to other parts of the body (metastatic disease) and surgery to remove the tumor is not possible.

"Many FDA staff who review drug applications are clinicians as well, so it's especially rewarding when we are able to expedite access to new treatments for patients with unmet needs," said Richard Pazdur, M.D., director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research. "By using the Priority Review designation for the application for Onivyde, patients will have earlier access to a drug that helps extend survival."

The FDA granted Priority Review and orphan drug designations for Onivyde. **Priority review (http://www.fda.gov/ForPatients/Approvals/Fast/ucm405405.htm)** status is granted to applications for drugs that, if approved, would be a significant improvement in safety or effectiveness in the treatment of a serious condition. **Orphan drug designation (http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/ucm2005525.htm)** provides incentives such as tax credits, user fee waivers, and eligibility for orphan drug exclusivity to assist and encourage the development of drugs for rare diseases.

The effectiveness of Onivyde was demonstrated in a three-arm, randomized, open label study of 417 patients with metastatic pancreatic adenocarcinoma whose cancer had grown after receiving the chemotherapeutic drug gemcitabine or a gemcitabine-based therapy. The study was designed to determine whether patients receiving Onivyde plus fluorouracil/leucovorin or Onivyde alone lived longer than those receiving fluorouracil/leucovorin. Patients treated with Onivyde plus

fluorouracil/leucovorin lived an average of 6.1 months, compared to 4.2 months for those treated with only fluorouracil/leucovorin. There was no survival improvement for those who received only Onivyde compared to those who received fluorouracil/leucovorin.

In addition, patients receiving Onivyde plus fluorouracil/leucovorin had a delay in the amount of time to tumor growth compared to those who received fluorouracil/leucovorin. The average time for those receiving Onivyde plus fluorouracil/leucovorin was 3.1 months compared to 1.5 months for those receiving fluorouracil/leucovorin.

The safety of Onivyde was evaluated in 398 patients who received either Onivyde with fluorouracil/leucovorin, Onivyde alone or fluorouracil/leucovorin. The most common side effects of treatment with Onivyde included diarrhea, fatigue, vomiting, nausea, decreased appetite, inflammation in the mouth (stomatitis) and fever (pyrexia). Onivyde was also found to result in low counts of infection-fighting cells (lymphopenia and neutropenia). Death due to sepsis following neutropenia has been reported in patients treated with Onivyde.

The labeling for Onivyde includes a boxed warning to alert health care professionals about the risks of severe neutropenia and diarrhea. Onivyde is not approved for use as a single agent for the treatment of patients with metastatic pancreatic cancer.

Onivyde is marketed by Merrimack Pharmaceuticals Inc. of Cambridge, Massachusetts.

The FDA, an agency within the U.S. Department of Health and Human Services, promotes and protects the public health by, among other things, assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

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Consumers

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Related Information

- FDA: Office of Hematology and Oncology Products (/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm091745.htm)

- **FDA Approved Drugs: Questions and Answers (/Drugs/ResourcesForYou/Consumers/ucm054420.htm)**
- **NCI: Pancreatic Cancer (http://www.cancer.gov/types/pancreatic/patient/pancreatic-treatment-pdq)**

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Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial

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Summary

Background Nanoliposomal irinotecan showed activity in a phase 2 study in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapies. We assessed the effect of nanoliposomal irinotecan alone or combined with fluorouracil and folinic acid in a phase 3 trial in this population.

Methods We did a global, phase 3, randomised, open-label trial at 76 sites in 14 countries. Eligible patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy were randomly assigned (1:1) using an interactive web response system at a central location to receive either nanoliposomal irinotecan monotherapy (120 mg/m² every 3 weeks, equivalent to 100 mg/m² of irinotecan base) or fluorouracil and folinic acid. A third arm consisting of nanoliposomal irinotecan (80 mg/m², equivalent to 70 mg/m² of irinotecan base) with fluorouracil and folinic acid every 2 weeks was added later (1:1:1), in a protocol amendment. Randomisation was stratified by baseline albumin, Karnofsky performance status, and ethnic origin. Treatment was continued until disease progression or intolerable toxic effects. The primary endpoint was overall survival, assessed in the intention-to-treat population. The primary analysis was planned after 305 events. Safety was assessed in all patients who had received study drug. This trial is registered at ClinicalTrials.gov, number NCT01494506.

Findings Between Jan 11, 2012, and Sept 11, 2013, 417 patients were randomly assigned either nanoliposomal irinotecan plus fluorouracil and folinic acid (n=117), nanoliposomal irinotecan monotherapy (n=151), or fluorouracil and folinic acid (n=149). After 313 events, median overall survival in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid was 6·1 months (95% CI 4·8–8·9) *vs* 4·2 months (3·3–5·3) with fluorouracil and folinic acid (hazard ratio 0·67, 95% CI 0·49–0·92; p=0·012). Median overall survival did not differ between patients assigned nanoliposomal irinotecan monotherapy and those allocated fluorouracil and folinic acid (4·9 months [4·2–5·6] *vs* 4·2 months [3·6–4·9]; 0·99, 0·77–1·28; p=0·94). The grade 3 or 4 adverse events that occurred most frequently in the 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were neutropenia (32 [27%]), diarrhoea (15 [13%]), vomiting (13 [11%]), and fatigue (16 [14%]).

Interpretation Nanoliposomal irinotecan in combination with fluorouracil and folinic acid extends survival with a manageable safety profile in patients with metastatic pancreatic ductal adenocarcinoma who previously received gemcitabine-based therapy. This agent represents a new treatment option for this population.

Funding Merrimack Pharmaceuticals.

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Introduction

Pancreatic ductal adenocarcinoma is typically diagnosed late, when curative resection is impossible and prognosis is poor, with only 1–2% of patients surviving at 5 years.[1,2] Gemcitabine-based therapies have been the standard of care for patients with locally advanced or metastatic pancreatic ductal adenocarcinoma for the past two decades.[3–5] However, two combination regimens—FOLFIRINOX (a combination of oxaliplatin, folinic acid, irinotecan, and fluorouracil) and albumin-bound paclitaxel in combination with gemcitabine—have gained acceptance as front-line treatments.[6,7] Despite these advances, progression after front-line therapy is inevitable, leaving patients and clinicians with few options and no universally accepted standard treatment—showing the unmet need in this population.[8]

Irinotecan has been investigated in several small monotherapy[9] and combination therapy[10–20] studies. The findings have provided initial evidence of the activity of irinotecan in the second-line setting. 1 mg of irinotecan hydrochloride trihydrate salt is equivalent to 0·86 mg of irinotecan free base. Nanoliposomal irinotecan comprises irinotecan free base encapsulated in liposome nanoparticles. The liposome is designed to keep irinotecan in the circulation—sheltered from conversion

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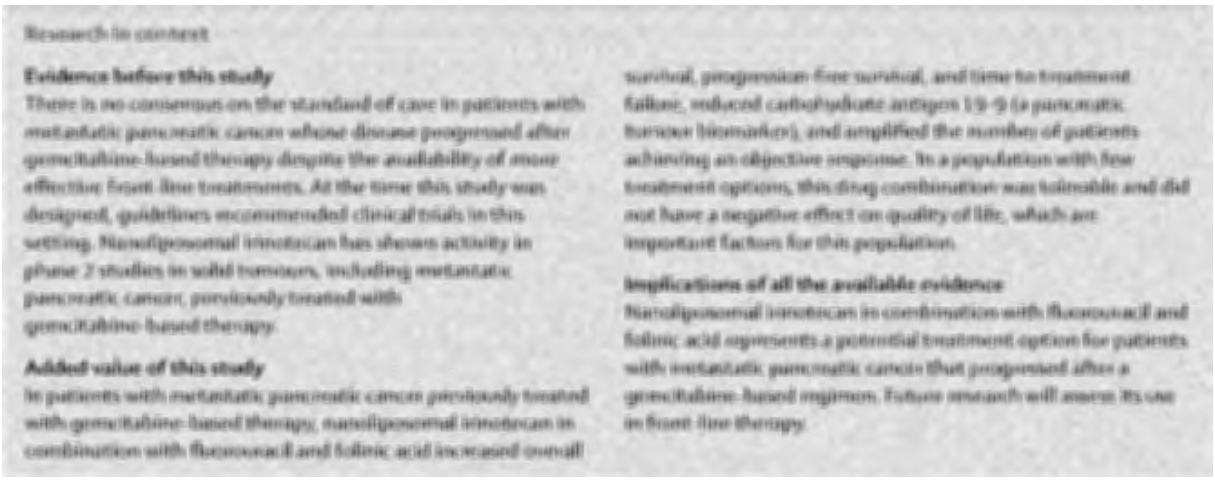

Research in context

Evidence before this study

There is no consensus on the standard of care in patients with metastatic pancreatic cancer whose disease progressed after gemcitabine-based therapy despite the availability of more effective front-line treatments. At the time this study was designed, guidelines recommended clinical trials in this setting. Nanoliposomal irinotecan has shown activity in phase 2 studies in solid tumours, including metastatic pancreatic cancer, previously treated with gemcitabine-based therapy.

Added value of this study

In patients with metastatic pancreatic cancer previously treated with gemcitabine-based therapy, nanoliposomal irinotecan in combination with fluorouracil and folinic acid increased overall survival, progression-free survival, and time to treatment failure, reduced carbohydrate antigen 19-9 (a pancreatic tumour biomarker), and amplified the number of patients achieving an objective response. In a population with few treatment options, this drug combination was tolerable and did not have a negative effect on quality of life, which are important factors for this population.

Implications of all the available evidence

Nanoliposomal irinotecan in combination with fluorouracil and folinic acid represents a potential treatment option for patients with metastatic pancreatic cancer that progressed after a gemcitabine-based regimen. Future research will assess its use in front-line therapy.

to its active metabolite (SN-38)—longer than free (unencapsulated) irinotecan, which would increase and prolong intratumoral levels of both irinotecan and SN-38 compared with free irinotecan.[21–23] The roughly 5·6-fold higher level of SN-38 found in tumours compared with plasma at 72 h suggests local metabolic activation of irinotecan, which was contained in the liposomal nanoparticles, to SN-38.[21] In a phase 2 study of 40 patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy, nanoliposomal irinotecan at 120 mg/m² every 3 weeks resulted in a median overall survival of 5·2 months, 1-year survival of 25%, and a manageable toxicity profile.[24] The aim of this study (NAPOLI-1) was to assess the effect of nanoliposomal irinotecan, alone and in combination with fluorouracil and folinic acid, compared with a common control (fluorouracil and folinic acid), for patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy.

Methods

Study design and participants

We designed a global, multicentre, open-label, phase 3 study conducted at 76 sites in 14 countries (Argentina, Australia, Brazil, Canada, Czech Republic, France, Germany, Hungary, Italy, South Korea, Spain, Taiwan, the UK, and USA). We included patients aged 18 years or older with histologically or cytologically confirmed pancreatic ductal adenocarcinoma and documented measurable or non-measurable distant metastatic disease. The disease must have progressed after previous gemcitabine-based therapy given in a neoadjuvant, adjuvant (only if distant metastases occurred within 6 months of completing adjuvant therapy), locally advanced, or metastatic setting. Other key inclusion criteria were a Karnofsky performance status score of 70 or more and adequate haematological (including absolute neutrophil count >1·5×10⁹ cells per L), hepatic (including normal serum total bilirubin, according to local institutional standards, and albumin levels ≥30 g/L), and renal function. We also enrolled patients who had previously received irinotecan or fluorouracil, or both.

All versions of the protocol and informed consent form were approved by the institutional review board or ethics committees for every site. The study was done according to the principles of the Declaration of Helsinki, the International Conference on Harmonisation Guidance on Good Clinical Practice, and the requirements of the US Food and Drug Administration and local regulatory authorities regarding the conduct of human clinical trials. All patients provided written informed consent.

Randomisation and masking

We initially randomly assigned patients in a 1:1 ratio to receive either nanoliposomal irinotecan monotherapy or a control of fluorouracil and folinic acid (protocol version 1). Following clinical interest in the combination of nanoliposomal irinotecan with other agents, we amended the protocol to add a third arm (1:1:1 ratio) of nanoliposomal irinotecan plus fluorouracil and folinic acid (protocol version 2) after safety data on this combination became available from an ongoing study in metastatic colorectal cancer.[25] Sites continued to enrol patients under protocol version 1 until protocol version 2 was approved at that site.

We randomised patients according to a prespecified scheme generated by an independent statistician within the funder-designated contract research organisation. On confirmation of a patient's eligibility, investigators used a computerised interactive web response system to obtain a patient number, which was associated with a random assignment. We stratified the randomisation by baseline albumin levels (≥40 g/L *vs* <40 g/L), Karnofsky performance status (70 and 80 *vs* ≥90), and ethnic origin (white *vs* east Asian *vs* all others).

Procedures

All patients underwent *UGT1A1* genotype testing. Patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid (combination therapy arm) received an intravenous infusion of nanoliposomal irinotecan over 90 min at a dose of 80 mg/m² (equivalent to 70 mg/m² of irinotecan free base), followed by folinic acid 400 mg/m² over 30 min, then fluorouracil 2400 mg/m² over 46 h, every 2 weeks. For those allocated to the monotherapy arm, nanoliposomal irinotecan was administered at a dose of 120 mg/m² (equivalent to 100 mg/m² of irinotecan free base), every 3 weeks.[24,26] We reduced the initial nanoliposomal irinotecan dose for patients homozygous for the *UGT1A1*28* allele by 20 mg/m² then increased it to the standard dose after the first cycle in the absence of drug-related toxic effects.[27] Patients who were assigned fluorouracil and folinic acid (control arm) received 200 mg/m² of folinic acid as a 30-min infusion followed by an infusion of 2000 mg/m² fluorouracil over 24 h, every week for the first 4 weeks of each 6-week cycle. We based the fluorouracil and folinic acid schedule of the control arm on that used in the CONKO-003 trial[28] and of the combination therapy arm on that used in the PEPCOL study,[25] with expected dose intensities of fluorouracil over a 6-week period of 8000 mg/m² in the control arm and 7200 mg/m² in the combination therapy arm. Treatment continued until disease progression or intolerable toxic effects arose.

We did serial imaging studies and measured amounts of carbohydrate antigen 19-9 (CA19-9) at baseline and every 6 weeks until either disease progression, a new antineoplastic treatment was started, or withdrawal of consent. We did radiographic tumour response assessment according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and we assessed safety by grading adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. We measured quality of life at baseline and every 6 weeks with the European Organization for Research and Treatment of Cancer Quality-of-Life Core Questionnaire (EORTC-QLQ-C30). We assessed clinical benefit response as described elsewhere.[3] We followed up patients every month after treatment termination for survival until death or study completion. An independent data safety monitoring board assessed cumulative safety and other trial-related data at regular intervals.

Outcomes

The primary efficacy endpoint was overall survival. Secondary endpoints included progression-free survival; time to treatment failure; the proportion of patients achieving an objective response; serum CA19-9 response (ie, ≥50% decrease in amount of CA19-9 from baseline at least once during the treatment period); clinical benefit response (ie, either achievement of pronounced and sustained [≥4 weeks contiguous] improvement in pain intensity, analgesic consumption, or performance status, or a combination of these, without any worsening in any of the other factors, or stability in pain intensity, analgesic consumption, and performance status with pronounced and sustained [≥4 weeks contiguous] weight gain); quality of life; and safety. A secondary objective, the pharmacokinetics of nanoliposomal irinotecan as a single agent and in combination with fluorouracil and folinic acid, will be reported separately.

Statistical analysis

We calculated the sample size for the three-arm study through a simulation as part of this study. In the protocol, we planned to enrol 405 patients, for a primary analysis of overall survival after 305 events, to provide at least 98% power to detect a hazard ratio (HR) for death with nanoliposomal irinotecan plus fluorouracil and folinic acid relative to fluorouracil and folinic acid of 0·5, and at least 85% power to detect a HR for death with nanoliposomal irinotecan monotherapy relative to fluorouracil and folinic acid of 0·67.

We did efficacy analyses in the intention-to-treat population (ie, all randomised patients). We analysed safety in patients who received one dose or more (including a partial dose) of study treatment. For the primary efficacy analysis, the null hypotheses tested were: no effect of nanoliposomal irinotecan monotherapy on overall survival relative to control; and no effect of nanoliposomal irinotecan plus fluorouracil and folinic acid on overall survival relative to control. We controlled the family-wise error at a two-sided 0·05 level with the Bonferroni-Holm procedure. For all efficacy and quality-of-life comparisons, the patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were compared with those allocated the fluorouracil and folinic acid control under the amended protocol (version 2), whereas patients assigned nan] oliposomal irinotecan monotherapy were compared with those allocated the fluorouracil and folinic acid control under either version of the protocol (versions 1 and 2).

We did Kaplan-Meier analyses on each treatment group to obtain non-parametric estimates of median overall survival and progression-free survival and time to treatment failure. We calculated corresponding 95% CIs with the log–log method. We used unstratified Cox proportional hazards regression to estimate HRs and their corresponding 95% CIs. We did two pairwise comparisons of overall survival and progression-free survival between the study treatments by unstratified log-rank test. To assess the robustness of the primary endpoint results, we used a Cox regression model with stepwise selection (p to enter <0·25, p to remain <0·15), with treatment and baseline potential prognostic factors as candidates for inclusion in the model for overall survival. We also did a supportive stratified analysis for overall survival, accounting for randomisation strata.

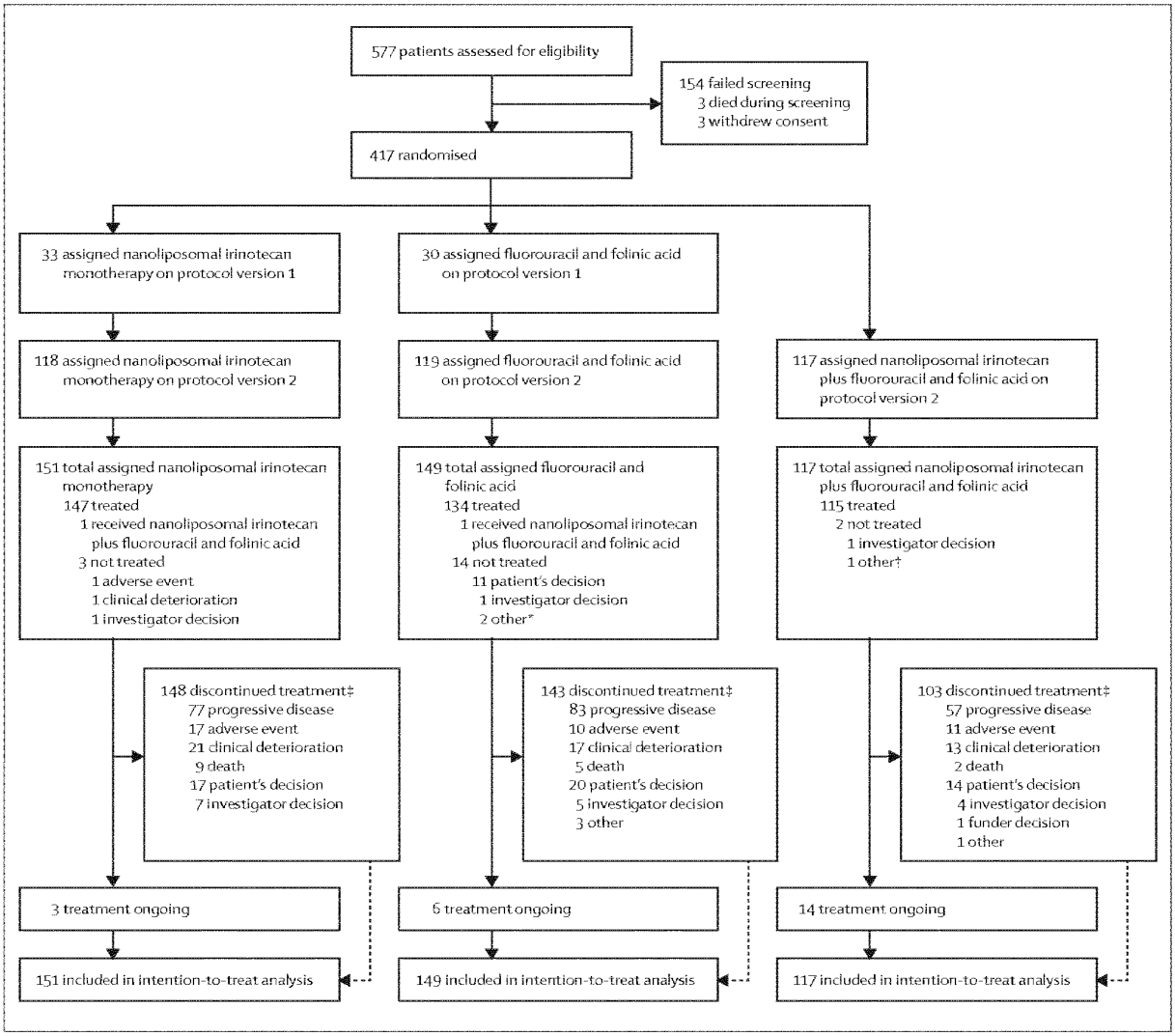

Figure 1: **Trial profile**
*One patient became ineligible after randomisation; one patient had an adverse event that delayed dosing more than 7 days from randomisation. †One patient became ineligible after randomisation. ‡The primary reason for discontinuation was at the discretion of the investigator.

We used Fisher's exact test for pairwise comparisons of objective response, clinical benefit response (we only included treated patients with a baseline pain intensity ≥20/100, baseline opioid pain medication consumption ≥10 mg/day of oral morphine equivalents, and a baseline Karnofsky performance status score of 70–90), and CA19-9 response (we only included treated patients with a baseline CA19-9 value >30 U/mL). We based analyses of progression-free survival and response on tumour and disease progression assessments per the investigator. We did pairwise treatment group comparisons for response classification for each quality-of-life subscale with the Cochran-Mantel-Haenszel test, and we used the Benjamini-Hochberg method to control type I error for comparisons on multiple subscales. We did all analyses and summaries with SAS, version 9.2 (or higher).

This study is registered with ClinicalTrials.gov, number NCT01494506.

Role of the funding source

This study was funded by Merrimack Pharmaceuticals. The study protocol was designed by the funder and external consultants, and data were analysed by a statistician employed by the funder (BB). All authors gathered data and were assisted in writing of the report by a medical writer employed by the funder. L-TC, AW-G, and DDVH had full access to all data in the study, participated in data interpretation, and had final responsibility for the

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)	Fluorouracil and folinic acid combination therapy control (n=119*)	Nanoliposomal irinotecan monotherapy (n=151)	Fluorouracil and folinic acid monotherapy control (n=149)
Men	69 (59%)	67 (56%)	87 (58%)	81 (54%)
Women	48 (41%)	52 (44%)	64 (42%)	68 (46%)
Age (years)	63 (57–70)	62 (55–69)	65 (58–70)	63 (55–69)
Ethnic origin				
East Asian	34 (29%)	36 (30%)	52 (34%)	50 (34%)
Black or African American	4 (3%)	3 (3%)	3 (2%)	3 (2%)
White	72 (62%)	76 (64%)	89 (59%)	92 (62%)
Other	7 (6%)	4 (3%)	7 (5%)	4 (3%)
Region				
Asia	34 (29%)	35 (29%)	50 (33%)	48 (32%)
Europe	47 (40%)	49 (41%)	54 (36%)	55 (37%)
North America	19 (16%)	19 (16%)	26 (17%)	25 (17%)
Other	17 (15%)	16 (13%)	21 (14%)	21 (14%)
Karnofsky performance status score†				
100	18 (15%)	17 (14%)	22 (15%)	22 (15%)
90	51 (44%)	40 (34%)	64 (42%)	54 (36%)
80	38 (32%)	51 (43%)	50 (33%)	61 (41%)
70	7 (6%)	10 (8%)	15 (10%)	11 (7%)
50–60	3 (3%)	0	0	0
Pancreatic tumour location				
Head	76 (65%)	69 (58%)	99 (66%)	81 (54%)
Other	41 (35%)	50 (42%)	52 (34%)	68 (46%)
Amount of CA19-9‡				
≥40 U/mL	92/114 (81%)	91/114 (80%)	125/146 (86%)	116/144 (81%)
<40 U/mL	22/114 (19%)	23/114 (20%)	21/146 (14%)	28/144 (39%)
Site of metastatic lesions§				
Liver	75 (64%)	83 (70%)	101 (67%)	108 (72%)
Lung	36 (31%)	36 (30%)	49 (32%)	44 (30%)
Lymph node, distant	32 (27%)	31 (26%)	44 (29%)	40 (27%)
Lymph node, regional	13 (11%)	14 (12%)	19 (13%)	20 (13%)
Pancreas	75 (64%)	72 (61%)	99 (66%)	97 (65%)
Peritoneum	28 (24%)	32 (27%)	48 (32%)	39 (26%)
Other	27 (23%)	39 (33%)	38 (25%)	48 (32%)

(Table 1 continues on next page)

decision to submit for publication. All authors agreed to submit the report.

Results

Between Jan 11, 2012, and Sept 11, 2013, 417 patients at 76 sites from 14 countries worldwide were randomly assigned to nanoliposomal irinotecan plus fluorouracil and folinic acid (n=117), nanoliposomal irinotecan monotherapy (n=151), or fluorouracil and folinic acid (n=149). 63 patients were enrolled under protocol version 1 before all sites switched to version 2 (figure 1). Patients' demographics and baseline clinical characteristics were similar among the three treatment groups (table 1). 51 (12%) patients received gemcitabine-based therapy in the adjuvant, neoadjuvant, or locally advanced setting but had not had previous treatment for metastatic disease, 234 (56%) had received one previous line of metastatic treatment, and 132 (32%) patients had previously received two or more lines of metastatic treatment.

Seven patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and seven individuals allocated nanoliposomal irinotecan as monotherapy were homozygous for the *UGT1A1*28* allele. Three patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were able to escalate to the standard starting dose of 80 mg/m² without need for subsequent dose reduction. One additional patient needed a dose reduction to 40 mg/m², and one discontinued because of an adverse event (grade 3 vomiting). Two of seven patients allocated nanoliposomal irinotecan were able to increase the dose, to 100 mg/m² and 120 mg/m². One patient needed a dose

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)	Fluorouracil and folinic acid combination therapy control (n=119*)	Nanoliposomal irinotecan monotherapy (n=151)	Fluorouracil and folinic acid monotherapy control (n=149)
(Continued from previous page)				
Measurable metastatic sites (n)				
1	19 (16%)	22 (18%)	36 (24%)	26 (17%)
2	49 (42%)	58 (49%)	63 (42%)	72 (48%)
3	22 (19%)	15 (13%)	22 (15%)	21 (14%)
≥4	7 (6%)	8 (7%)	7 (5%)	10 (7%)
Previous therapies or procedures				
Radiotherapy	24 (21%)	27 (23%)	40 (26%)	33 (22%)
Whipple procedure	30 (26%)	33 (28%)	47 (31%)	36 (24%)
Biliary stent	15 (13%)	8 (7%)	13 (9%)	9 (6%)
Previous lines of metastatic therapy				
0¶	15 (13%)	15 (13%)	17 (11%)	19 (13%)
1	62 (53%)	67 (56%)	86 (57%)	86 (58%)
≥2	40 (34%)	37 (31%)	48 (32%)	44 (30%)
Previous anticancer therapy‖				
Gemcitabine alone	53 (45%)	55 (46%)	67 (44%)	66 (44%)
Gemcitabine combination	64 (55%)	64 (54%)	84 (56%)	83 (56%)
Fluorouracil based	50 (43%)	52 (44%)	70 (46%)	63 (42%)
Irinotecan based	12 (10%)	17 (14%)	17 (11%)	17 (11%)
Platinum based	38 (32%)	41 (34%)	54 (36%)	45 (30%)

Data are number of patients (%) or median (IQR). CA19-9=carbohydrate antigen 19-9. *Fluorouracil and folinic acid combination control group based on protocol version 2. †Baseline Karnofsky performance status score was missing for one patient in the fluorouracil and folinic acid group (enrolled under protocol 2) who was subsequently stratified as having a score ≥90. ‡Data were missing for three patients in the nanoliposomal iinotecan plus fluorouracil and folinic acid group and in five patients each in the nanoliposomal irinotecan monotherapy and fluorouracil and folinic acid groups (enrolled under protocol 2). §Investigator-reported with review by the funder's medical team. Some patients had multiple metastatic sites and are listed in more than one group. ¶Patients received neoadjuvant, adjuvant, or locally advanced treatment, but no previous therapy for metastatic disease. ‖Columns add up to greater than 100% because some patients received more than one line of therapy and are listed in more than one group, and regimens might include multiple drug classes, but at least one gemcitabine based.

Table 1: **Baseline characteristics**

reduction to 40 mg/m², but none discontinued because of an adverse event.

The survival analysis was based on 313 deaths, with a cutoff date of Feb 14, 2014. Deaths were recorded in 75 (64%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, 80 (67%) of 119 individuals allocated the fluorouracil and folinic acid combination control, 129 (85%) of 151 patients assigned nanoliposomal irinotecan monotherapy, and 109 (73%) of 149 individuals allocated the fluorouracil and folinic acid monotherapy control. Median overall survival was 6·1 months (95% CI 4·8–8·9) in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 4·2 months (3·3–5·3) in those allocated fluorouracil and folinic acid (unstratified HR 0·67, 95% CI 0·49–0·92; p=0·012; figure 2A). Median overall survival was 4·9 months (95% CI 4·2–5·6) for patients allocated nanoliposomal irinotecan monotherapy compared with 4·2 months (3·6–4·9) for those assigned fluorouracil and folinic acid (unstratified HR 0·99, 95% CI 0·77–1·28; p=0·94; figure 2B).

Preplanned subgroup analyses showed that the survival benefit of nanoliposomal irinotecan plus fluorouracil and folinic acid was homogeneous across most subgroups (figure 3). In the stepwise Cox regression analysis of nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid, an association of overall survival was identified between treatment and the following prognostic factors: baseline Karnofsky performance status, albumin, time since receiving most recent anticancer therapy, tumour stage at diagnosis, status of liver metastases, and baseline CA19-9. Adjusting for these prognostic factors, the combination of nanoliposomal irinotecan plus fluorouracil and folinic acid maintained a strong treatment effect on overall survival (HR 0·58, 95% CI 0·42–0·81).

In patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, median progression-free survival was 3·1 months (95% CI 2·7–4·2) compared with 1·5 months (1·4–1·8) in those allocated fluorouracil and folinic acid (unstratified HR 0·56, 95% CI 0·41–0·75; p=0·0001; figure 2C). In patients allocated nanoliposomal irinotecan monotherapy, median progression-free survival was 2·7 months (95% CI 2·1–2·9) versus 1·6 months (1·4–1·8) for those assigned fluorouracil and

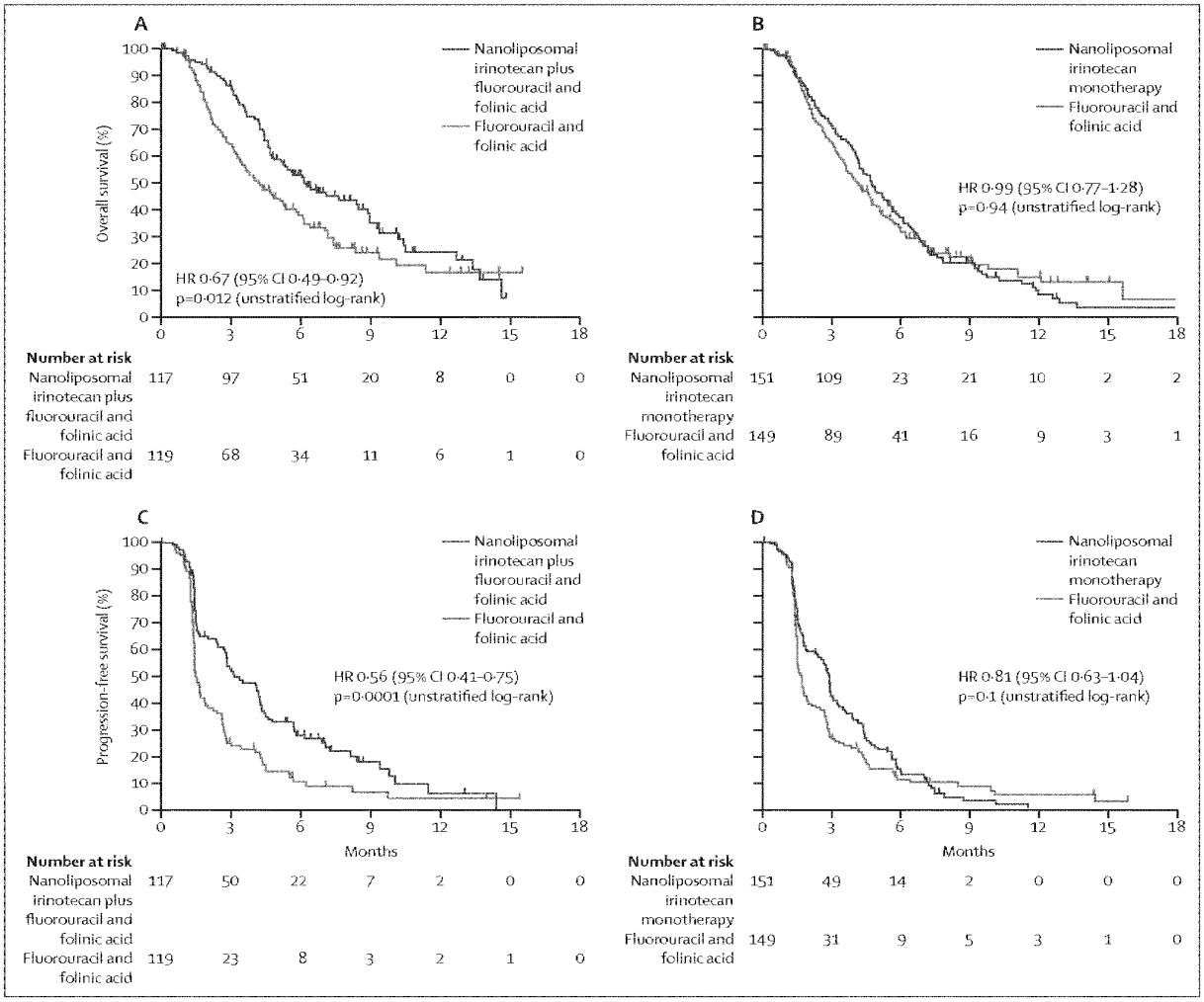

Figure 2: **Kaplan-Meier survival analyses**

HR=hazard ratio. (A) Overall survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (B) Overall survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid. (C) Progression-free survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (D) Progression-free survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid.

folinic acid (unstratified HR 0·81, 95% CI 0·63–1·04; p=0·1; figure 2D).

Median time to treatment failure was 2·3 months (95% CI 1·6–2·8) in patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid compared with 1·4 months (1·3–1·4) in those assigned fluorouracil and folinic acid (HR 0·6, 95% CI 0·45–0·78; p=0·0002). Time to treatment failure did not differ significantly between patients assigned nanoliposomal irinotecan monotherapy and those allocated fluorouracil and folinic acid (1·7 months [95% CI 1·5–2·7] *vs* 1·4 months [1·3–1·4]; HR 0·82, 95% CI 0·65–1·03; p=0·1).

19 (16%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid achieved an objective response compared with one (1%) of 119 individuals allocated fluorouracil and folinic acid (difference 15·4 percentage points, 95% CI 8·5–22·3; p<0·0001). Nine (6%) of 151 patients allocated nanoliposomal irinotecan monotherapy achieved an objective response compared with one (1%) of 149 assigned fluorouracil and folinic acid (difference 5·3 percentage points, 95% CI 1·3–9·3; p=0·02).

28 (29%) of 97 patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid achieved a CA19-9 response (≥50% decrease from abnormal baseline) versus seven (9%) of 81 assigned fluorouracil and folinic acid (p=0·0006). 29 (24%) of 123 patients allocated nanoliposomal irinotecan monotherapy had a CA19-9 response versus 12 (11%) of 105 assigned fluorouracil and folinic acid (p=0·024).

At baseline, median scores for quality-of-life measures (global health status, functional scale, and symptoms

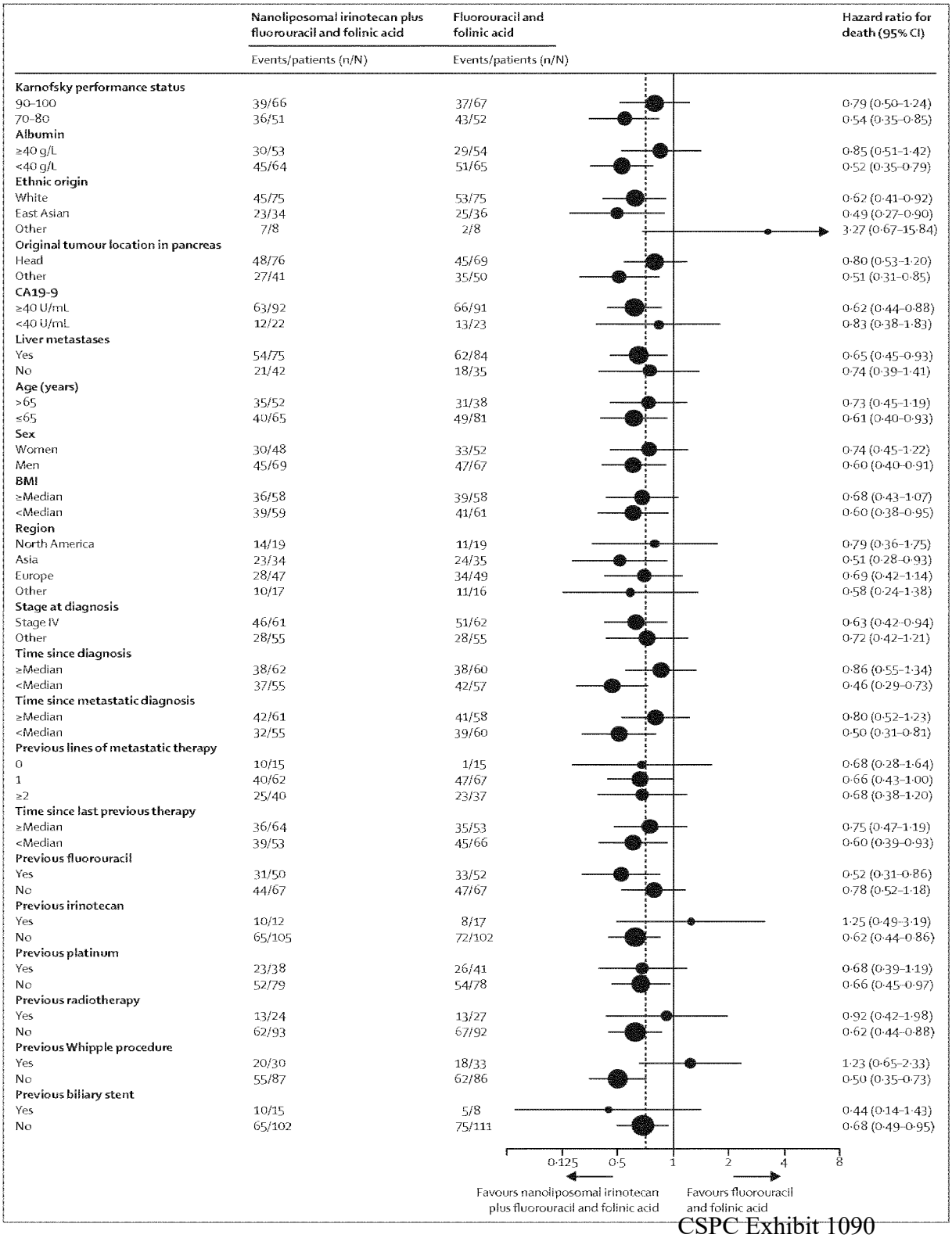

Figure 3: **Forest plot of treatment effect on survival in prespecified subgroups**
Hazard ratios are depicted by filled circles and 95% CIs by horizontal lines. The size of the circle reflects the size of the subgroup relative to the intention-to-treat population. BMI=body-mass index. CA19-9=carbohydrate antigen 19-9.

scores) were similar between groups. At 6 and 12 weeks, the median functional scale scores did not differ appreciably from baseline, suggesting that the effects of the treatments on functional scale scores were negligible. Clinical benefit response was less than 20% and did not differ significantly between treatment groups (appendix pp 1–3).

In patients allocated nanoliposomal irinotecan plus fluorouracil and folinic acid and nanoliposomal irinotecan monotherapy, median duration of exposure to nanoliposomal irinotecan was 8·7 weeks (IQR 5·4–22·0) and 8·9 weeks (6·0–16·0), respectively, and mean dose intensities were 167·5 mg/m² (SD 44·8) over 6 weeks and 188·0 mg/m² (52·0) over 6 weeks, respectively. Median exposure to fluorouracil was 8·7 weeks (IQR 5·4–22·0) in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 6·0 weeks (5·9–12·1) in those allocated fluorouracil and folinic acid (n=105, protocol version 2); mean dose intensities of fluorouracil were, respectively, 5065·0 mg/m² (SD 1539·1) over 6 weeks and 6710·2 mg/m² (1719·2) over 6 weeks.

Post-progression anticancer therapy was given to 36 (31%) of 117 patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid and 45 (38%) of 119 patients allocated fluorouracil and folinic acid. The use of treatment after progression and the type of therapy used was generally similar between groups. Eight patients assigned the combination therapy and nine patients allocated the control received irinotecan as part of post-progression therapy.

398 (95%) of the 417 patients randomly assigned received at least one dose of study drug and were included in the safety analysis population. The most common treatment-emergent adverse events of all grades in patients whose treatment included nanoliposomal irinotecan were diarrhoea, nausea, and vomiting (table 2). Alopecia occurred in 16 (14%) of 117 patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, 32 (22%) of 147 individuals who received nanoliposomal irinotecan as monotherapy, and six (5%) of 134 patients who received fluorouracil and folinic acid. Adverse events that resulted in a dose reduction occurred in 39 (33%) patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, 46 (31%) individuals given nanoliposomal irinotecan monotherapy, and five (4%) patients who received fluorouracil and folinic acid. Grade 3 or 4 neutropenic sepsis (including febrile neutropenia) was noted in three (3%) patients in the combination therapy group and six (4%) individuals who had monotherapy, with no events of this type reported in the control group. Granulocyte colony-stimulating factor was administered to 20 (17%) patients receiving nanoliposomal irinotecan plus fluorouracil and folinic acid and 17 (12%) of those treated with nanoliposomal irinotecan monotherapy, compared with one (1%) patient in the fluorouracil and folinic acid group. Grade 4 treatment-emergent adverse events were reported in 12 (10%) patients given nanoliposomal irinotecan plus fluorouracil and folinic acid, 24 (16%) individuals who received nanoliposomal irinotecan monotherapy, and nine (7%) of those in the fluorouracil and folinic acid group. Of these, only three patients in the monotherapy group and one individual receiving control had a gastrointestinal event. 30-day mortality was low in all groups (three [3%] of 117 in the nanoliposomal irinotecan plus fluorouracil and folinic acid group, three [2%] of 151 in the monotherapy group, and four [3%] of 149 with control). Adverse events leading to discontinuation of study drug arose in 13 (11%) patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, 17 (12%) individuals allocated nanoliposomal irinotecan monotherapy, and ten (7%) patients assigned fluorouracil and folinic acid.

Of 47 patients who died during the study or within 30 days from the last dose of study drug, 30 deaths were attributed to pancreatic cancer, 16 were due to an adverse event (five related to treatment, according to the investigator), and the cause was unknown for one. The treatment-related adverse events that resulted in death were gastrointestinal toxic effects (n=1, nanoliposomal irinotecan monotherapy), infectious enterocolitis (n=1, nanoliposomal irinotecan monotherapy), septic shock (n=1, nanoliposomal irinotecan monotherapy; n=1, nanoliposomal irinotecan plus fluorouracil and folinic acid), and disseminated intravascular coagulation with pulmonary embolism (n=1, nanoliposomal irinotecan monotherapy). 90 (61%) of 147 patients assigned nanoliposomal irinotecan monotherapy had a treatment-emergent serious adverse event compared with 56 (48%) of 117 individuals allocated nanoliposomal irinotecan plus fluorouracil and folinic acid and 60 (45%) of 134 patients assigned fluorouracil and folinic acid.

	Nanoliposomal irinotecan plus fluorouracil and folinic acid combination therapy (n=117)		Nanoliposomal irinotecan monotherapy (n=147)		Fluorouracil and folinic acid control (n=134)	
	Any grade	Grades 3–4	Any grade	Grades 3–4	Any grade	Grades 3–4
Diarrhoea	69 (59%)	15 (13%)	103 (70%)	31 (21%)	35 (26%)	6 (4%)
Vomiting	61 (52%)	13 (11%)	80 (54%)	20 (14%)	35 (26%)	4 (3%)
Nausea	60 (51%)	9 (8%)	89 (61%)	8 (5%)	46 (34%)	4 (3%)
Decreased appetite	52 (44%)	5 (4%)	72 (49%)	13 (19%)	43 (32%)	3 (2%)
Fatigue	47 (40%)	16 (14%)	54 (37%)	9 (6%)	37 (28%)	5 (4%)
Neutropenia*	46 (39%)	32 (27%)	37 (25%)	22 (15%)	7 (5%)	2 (1%)
Anaemia	44 (38%)	11 (9%)	48 (33%)	16 (11%)	31 (23%)	9 (7%)
Hypokalaemia	14 (12%)	4 (3%)	32 (22%)	17 (12%)	12 (9%)	3 (2%)

Data are number of patients (%). The table shows grade 3 and 4 adverse events reported in ≥5% of patients whose treatment included nanoliposomal irinotecan with ≥2% incidence versus fluorouracil and folinic acid. *Includes agranulocytosis, febrile neutropenia, granulocytopenia, neutropenia, neutropenic sepsis, decreased neutrophil count, and pancytopenia.

Table 2: Adverse events

See Online for appendix

Discussion

The results of this international, multicentre, randomised, phase 3 study (NAPOLI-1) showed that nanoliposomal irinotecan plus fluorouracil and folinic acid significantly improved the overall survival of patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based therapy. Progression-free survival, objective tumour response, time to treatment failure, and CA19-9 tumour marker response in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid were also significantly superior to fluorouracil and folinic acid control. In the preplanned analyses of each subgroup, overall survival was increased in patients with a Karnofsky performance score less than 90, a concentration of albumin less than 40 g/L, CA19-9 greater than 40 IU/mL, and liver metastases who were assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, versus those allocated fluorouracil and folinic acid. Furthermore, patients with unfavourable prognostic factors who were assigned nanoliposomal irinotecan plus fluorouracil and folinic acid achieved lower HRs compared with patients assigned fluorouracil and folinic acid, supporting the possible use of nanoliposomal irinotecan plus fluorouracil and folinic acid in this population. Use of post-progression anticancer therapy was generally similar between treatment groups.

Patients assigned nanoliposomal irinotecan monotherapy achieved a median overall survival of 4·9 months, which was consistent with the 5·2 months recorded in a previous phase 2 study of nanoliposomal irinotecan in 40 patients with similar demographics and baseline disease characteristics.[24] Although patients assigned nanoliposomal irinotecan monotherapy did not show superiority in overall survival or progression-free survival compared with those allocated fluorouracil and folinic acid, they had better objective and CA19-9 responses, suggesting that nanoliposomal irinotecan alone has some activity against pancreatic cancer. However, nanoliposomal irinotecan as monotherapy was administered at a higher dose and a lower frequency, which resulted in patients assigned to this group having a higher incidence of gastrointestinal adverse events compared with those allocated the nanoliposomal irinotecan combination regimen.

The choice of fluorouracil and folinic acid as control was based on several factors. First, there is no universally accepted standard treatment for metastatic pancreatic ductal adenocarcinoma following gemcitabine-based therapy. Second, there is a preference for using approved drugs as controls in registration trials, and fluorouracil is an approved agent for treatment of pancreatic cancer. Third, fluorouracil and folinic acid served as the control in CONKO-003,[28] a controlled study in patients with advanced and metastatic pancreatic ductal adenocarcinoma following gemcitabine therapy, thereby setting a precedent. We did not change the control after adding the third arm of nanoliposomal irinotecan plus fluorouracil and folinic acid to our study because 63 patients had been treated with the original schedule and changing it would render the data not available for inclusion in the final analysis. Fourth, no data existed on use of the fluorouracil and folinic acid dose and schedule (FOLFIRI.3) given without irinotecan for treatment of metastatic pancreatic ductal adenocarcinoma, so although it would have been an ideal control arm it would not have historical precedence. Although we acknowledge that the fluorouracil and folinic acid regimen used in the combination arm was different from the control, which is not a standard design with the study drug being added to the same control regimen, the fluorouracil and folinic acid regimen given with nanoliposomal irinotecan was optimised for the combination. It is highly unlikely that the difference in dosing created bias in favour of the investigational arm, because the planned and recorded fluorouracil dose intensities were lower in the investigational arm compared with the control arm. The significantly improved overall survival in patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid, particularly in view of the lower fluorouracil dose intensity compared with the fluorouracil and folinic acid control, supports the benefit of combining nanoliposomal irinotecan with fluorouracil and folinic acid. Finally, it should be noted that the control arm in NAPOLI-1 performed better than did the historical control (CONKO-003)[28] with respect to overall survival (3·3 months in CONKO-003 *vs* 4·2 months in NAPOLI-1).

The NAPOLI-1 and CONKO-003 studies used the same dose and schedule of fluorouracil and folinic acid as control. Moreover, relatively similar median overall survival was reported in both studies (6·1 months with nanoliposomal irinotecan plus fluorouracil and folinic acid in NAPOLI-1 *vs* 5·9 months with oxaliplatin plus fluorouracil and folinic acid in CONKO-003). However, findings of a more recent study comparing an oxaliplatin plus fluorouracil and folinic acid regimen failed to show superiority of overall survival over fluorouracil and folinic acid (n=54 in each arm; 6·1 months *vs* 9·9 months; p=0·02).[29] The reasons for these contradictory results are not clear, but they show the hazards of cross-study comparisons. Despite the similarities between NAPOLI-1 and CONKO-003, there are many differences. For example, the study population in NAPOLI-1 consisted of patients with metastatic pancreatic ductal adenocarcinoma who had progressed after previous gemcitabine-based therapy in a neoadjuvant, adjuvant, locally advanced, or metastatic setting. Patients in CONKO-003 had advanced cancer, which included a heterogeneous group of both metastatic and locally advanced disease (12%)—those with locally advanced disease having a historically documented better survival.[28] Moreover, patients in CONKO-003 had disease that had progressed with first-line gemcitabine monotherapy, and they had to start on the study within 4 weeks of disease

progression. On the other hand, patients in NAPOLI-1 had received gemcitabine either as monotherapy or in combination, for any stage of disease at any time in the past, in many cases having received multiple lines of different treatments; their disease had to be progressing with distant metastases only, irrespective of the previous line of therapy for inclusion. Patients' enrolment for CONKO-003 (n=168) occurred between 2004 and 2007 at 16 sites in Germany.[28] The larger, global, NAPOLI-1 study (n=117 study arm, n=119 control arm after protocol amendment) was conducted between 2012 and 2013 at 76 sites worldwide: more than a third of patients in NAPOLI-1 were not of white ethnic origin. The consistency of the results of NAPOLI-1 in a diverse population at multiple medical centres supports the robustness of the positive outcome. The biggest difference between CONKO-003 and NAPOLI-1 with respect to safety is that, unlike with the oxaliplatin plus fluorouracil and folinic acid regimen, nanoliposomal irinotecan plus fluorouracil and folinic acid is not associated with neuropathy and should be considered as a treatment option for patients who fail the albumin-bound paclitaxel and gemcitabine combination.

With respect to combination therapies containing unencapsulated irinotecan plus fluorouracil and folinic acid in second-line pancreatic cancer, Yoo and colleagues[10] did a randomised phase 2 trial comparing modified versions of FOLFOX (folinic acid, fluorouracil, and oxaliplatin) and FOLFIRI (folinic acid, fluorouracil, and irinotecan) regimens for treatment of gemcitabine-refractory advanced pancreatic cancer. However, in that study, the median overall survival was short (3·5 months with FOLFOX and 3·9 months with FOLFIRI). Various other prospective and retrospective studies of FOLFIRI had small sample sizes and were of single-arm design. Of these, the longest median overall survival—6·6 months—was reported in a small (n=63) non-randomised study that included use of either the FOLFIRI.1 or FOLFIRI.3 regimens.[14] These two regimens differ in that FOLFIRI.3 does not include a fluorouracil bolus and divides the irinotecan dose in two, with the second irinotecan dose being given after fluorouracil and folinic acid administration. The next most effective study was a retrospective analysis of a small population (n=40) of patients with gemcitabine-refractory locally advanced and metastatic cancer, which showed an overall survival of 6·0 months.[11] Although promising, these studies show the need for large randomised, multicentre studies to clearly identify optimum therapy for patients with previously treated metastatic pancreatic cancer. Irinotecan-containing regimens have not been a standard until the advent of the FOLFIRINOX regimen in front-line metastatic pancreatic cancer.

Despite additional toxicity, the quality of life of patients assigned nanoliposomal irinotecan plus fluorouracil and folinic acid was not appreciably different from those allocated the fluorouracil and folinic acid control, which is an important measure in patients with metastatic pancreatic ductal adenocarcinoma, who are generally in poor health from the effects of the underlying disease and previous treatments. Little difference between study treatments was reported in clinical benefit response; however, assessment of this outcome is limited because of the burdensome requirements of data collection from these very sick patients. The pain component, based on patient-reported daily diary data, had low compliance (60% [250/417] of intention-to-treat patients eligible). The precision of the clinical benefit response classification rules, which call for 4 consecutive weeks with robust criteria for improvement and less robust criteria for negative clinical benefit, also restricted the assessment.

Adverse events in our study were consistent with those in previous studies of nanoliposomal irinotecan.[22,24,26] Despite a lower delivered dose per cycle and lower observed mean dose intensity of nanoliposomal irinotecan compared with the monotherapy arm, patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group had a higher incidence of grade 3 or 4 neutropenia than did those receiving nanoliposomal irinotecan monotherapy, which could be attributable to the addition of fluorouracil and folinic acid. However, the incidence of neutropenic sepsis was low in all treatment groups. Conversely, patients in the nanoliposomal irinotecan plus fluorouracil and folinic acid group had less frequent severe diarrhoea than did those receiving nanoliposomal irinotecan monotherapy. The incidence of alopecia was also lower in patients receiving nanoliposomal irinotecan plus fluorouracil and folinic acid than in those in the nanoliposomal irinotecan monotherapy group. The lower nanoliposomal irinotecan dose every 2 weeks—even in combination with fluorouracil and folinic acid—showed a better therapeutic index for severe gastrointestinal events than did nanoliposomal irinotecan at a higher dose every 3 weeks. Most patients tolerated the gastrointestinal adverse events, with around 11% of patients in each nanoliposomal irinotecan-containing treatment group discontinuing treatment because of any adverse event. Of note, there were no reports of hand-foot syndrome, which can be associated with irinotecan and pegylated liposomal doxorubicin therapy, in any study group.

The value of using this nanoliposomal irinotecan-containing regimen immediately after FOLFIRINOX treatment is still not clear, because very few patients received previous irinotecan in this study. Further investigation is needed to answer this question with confidence. Aside from this consideration, nanoliposomal irinotecan plus fluorouracil and folinic acid could potentially become a new standard of care for patients with metastatic pancreatic ductal adenocarcinoma whose disease has progressed following treatment with gemcitabine-based therapy.

In conclusion, the results of this phase 3 study show that nanoliposomal irinotecan in combination with

fluorouracil and folinic acid extends survival and improves other efficacy variables in patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based regimens, and has a manageable and mostly reversible safety profile. However, it is not possible to generalise the results of this study to patients with low performance status, as shown by a Karnofsky performance status less than 70 and albumin less than 30 g/L, and those with increased bilirubin, all of which occur in this disease. Future studies will assess the use of nanoliposomal irinotecan in other settings, including first-line therapy and the role of sequencing various regimens for pancreatic cancer.

Contributors

DDVH, L-TC, AW-G, VM, BB, ND, and EB led and coordinated the study design. All authors recruited patients and contributed to data collection. BB and EB analysed data, which was interpreted by all authors. L-TC, AW-G, DDVH, GB, AD, BB, and EB drafted the manuscript with input from all other authors. All authors have seen and approved the final report.

Declaration of interests

GB, C-FC, JFB, RAH, K-HL, C-PL, GS, TM, and Y-SS declare no competing interests. FB and JTS report personal fees from Merrimack Pharmaceuticals advisory boards, outside the submitted work. L-TC reports other funding from Merrimack Pharmaceuticals, during the conduct of the study; and personal fees from PharmaEngine, outside the submitted work. DC reports grants from AstraZeneca, Amgen, Celgene, Merck Serono, Sanofi, Merrimack Pharmaceuticals, and Medimmune, outside the submitted work. AD reports personal fees from AstraZeneca and Specialized Therapeutics, outside the submitted work; grants and personal fees from Roche, outside the submitted work; and grants from Boehringer Ingelheim, outside the submitted work. GJ reports grants from Merrimack Pharmaceuticals, during the conduct of the study. DDVH reports grants from Merrimack Pharmaceuticals, during the conduct of the study; and personal fees from AlphaMed Consulting, outside the submitted work. AW-G reports grants from Newlink, EMD, Pfizer, AstraZeneca, Precision Biological, BioMed Valley, Halozyme, ChemoCentryx, OncoMEd, ADURO, and Millennium, outside the submitted work; other fees from Pfizer and Merrimack Pharmaceuticals, outside the submitted work; and grants from Merrimack Pharmaceuticals, Prometheus, and CTI, outside the submitted work. EB, ND, and VM are employees of Merrimack Pharmaceuticals and have a patent (Methods for treating pancreatic cancer using combination therapies comprising liposomal irinotecan) issued to Merrimack Pharmaceuticals. BB is employed as statistician at Merrimack Pharmaceuticals.

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Safeguarding public health

MHRA

Public Assessment Report

Fluorouracil 50mg/ml Solution for Injection or Infusion

PL 20851/0010
PL 20851/0011
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FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010
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LAY SUMMARY

The Medicines and Healthcare products Regulatory Agency (MHRA) has granted Wockhardt UK Limited Marketing Authorisations (licences) for the medicinal products Fluorouracil 50mg/ml Solution for Injection or Infusion (PLs 20851/0010-3). These are prescription only medicines [POMs] used to treat various types of cancer, including breast cancer and lung cancer.

The active ingredient fluorouracil interferes with the production of DNA in cells.

The clinical data presented to the MHRA, before licensing, demonstrated that Fluorouracil 50mg/ml Solution for Injection or Infusion is essentially similar or equivalent to the approved product, Fluorouracil 50mg/ml Injection, and as such can be used interchangeably.

No new or unexpected safety concerns arose from these applications and it was decided that the benefits of using Fluorouracil 50mg/ml Solution for Injection or Infusion outweigh the risks, hence Marketing Authorisations have been granted.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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SCIENTIFIC DISCUSSION

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INTRODUCTION

Based on the review of the data on quality, safety and efficacy, the UK granted marketing authorisations for the medicinal product Fluorouracil 50mg/ml Solution for Injection or Infusion (PLs 20851/0010-3) to Wockhardt UK Limited on 19 September 2006. The product is a prescription only medicine.

The applications were submitted as abridged applications according to Article 10.1(a)(iii) of Directive 2001/83/EC, claiming essential similarity to Fluorouracil 50mg/ml Injection (PL 04515/0088), which was authorised in January 1996.

Fluorouracil 50mg/ml Solution for Injection or Infusion may be used alone, or in combination, for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

PHARMACEUTICAL ASSESSMENT

PL NUMBER:	**PLs 20851/0010-0013**
PRODUCT:	**Fluorouracil 50mg/ml Solution for Injection or Infusion**
ACTIVE:	**Fluorouracil**
COMPANY:	**Wockhardt UK Limited**
E.C. ARTICLE:	**10.1(a)(iii) of Directive 2001/83/EC**
LEGAL STATUS:	**POM**

INTRODUCTION

These are generic applications for Marketing Authorisations in the UK submitted under Article 10.1(a)(iii) of Directive 2001/83/EC, as amended, first paragraph (so-called generic application). The UK reference product is Fluorouracil 50mg/ml Injection, PL 04515/0088 licensed to Mayne Pharma plc on 4 January 1996.

DRUG SUBSTANCE

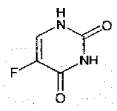
General information

Nomenclature

Chemical name: 5-fluoropyrimidine-2,4(1H,3H)-dione

INN: Fluorouracil

Structure



Molecular formula: $C_4H_3FN_2O_2$

Relative molecular mass: 130.1

General properties

Description: White to almost white crystalline powder

Manufacture

Manufacturer

Suitable manufacturing sites of the active substance have been named.

Manufacturing process

The manufacturing process is referenced to the Certificates of Suitability.

Characterisation

Referenced to the Certificates of Suitability.

Control of active substance

Specification

The finished product manufacturer specification provided covers the requirements of the European Pharmacopoeia. Additional limits are also included in the respective Certificates of Suitability.

Analytical test methods

Relevant details have been provided on the pharmacopoeial and non-pharmacopoeial test methods used.

Analytical test method validation

No validation data has been provided for the pharmacopoeial methods. Other methods are suitably validated.

Batch analyses

Reference has been made to the Certificate of Suitability. In addition Certificates of Analysis has been provided on a batch from each supplier as tested by the finished product manufacturer. All parameters are within specification and show a reasonable degree of comparability.

Reference standards

Details of appropriate reference standards have been provided.

Container closure system

Referenced to the Certificates of Suitability.

Stability

Stability data have been provided from batches manufactured at each of the active substance manufacturing sites. The batches show acceptable stability and support the proposed re-test period and shelf-life.

DRUG PRODUCT

Description and composition of the drug product

The composition of the product is summarised in the table below. The product is a sterile solution filled into colourless glass (type I) vials with a nominal volume of 6ml (250mg/5ml), 10ml (500mg/10ml), 20ml (1000mg/20ml), 50ml (2500mg/50ml) and 100ml (5000mg/100ml). The vials are closed with grey halobutyl rubber stoppers.

Ingredient	Function	Reference
Fluorouracil	Active	Ph.Eur.
Sodium hydroxide	pH adjustment	Ph.Eur.
Water for injections	Solvent	Ph.Eur.
Nitrogen	Inert gas	Ph.Eur.

Pharmaceutical development

Physicochemical and biological properties

It has been stated that the physicochemical and biological parameters are controlled with the specification. Sterility has been identified as an important biological property with data presented on seven batches covering the different presentations demonstrating compliance to sterility.

Manufacturing development

Data has been provided to justify the method of manufacture.

Manufacture

Batch formula

The batch formula is provided for suitable batch sizes.

Manufacturing process

A flow diagram detailing the manufacturing process and in-process control testing has been provided. A written summary of the process has been included.

Control of critical steps

The critical steps are controlled by the proposed in-process controls.

Process validation or evaluation

Satisfactory data provided.

Control of excipients

Specification

Sodium hydroxide, water for injections and nitrogen have monographs in the European Pharmacopoeia. Batch analysis shows compliance with the respective monographs.

No excipients of human or animal origin have been used in the manufacture of the finished product.

Control of drug product

Specification

An acceptable finished product specification has been provided.

Analytical procedures

Details have been provided for the pharmacopoeial and non-pharmacopoeial methods.

Validation

Relevant validation data has been provided and is satisfactory.

Batch analyses

Batch analyses have been provided for six batches. All parameters are within specification and comparable.

Reference standards

Details of appropriate reference standards have been provided.

Container closure system

The injection vials are colourless glass, hydrolytic type I complying with the European Pharmacopoeia and the manufacturer's specification. The vials used have a 6ml, 10ml, 20ml, 50ml and 100ml capacity.

The closures are grey halobutyl rubber. The quality is in compliance with the European Pharmacopoeia and the manufacturer's specification.

The metallic cap is aluminium sheet. This item is not in contact with the finished product.

Relevant specifications have been provided which are considered acceptable. Relevant details of the methods have been provided. Drawings have also been supplied.

Stability

All dosage strengths have the same composition and only differ on fill volume. Consequently, data from one strength can be used as supporting data for the others.

Stability data has been presented on batches of the product packed in the proposed packaging. All vials have been stored upside down at 25°C/60%RH and at 40°C/75%RH. The data support a two year shelf-life with storage below 25°C.

Stability in the infusion fluids has been determined in glucose 5% and sodium chloride 0.9% in glass bottles and polyethylene bags at concentrations of 0.35mg/ml and 15.0mg/ml. All parameters remained constant and within specification under all conditions in all packaging for up to 28 days at 25°C.

ESSENTIAL SIMILARITY

Comparable impurity profiles have been provided for the finished product in comparison to the UK reference product.

SUMMARY OF PRODUCT CHARACTERISTICS
LABELLING
PACKAGE LEAFLETS

Satisfactory.

ASSESSOR'S OVERALL CONCLUSIONS ON QUALITY

Marketing authorisations can be granted.

PRECLINICAL ASSESSMENT

No new preclinical data have been supplied with these applications and none are required.

CLINICAL ASSESSMENT

PL NUMBER:	**PLs 20851/0010-0013**
PRODUCT:	**Fluorouracil 50mg/ml Solution for Injection or Infusion**
ACTIVE:	**Fluorouracil**
COMPANY:	**Wockhardt UK Limited**
E.C. ARTICLE:	**10.1(a)(iii) of Directive 2001/83/EC**
LEGAL STATUS:	**POM**

INTRODUCTION

These are generic applications for UK marketing authorisations.

Fluorouracil injection has been available on the UK market for decades. The product was first licensed in September 1972 to Roche Products Ltd. This licence has since been cancelled although there are a number of generic products currently available.

The applicant has submitted these applications under Article 10.1(a)(iii) of Directive 2001/83/EC, claiming essential similarity to one of the generic products as the reference medicinal product. The reference medicinal product in the UK is:

Product Name:	Fluorouracil 50mg/ml Injection
MAH:	Mayne Pharma plc
MA Number:	PL 04515/0088
Date approved:	4 January 1996

Fluorouracil is a fluorinated derivative of the pyrimidine base, uracil, and belongs to the antimetabolite group of cytostatic agents. This current application is indicated for use either alone or in combination, for its palliative action in the management of common malignancies particularly cancer of the colon and breast, either as single agent or in combination with other cytotoxic agents.

ASSESSMENT

The Summaries of Product Characteristics are satisfactory and compare well with the Summary of Product Characteristics of the generic reference product in the UK.

BIOEQUIVALENCE

Since these products are for parenteral (intravenous or intra-arterial) administration, there are no issues relevant to bioequivalence.

PATIENT INFORMATION LEAFLETS

These are satisfactory and in compliance with Directive 2001/83/EEC, as amended.

RECOMMENDATION

The recommendation is to grant marketing authorisations for these preparations.

OVERALL CONCLUSION AND RISK-BENEFIT ASSESSMENT

QUALITY

The important quality characteristics of Fluorouracil 50mg/ml Solution for Injection or Infusion are well defined and controlled. The specifications and batch analytical results indicate consistency from batch to batch. There are no outstanding quality issues that would have a negative impact on the benefit/risk balance.

PRECLINICAL

No new preclinical data were submitted and none are required for applications of this type.

EFFICACY

No clinical pharmacology data or clinical trials data have been submitted to directly support the claim of essential similarity of the proposed product to the reference product Fluorouracil 50mg/ml Injection (PL 04515/0088). This is acceptable as the formulations are similar and the same routes of administration are proposed.

No new or unexpected safety concerns arise from these applications.

The SPC, PIL and labelling are satisfactory and consistent with those of Fluorouracil 50mg/ml Injection.

RISK-BENEFIT ASSESSMENT

The quality of the products is acceptable and no new preclinical or clinical safety concerns have been identified. The risk-benefit assessment is therefore considered to be favourable.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010
PL 20851/0011
PL 20851/0012
PL 20851/0013

STEPS TAKEN FOR ASSESSMENT

1	The MHRA received the marketing authorisation applications for Fluorouracil 50mg/ml Solution for Injection or Infusion on 19 August 2005.
2	The MHRA's assessment of the submitted quality data was completed on 25 February 2006.
3	Further information (quality) was requested from the company on 27 February 2006.
4	The MHRA's assessment of the submitted clinical data was completed on 2 March 2006.
5	Further information (clinical) was requested from the company on 2 March 2006.
6	The applicant's response to further information request (clinical) was received on 6 March 2006.
7	The applicant's response to further information request (quality) was sent in a letter dated 7 July 2006.
8	The MHRA completed its assessment of the applications on 19 September 2006.
9	The applications were determined on 19 September 2006.

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010
PL 20851/0011
PL 20851/0012
PL 20851/0013

STEPS TAKEN AFTER AUTHORISATION - SUMMARY

Date submitted	Application type	Scope	Outcome

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 250mg of fluorouracil in 5ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: l2mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4 Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 6ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0010

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 500mg of fluorouracil in 10ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: l2mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4 Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole,

leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 10ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0011

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 1000mg of fluorouracil in 20ml of solution

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: l2mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4 Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5 Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

Marked elevations of prothrombin time and INR have been reported in a few patients stabilised on warfarin therapy following initiation of fluorouracil regimes.

A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

Incidences of excessive lacrimation, dacryostenosis, visual changes and photophobia.

Palmar-Plantar Erythrodysesthesia Syndrome has been reported as an unusual complication of high dose bolus or protracted continuous therapy for 5-fluorouracil.

Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2 Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 20ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with a absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0012

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

Fluorouracil 50mg/ml Solution for Injection or Infusion.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Each 1ml contains 50 mg of fluorouracil.

Each vial contains 5000mg of fluorouracil in 100ml of solution

For full list of excipients, see section 6.1

3. PHARMACEUTICAL FORM

Solution for injection or infusion.

Clear and colourless solution.

4 CLINICAL PARTICULARS

4.1. Therapeutic indications

Fluorouracil may be used alone, or in combination for its palliative action in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents.

4.2 Posology and method of administration

Selection of an appropriate dose and treatment regime will depend upon the condition of the patient, the type of carcinoma being treated and whether fluorouracil is to be administered alone or in combination with other therapy. Initial treatment should be given in hospital and the total daily dose should not exceed one gram. It is customary to calculate the dose in accordance with patient's actual weight unless there is obesity, oedema or some other form of abnormal fluid retention such as ascites. In this case, ideal weight should be used as the basis for the calculation. Reduction of the dose is advisable in patients with any of the following:

1) Cachexia

2) Major surgery within preceding 30 days

3) Reduced bone marrow function

4) Impaired hepatic or renal function

Fluorouracil injection can be given by intravenous injection or, intravenous or intra-arterial infusion.

Adult Dose:

The following regimen have been recommended for use as a single agent:

Initial Treatment: This may be in the form of an infusion or an injection, the former usually being preferred because of lesser toxicity.

Intravenous infusion: 15mg/kg bodyweight but not more than 1g per infusion, diluted in 300 - 500ml of 5% glucose or 9% sodium chloride injection and given by intravenous infusion at a rate of 40 drops per minute over four hours. Alternatively the daily dose may be infused over 30 - 60 minutes or may be given as a continuous infusion over 24 hours. The infusion may be repeated daily until there is evidence of toxicity or a total dose of 12 - 15g has been reached.

Intravenous Injection: l2mg/kg bodyweight may be given daily for three days and then, if there is no evidence of toxicity, 6mg/kg on alternate days for three further doses. An alternative regimen is 15mg/kg as a single intravenous injection once a week throughout the course.

Intra-arterial Infusion: 5/7.5mg/kg may be given by 24 hour continuous intra-arterial infusion.

Maintenance Therapy: An initial intensive course may be followed by maintenance therapy providing there are no significant toxic effects. In all instances, toxic side effects must disappear before maintenance therapy is started.

The initial course of fluorouracil can be repeated after an interval of four to six weeks from the last dose or, alternatively, treatment can be continued with intravenous injections of 5-15mg/kg bodyweight at weekly intervals.

This sequence constitutes a course of therapy. Some patients have received up to 30g at a maximum rate of 1g daily. A more recent alternative method is to give 15mg/kg IV once a week throughout the course of treatment. This obviates the need for an initial period of daily administration.

In combination with Irradiation: Irradiation combined with fluorouracil has been found to be useful in the treatment of certain types of metastatic lesions in the lungs and for the relief of pain caused by recurrent, inoperable growth. The standard dose of fluorouracil should be used.

Children:

No recommendations are made regarding the use of fluorouracil in children.

Elderly:

Fluorouracil should be used in the elderly with similar considerations as with normal adult dosages.

4.3 Contraindications

Fluorouracil is contraindicated in seriously debilitated patients or those with bone marrow depression after radiotherapy or treatment with other antineoplastic agents.

Fluorouracil is strictly contraindicated in pregnant or breast feeding women.

Fluorouracil should not be used in the management of non-malignant disease.

4.4. Special warnings and precautions for use

It is recommended that fluorouracil be given only by, or under the strict supervision of, a qualified physician who is conversant with the use of potent antimetabolites.

All patients should be admitted to hospital for initial treatment.

Adequate treatment with fluorouracil is usually followed by leucopenia, the lowest white blood cell (W.B.C) count commonly being observed between the seventh and fourteenth day of the first course, but occasionally being delayed for as long as 20 days. The count usually returns to normal by the thirtieth day. Daily monitoring of platelet and W.B.C count is recommended and treatment should be stopped if platelets fall below 100,000 per mm^3 or the W.B.C. count falls below 3,500 per mm^3. If the total count is less than $2000mm^3$, and especially if there is granulocytopenia, it is recommended that the patient be placed in protective isolation in the hospital and treated with appropriate measures to prevent systemic infection.

Treatment should be stopped at the first sign of oral ulceration or if there is evidence of gastrointestinal side effects such as stomatitis, diarrhoea, bleeding from the G.I. tract or haemorrhage at any site. The ratio between effective and toxic dose is small and therapeutic response is unlikely without some degree of toxicity. Care must be taken therefore, in the selection of patients and adjustment of dosage.

Fluorouracil should be used with caution in patients with reduced renal or liver function or jaundice. Isolated cases of angina, ECG abnormalities and rarely, myocardial infarction have been reported following administration of fluorouracil. Care should be therefore be exercised in treating patients who experience chest pain during courses of treatment, or patients with a history of heart disease.

There have been reports of increased toxicity in patients who have reduced activity/deficiency of the enzyme dihydropyrimidine dehydrogenase (DPD).

4.5. Interaction with other medicinal products and other forms of interaction

Various agents have been reported to biochemically modulate the antitumour efficacy or toxicity of fluorouracil - common drugs include methotrexate, metronidazole, leucovorin as well as allopurinol and cimetidine which can affect the availability of the active drug.

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A clinically significant interaction has been reported between the antiviral sorivudine and fluorouracil , resulting from inhibition of dihydropyrimidine dehydrogenase by sorivudine or chemically related analogues. Caution should be taken when using fluorouracil in conjunction with medications which may affect dihydropyrimidine dehydrogenase activity.

4.6 Pregnancy and lactation

Fluorouracil is strictly contraindicated in pregnant and breast feeding women.

4.7 Effects on ability to drive and use machines

Not applicable.

4.8 Undesirable effects

Diarrhoea, nausea and vomiting are observed quite commonly during therapy and may be treated symptomatically. An anti-emetic may be given for nausea and vomiting.

Alopecia may be seen in a substantial number of cases, particularly females, but is reversible. Other side effects include dermatitis, pigmentation, changes in nails, ataxia and fever.

There have been reports of chest pain, tachycardia, breathlessness and E.C.G. changes after administration of Fluorouracil. Special attention is advisable in treating patients with a history of heart disease or those who develop chest pain during treatment.

Leucopenia is common and the precautions described above should be followed.

Systemic fluorouracil treatment has been associated with various types of ocular toxicity.

A transient reversible cerebellar syndrome has been reported following fluorouracil treatment. Rarely, a reversible confusional state may occur. Cases of leucoencephalopathy have also been reported.

Additionally, several other reports have been noted including:

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Thrombophlebitis / Vein Tracking.

4.9 Overdose

The symptoms and signs of overdosage are qualitatively similar to the adverse reactions and should be managed as indicated under "Other Undesirable Effects" and "Special Warnings and Precautions".

5 PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

ATC Code: L01B C02, Pyrimidine Analogue

Fluorouracil is an analogue of uracil, a component of ribonucleic acid. The drug is believed to function as an antimetabolite. After intracellular conversion to the active deoxynucleotide, it interferes with the synthesis of DNA by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate synthetase. Fluorouracil may also interfere with RNA synthesis.

5.2 Pharmacokinetic properties

After intravenous administration, fluorouracil is distributed through the body water and disappears from the blood within three hours. It is preferentially taken up by actively dividing tissues and tumours after conversion to its nucleotide. Fluorouracil ready enters the C.S.F and brain tissue.

Following IV administration, the plasma elimination half-life averages about 16 minutes and is dose dependent. Following a single intravenous dose of fluorouracil approximately 15% of the dose is excreted unchanged in the urine within six hours; over 90% of this is excreted in the first hour. The remainder is mostly metabolised in the liver by the usual body mechanisms for uracil.

5.3 Preclinical safety data

Preclinical information has not been included because the toxicity profile of fluorouracil has been established after many years of clinical use. Please refer to section four.

6 PHARMACEUTICAL PARTICULARS

6.1. List of excipients

Sodium hydroxide (for pH adjustment)
Water for injections

6.2. Incompatibilities

Fluorouracil is incompatible with carboplatin, cisplatin, cytarabine, diazepam, doxorubicin, other anthracyclines and possibly methotrexate.

Formulated solutions are alkaline and it is recommended that admixture with acidic drugs or preparations should be avoided.

6.3 Shelf life

24 months

After dilution, see section 6.4 Special precautions for storage.

Discard any unused solution immediately after use.

6.4 Special precautions for storage

Unopened: Do not store above 25°C
After dilution: Do not store above 25°C (see 6.3 Shelf Life).

Chemical and physical in use stability of fluorouracil solutions with a concentration of 0.35mg/ml and 15.0mg/ml prepared in glucose 5% or sodium chloride 0.9% has been demonstrated for 28 days at 25°C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

6.5 Nature and contents of container

Packs* of one, five or ten Type I colourless glass 20ml vials stoppered with a halobutyl stopper sealed with an aluminium cap.

*Not all pack sizes may be marketed

6.6 Special precautions for disposal

Cytotoxic Handling Guidelines

Should be administered only by or under the direct supervision of a qualified physician who is experienced in the use of cancer chemotherapeutic agents.

Fluorouracil injection should only be prepared for administration by professionals who have been trained in the safe use of the preparation. Preparation should only be carried out in an aseptic cabinet or suite dedicated for the assembly of cytotoxics.

In the event of spillage, operators should put on gloves, face mask, eye protection and disposable apron and mop up the spilled material with an absorbent material kept in the area for that purpose. The area should then be cleaned and all contaminated material transferred to a cytotoxic spillage bag or bin and sealed for incineration.

Contamination

Fluorouracil is an irritant, contact with skin and mucous membranes should be avoided.

In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of the skin. Medical advice should be sought if the eyes are affected or if the preparation is inhaled or ingested.

Preparation Guidelines

a) Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.

b) Operations such as reconstitution of powder and transfer to syringes should be carried out only under aseptic conditions in a suite or cabinet dedicated for the assembly of cytotoxics.

c) The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.

d) Pregnant personnel are advised not to handle chemotherapeutic agents.

Disposal

Syringes, vials and adaptors containing remaining solution, absorbent materials, and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated at 700°C.

Diluents

Fluorouracil injection may be diluted with glucose 5% injection or sodium chloride 0.9% injection or water for injections immediately before parenteral use.

7 MARKETING AUTHORISATION HOLDER

Wockhardt UK Limited
Ash Road North
Wrexham LL13 9UF
United Kingdom

8 MARKETING AUTHORISATION NUMBER(S)

PL 20851/0013

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

19/09/2006

10 DATE OF REVISION OF THE TEXT

19/09/2006

Patient Information Leaflet

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010
PL 20851/0011
PL 20851/0012
PL 20851/0013

PACKAGE LEAFLET: INFORMATION FOR THE USER

Fluorouracil 50mg/ml Solution for Injection or Infusion

Read all of this leaflet carefully before you start using this medicine.
- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or pharmacist.
- This medicine has been prescribed for you. Do not pass it on to others. It may harm them, even if their symptoms are the same as yours.
- If any of the side effects get serious, or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist.

In this leaflet:

1. WHAT FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION IS AND WHAT IT IS USED FOR

Fluorouracil belongs to a group of medicines known as cytotoxics, which are used in the treatment of cancer.

Fluorouracil is usually used to treat breast cancer and colon cancer. However, it may also be given to treat other types of cancer.

2. BEFORE YOU USE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Do not use Fluorouracil 50mg/ml Solution for Injection or Infusion

- if you are allergic to fluorouracil or any of the other ingredients
- if you are weakened after a long illness
- if your bone marrow has been damaged by other cytotoxic drugs or radiotherapy
- if you are pregnant, breast-feeding or trying for a baby
- if you have a tumour that is not malignant

Take special care with Fluorouracil 50mg/ml Solution for Injection or Infusion

Your doctor will take special care when giving you fluorouracil:

- if you have a low white blood cell count (you will have blood tests to check this)
- if you have liver or kidney problems
- if you have jaundice (yellowing of the skin)
- if you have angina or a history of heart disease (you should let your doctor know if you experience chest pain while you are receiving your treatment)
- if you have been told by your doctor that you have a low level of the enzyme dihydropyrimidine dehydrogenase (DPD)

Consult your doctor if any of the above warnings applies to you or has applied to you in the past.

Your doctor will also check your blood before, during and after every treatment. If the results of any of these tests are abnormal, treatment will only be resumed when all readings are back to normal.

Using other medicines

Please tell your doctor or pharmacist if you are taking or have recently taken any other medicines, including medicines obtained without a prescription.

Taking or being given another medicine while you are receiving fluorouracil can affect how it or the other medicine works. Please inform your doctor or pharmacist if you are taking or have recently taken any other medicines, even those you may have bought yourself without a prescription. Please check with your doctor if you are taking any of the following (or any other medication):

- Methotrexate, another cytotoxic drug
- Metronidazole, an antibiotic
- Calcium leucoverin (calcium folinate), used to reduce the harmful effects of cytotoxic drugs
- Allopurinol, used to treat gout
- Cimetidine, used to treat stomach ulcers
- Warfarin, used to treat blood clots
- Sorivudine, an antiviral drug

Pregnancy

Fluorouracil should not be given to you if you are pregnant, because it can cause serious birth defects.

Female patients should also avoid getting pregnant while being treated with fluorouracil and for at least six months afterwards. Male patients receiving fluorouracil should take adequate precautions to ensure that their partner does not become pregnant for the same period. If you are considering becoming parents after the treatment, you should discuss this with your doctor.

Men who wish to father children in the future should seek advice about freezing sperm before the fluorouracil treatment is started.

Breast-feeding

Fluorouracil should not be given to you if you are breast-feeding, as fluorouracil might pass into breast milk and affect the baby.

Driving and using machines:

Fluorouracil treatment should not affect your ability to drive, but if you feel unwell, you should not drive or operate machinery.

3. HOW TO USE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Fluorouracil injection can be given by intravenous injection (the solution is given directly into a vein) or, intravenous or intra-arterial infusion (the solution is diluted and given by a drip into a vein or artery).

Fluorouracil 50mg/ml Solution for Injection or Infusion will only be given to you under the supervision of a doctor specialised in this type of treatment, which should be started in hospital. It may be diluted with glucose solution, sodium chloride solution or water for injections before use. It is injected into a vein or artery. If it is given into an artery it must be diluted first.

The dosage of fluorouracil depends on the condition you are being treated for, your bodyweight, if you have had recent surgery, how well your liver and kidneys are working and results of your blood tests.

Your general condition and your response to the treatment will be closely observed before, during and after the fluorouracil treatment. This will include blood tests.

4. POSSIBLE SIDE EFFECTS

Like all medicines, Fluorouracil 50mg/ml Solution for Injection or Infusion can cause side effects, although not everybody gets them.

These include nausea, vomiting, temporary hair loss, skin problems, changes in your nails, feeling unsteady on your feet, quickening of the heart and breathlessness (following injection), painful and watery eyes, changes in vision and sensitivity to light, feeling confused and reddening of the palms of the hands and soles of the feet.

If you develop any of the following symptoms, **tell your doctor immediately:**

- chest pain
- diarrhoea
- blood stained or black bowel motions
- sore throat, sore mouth or mouth ulcers
- feeling generally unwell
- fever
- aching muscles and joints
- weakness,
- confusion
- difficulties with co-ordination, memory, thinking, or talking
- fits
- severe headache

Pain may occur temporarily at the injection site.

Allergic reactions to fluorouracil can occur, with wheezing, a skin rash or swelling of your lips, eyes or tongue. You should contact your doctor **immediately** if you develop such symptoms.

If any of the side effects gets serious, or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist.

5. HOW TO STORE FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

Keep out of the reach and sight of children.

Do not use Fluorouracil 50mg/ml Solution for Injection or Infusion after the expiry date which is stated on the label or carton. The expiry date refers to the last day of that month.

<o not use Fluorouracil 50mg/ml Solution for Injection or Infusion if you notice signs of discoloration {description of the visible signs of deterioration}.

After first opening or following dilution, from a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2-8°C, unless opening and dilution has taken place in controlled and validated aseptic conditions.

Medicines should not be disposed of via wastewater or household waste. Ask your pharmacist how to dispose of medicines no longer required. These measures will help to protect the environment.

6. FURTHER INFORMATION

What Fluorouracil 50mg/ml Solution for Injection or Infusion contains

- The active substance is fluorouracil
  The other ingredients are sodium hydroxide and water for injections.

What X looks like and contents of the pack

Fluorouracil 50mg/ml Solution for Injection or Infusion is a clear and colourless solution free from particles.

Fluorouracil 50mg/ml Solution for Injection or Infusion is available in single packs containing:-

- 250mg of fluorouracil in 5ml of solution
- 500mg of fluorouracil in 10ml of solution
- 1000mg of fluorouracil in 20ml of solution
- 5000mg of fluorouracil in 100ml of solution

Marketing Authorisation Holder and Manufacturer

Fluorouracil 50mg/ml Solution for Injection or Infusion is manufactured by:-

EBEWE Pharma
Ges.m.b.H. Nfg. KG, A-4866
Unterach
Austria

for the Marketing Authorisation holder

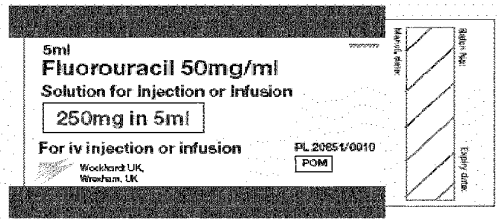
Wockhardt UK Limited
Ash Road North
Wrexham
LL13 9UF

This leaflet was last approved on .....

Labels/Packaging

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0010



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FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

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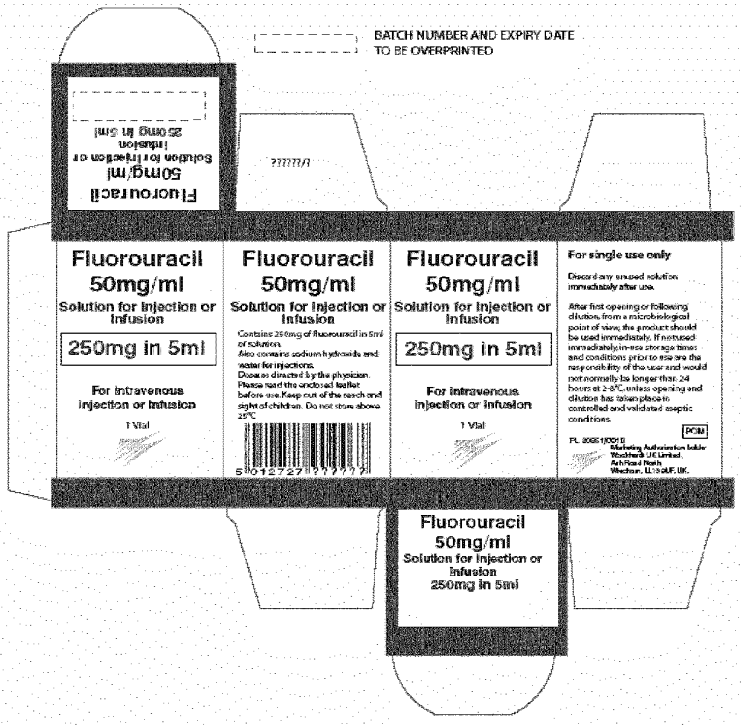
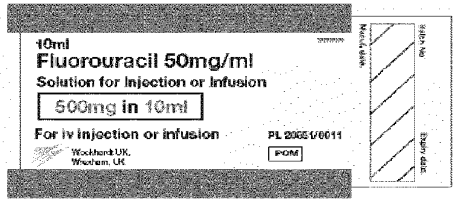


Fluorouracil 50mg/ml
1 Vial
(250mg in 5ml)
Colour: Red 221, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0011



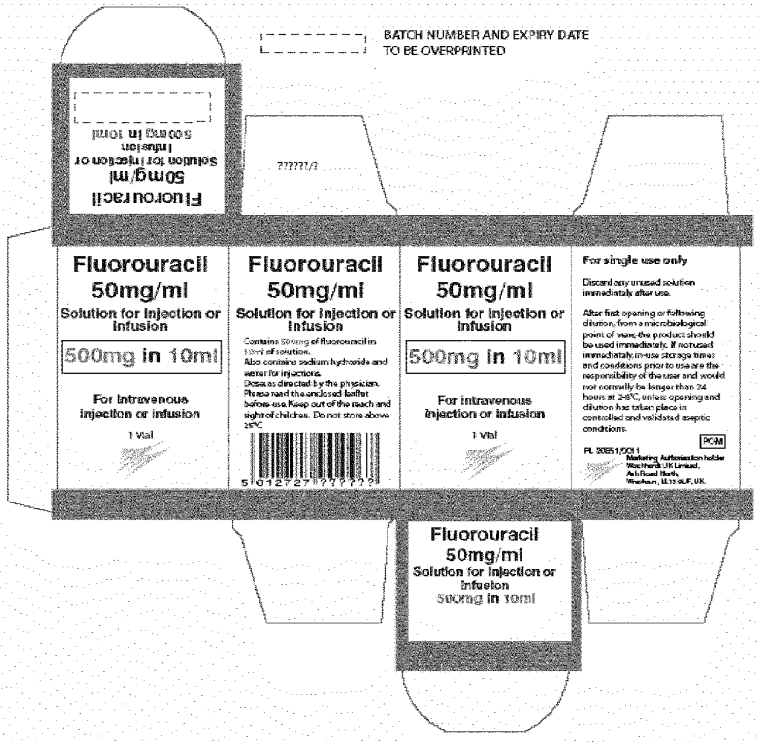
100% size

Fluorouracil 50mg/ml
1 Vial
(500mg in 10ml)
Colour: Green 370, Black

Mock-up 29/06/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0011

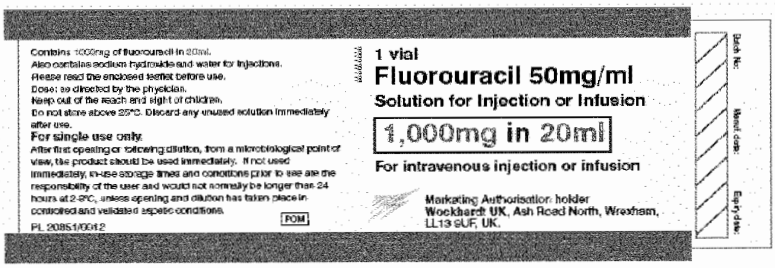


Fluorouracil 50mg/ml
1 Vial
(500mg in 10ml)
Colour: Green 370, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0012



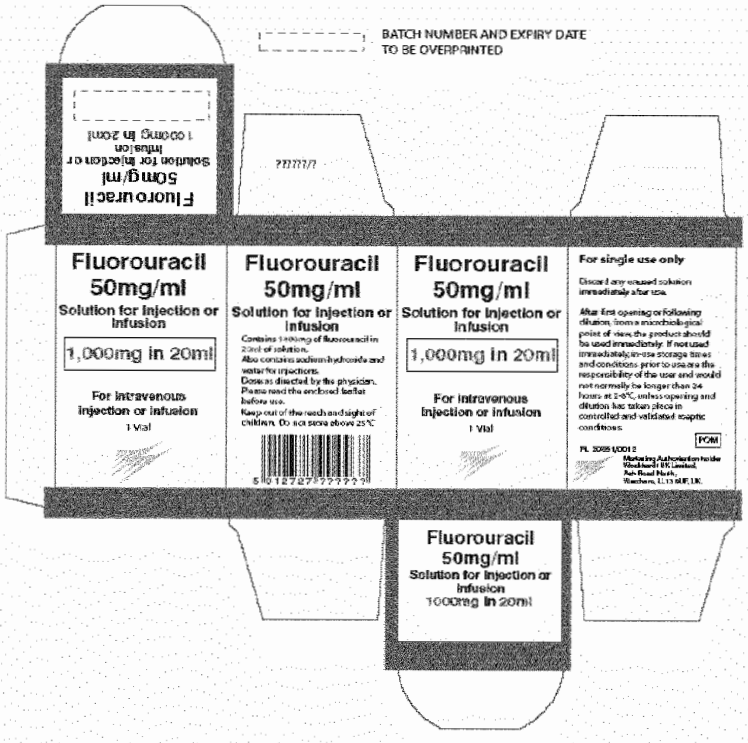
100%

Fluorouracil 50mg/ml
1 Vial
(1000mg in 20ml)
Colour: Blue 285, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0012

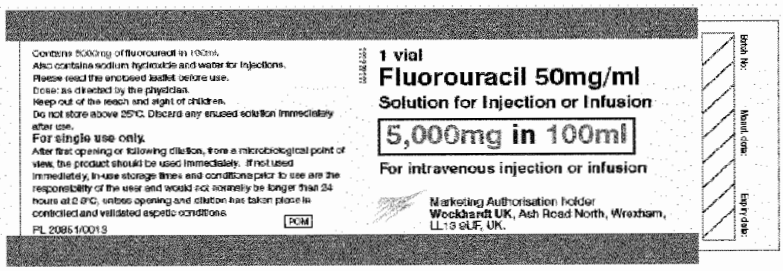


Fluorouracil 50mg/ml
1 Vial
(1000mg in 20ml)
Colour:Blue 285, Black

Mock-up 07/07/56

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0013



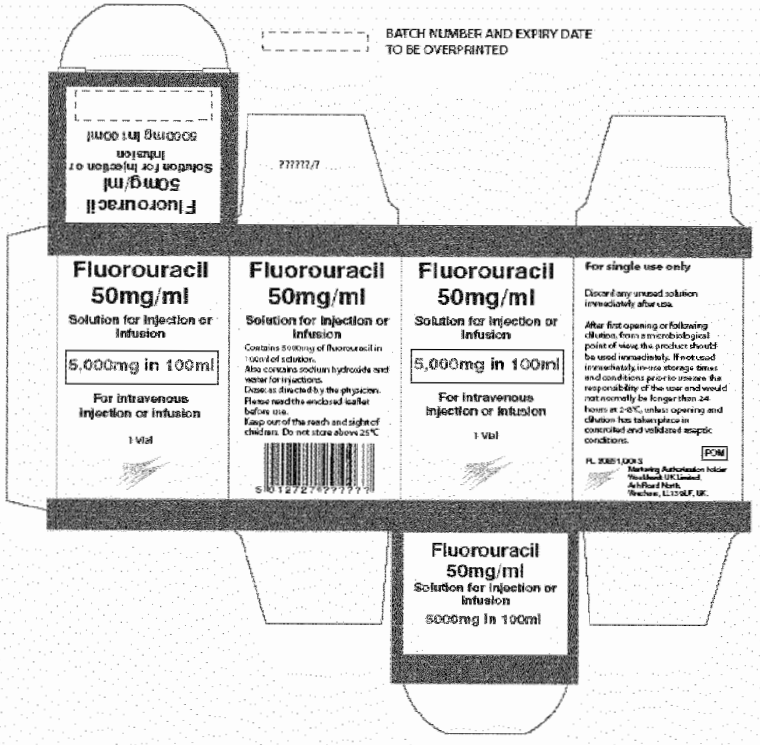
100%

Fluorouracil 50mg/ml
1 Vial
(5000mg in 100ml)
Colour: Red 186, Black

Mock-up 07/07/06

FLUOROURACIL 50MG/ML SOLUTION FOR INJECTION OR INFUSION

PL 20851/0013



Fluorouracil 50mg/ml
1 Vial
(5000mg in 100ml)
Colour: Red 186, Black

Mock-up 07/07/06

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Masserut, Marilú

Oates, Edward Christopher
Carpmaels & Ransford LLP
One Southampton Row
London WC1B 5HA
ROYAUME UNI

Date
30.01.2019

Reference	Application No./Patent No.
P067376EP:ECO	13731230.2 - 1109 / 2861210
Applicant/Proprietor	
Ipsen Biopharm Ltd.	

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent.

The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will be public, will take place before the opposition division

on 10.07.19 at 09.00 hrs in Room 0.6 at the EPO in Rijswijk.
Rijswijk New Main, Patentlaan 2, NL-2288 EE Rijswijk (ZH)

You are asked to report at the reception desk, Patentlaan 2, in good time before the starting time mentioned above. The dedicated waiting area will be indicated to you on site.

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 1/2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to the Special edition No. 3 OJ EPO 2007, 128, concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.

The final date for making written submissions and/or amendments (R. 116 EPC) is 10.05.19.

1st Examiner:	2nd Examiner:	Chairman:
Bazzanini, Rita	Gradassi, Giulia	Hoff, Philippe

For the Opposition Division

Annexes:
Confirmation of receipt (Form 2936)
Rule 4 EPC (EPC Form 2043)
Communication (EPO Form 2906)

Registered letter with advice of delivery

Europäisches Patentamt
European Patent Office
Office européen des brevets

Wichtige Hinweise zur mündlichen Verhandlung

Das Europäische Patentamt verfügt über keine eigenen Dolmetscher. Diese müssen im Bedarfsfall von außerhalb, teilweise sogar aus anderen Ländern, beigezogen werden, was mit einem hohen Aufwand an Kosten und organisatorischen Vorbereitungen verbunden ist. Muss ein Verhandlungstermin kurzfristig abberaumt werden, können Kosten für bestellte Dolmetscher nicht mehr vermieden werden.

Es wird daher gebeten, eine Simultanübersetzung nur bei wirklichem Bedarf in Anspruch zu nehmen. Es wäre wünschenswert, wenn sich die Beteiligten (zweckmäßigerweise gleichzeitig mit der Terminabstimmung) auf die Benutzung einer Amtssprache einigen könnten. Bei Verständigungsschwierigkeiten sind die Mitglieder der Einspruchsabteilung bereit zu helfen.

Die von den Verfahrensbeteiligten bevorzugte (abgestimmte) Verhandlungssprache und ggf. eine notwendige Simultanübersetzung sind dem Amt möglichst vor der in Regel 4(1) EPÜ angegebenen Frist mitzuteilen.

☐ Verfahrenssprache ist **Deutsch**

Von der/dem/den Einsprechenden wurde

☐ Englisch

☐ Französisch benutzt.

Es wird um eilige Mitteilung - möglichst per Telefax an den zuständigen Formalprüfer - gebeten,

möglichst bis

Datum **08.05.2019**

1. welche Sprache(n) Sie in der mündlichen Verhandlung verwenden (**Sprechen**)
2. aus welcher Sprache Sie eine Simultanübersetzung benötigen (**Hören**).

Important information concerning oral proceedings

The European Patent Office has no interpreters of its own. When interpreters are needed they have to be brought in from outside, sometimes even from other countries, which is costly and involves considerable organisation. If oral proceedings have to be cancelled at short notice, the cost of interpreters already engaged still has to be borne.

Please therefore make use of simultaneous interpreting facilities only where strictly necessary. If possible the parties should agree on an official language for the proceedings, preferably at the time when they arrange a date. The members of the Opposition Division will be willing to help should any communication problems arise.

The EPO should be told if possible before the period mentioned in Rule 4 (1) EPC which language the parties prefer (agree on) and whether simultaneous interpreting facilities are required.

☒ Language of the proceedings is **English**

The language used by the opponent/s was English

☐ German

☐ French.

Please inform us urgently - where possible by fax addressed to the formalities officer concerned -

if possible by

Date **08.05.2019**

1. which language(s) you intend to use during the oral proceedings (**Speaking**)
2. from which language you need simultaneous interpretation (**Listening**).

Très important Procédure orale

L'Office européen des brevets ne dispose pas de son propre service d'interprètes. Aussi faut-il appel le cas échéant à des interprètes de l'extérieur, qui viennent même parfois de l'étranger, ce qui occasionne de frais élevés et demande un grand travail d'organisation. Si la date d'une procédure orale doit être annulée au dernier moment, il n'est plus possible d'éviter les frais d'interprètes.

Les parties à une procédure sont donc priées de ne demander une traduction simultanée qu'en cas de réel besoin. Il serait souhaitable qu'elles puissent se mettre d'accord en même temps qu'elles conviennent de la date sur l'utilisation d'une langue officielle comme langue des débats. Si les parties éprouvent des difficultés de compréhension lors des débats, les membres de la division d'opposition sont disposés à leur prêter leur assistance.

L'Office doit être avisé si possible avant le début du délai mentionné dans la règle 4(1) CBE de la langue préférée par les parties pour le déroulement des débat (et sur laquelle elles se sont préalablement mises d'accord) et de la nécessité éventuelle d'une traduction simultanée.

☐ La langue de la procédure est le **français**

La langue utilisée par l'opposant/les opposants était

☐ l'allemand

☐ l'anglais.

Prière d'indiquer d'urgence à l'agent des formalités compétent si possible par téléfax

si possible jusqu'au

Date **08.05.2019**

1. quelle(s) langue(s) vous utiliserez au cours de la procédure orale (**pour parler**)
2. à partir de quelle langue vous aurez besoin d'une traduction simultanée (**pour écouter**).

Sollten Sie Ihren Antrag auf mündliche Verhandlung zurückziehen oder zum anberaumten Verhandlungstermin nicht erscheinen wollen bzw. aus wichtigem Grund daran gehindert sein, werden Sie gebeten,

- **unverzüglich das Amt - möglichst per Telefax - davon zu benachrichtigen, wobei das Schriftstück mit einem deutlichen Vermerk "Dringend, mündliche Verhandlung am ..." oder sinngemäß gekennzeichnet sein sollte;**
- **in dringenden Fällen** (weniger als 1 Monat vor dem Verhandlungstermin) zusätzlich **auch dem/die anderen Verfahrensbeteiligten** bzw. ihre(n) Vertreter auf schnellstem Weg **direkt zu unterrichten.**

In jedem solchen Fall obliegt der Einspruchsabteilung die Entscheidung, ob die Verhandlung durchgeführt oder abberaumt wird. Es wird jedoch darauf hingewiesen, dass einem Verfahrensbeteiligten, der eine nicht rechtzeitige oder unterbliebene Benachrichtigung zu verantworten hat, die dadurch den anderen Beteiligten verursachten Kosten auferlegt werden können (Art. 104 EPÜ).

Hinweis auf Regel 4 EPÜ

Regel 4
Sprache im mündlichen Verfahren

(1) Jeder an einem mündlichen Verfahren vor dem Europäischen Patentamt Beteiligte kann sich anstelle der Verfahrenssprache einer anderen Amtssprache des Europäischen Patentamts bedienen, sofern er dies dem Europäischen Patentamt spätestens einen Monat vor dem angesetzten Termin mitgeteilt hat oder selbst für die Übersetzung in die Verfahrenssprache sorgt. Jeder Beteiligte kann sich einer Amtssprache eines Vertragsstaats bedienen, sofern er selbst für die Übersetzung in die Verfahrenssprache sorgt. Von diesen Vorschriften kann das Europäische Patentamt Ausnahmen zulassen.

Should you decide to withdraw your request for oral proceedings or not wish to attend on the date set, or if for some special reason you are unable to do so, you are requested

- **to notify the EPO immediately, where possible by fax, marking the document clearly with the words "Urgent, oral proceedings on ..." or similar;**
- **in urgent cases** (less than one month before the date set for the proceedings), additionally to **notify the other party/parties** and/or their representative(s) **direct as rapidly as possible.**

In all such cases the Opposition Division will decide whether the proceedings are to go ahead or be cancelled. You should however note that costs incurred by the other parties may be charged to a party who either fails to notify them or does not do so in good time (Article 104 EPC).

Attention is drawn to Rule 4 EPC

Rule 4
Language in oral proceedings

(1) Any party to oral proceedings before the European Patent Office may use an official language of the European Patent Office other than the language of the proceedings, if such party gives notice to the European Patent Office at least one month before the date of such oral proceedings or provides for interpretation into the language of the proceedings. Any party may use an official language of a Contracting State, if he provides for interpretation into the language of the proceedings. The European Patent Office may permit derogations from these provisions.

Si vous retirez votre requête tendant à recourir à la procédure orale ou si vous ne souhaitez pas vous présenter à la date fixée pour la procédure orale ou ne pouvez vous y présenter pour une raison sérieuse, veuillez

- **en faire avis sans retard à l'Office, si possible par téléfax, en partant sur votre communication clairement la mention "Urgent, procédure orale le ..." ou une indication similaire;**
- **dans les cas urgents** (moins d'un mois avant la date fixée pour la procédure orale) **en faire avis également directement** pas la voie la plus rapide à **l'autre/aux autres partie(s)** ou bien à son/leurs mandataire(s).

Il appartient alors à la division d'opposition de décider si la procédure orale aura lieu ou non. Il est néanmoins souligné que les frais causés aux autres parties par une partie qui est responsable de l'omission d'un tel avis ou de ce que cet avis n'a pas été fait en temps utile peuvent être mis à la charge de cette partie (art. 104 CBE).

Rappel de la Règle 4 CBE

Règle 4
Langues admissibles lors de la procédure orale

(1) Toute partie à une procédure orale devant l'Office européen des brevets peut utiliser une langue officielle de l'Office européen des brevets autre que la langue de la procédure, à condition soit d'en aviser l'Office européen des brevets un mois au moins avant la date de la procédure orale, soit d'assurer l'interprétation dans la langue de la procédure. Toute partie peut utiliser une langue officielle de l'un des Etats contractants à condition d'assurer l'interprétation dans la langue de la procédure. L'Office européen des brevets peut autoriser des dérogations aux présentes dispositions.

(2) Die Bediensteten des Europäischen Patentamts können sich im mündlichen Verfahren anstelle der Verfahrenssprache einer anderen Amtssprache des Europäischen Patentamts bedienen.

(2) In the course of oral proceedings, employees of the European Patent Office may use an official language of the European Patent Office other than the language of the proceedings.

(2) Au cours de la procédure orale, les agents de l'Office européen des brevets peuvent utiliser une langue officielle de l'Office européen des brevets autre que la langue de la procédure.

(3) In der Beweisaufnahme können sich die zu vernehmenden Beteiligten, Zeugen oder Sachverständigen, die sich in einer Amtssprache des Europäischen Patentamts oder eines Vertragsstaats nicht hinlänglich ausdrücken können, einer anderen Sprache bedienen. Erfolgt die Beweisaufnahme auf Antrag eines Beteiligten, so werden die Beteiligten, Zeugen oder Sachverständigen mit Erklärungen, die sie in einer anderen Sprache als in einer Amtssprache des Europäischen Patentamts abgeben, nur gehört, sofern dieser Beteiligte selbst für die Übersetzung in die Verfahrenssprache sorgt. Das Europäische Patentamt kann jedoch die Übersetzung in eine seiner anderen Amtssprachen zulassen.

(3) Where evidence is taken, any party, witness or expert to be heard who is unable to express himself adequately in an official language of the European Patent Office or of a Contracting State may use another language. Where evidence is taken upon request of a party, parties, witnesses or experts expressing themselves in a language other than an official language of the European Patent Office shall be heard only if that party provides for interpretation into the language of the proceedings. The European Patent Office may, however, permit interpretation into one of its other official languages.

(3) Lors de l'instruction, les parties, témoins ou experts appelés à être entendus, qui ne possèdent pas une maîtrise suffisante d'une langue officielle de l'Office européen des brevets ou d'un Etat contractant, peuvent utiliser une autre langue. Si la mesure d'instruction est ordonnée sur requête d'une partie, les parties, témoins ou experts qui s'expriment dans une langue autre qu'une langue officielle de l'Office européen des brevets ne sont entendus que si cette partie assure l'interprétation dans la langue de la procédure. L'Office européen des brevets peut toutefois autoriser l'interprétation dans l'une de ses autres langues officielles.

(4) Mit Einverständnis aller Beteiligten und des Europäischen Patentamts kann jede Sprache verwendet werden.

(4) If the parties and the European Patent Office agree, any language may be used.

(4) Sous réserve de l'accord des parties et de l'Office européen des brevets, toute langue peut être utilisée.

(5) Das Europäische Patentamt übernimmt, soweit erforderlich, auf seine Kosten die Übersetzung in die Verfahrenssprache und gegebenenfalls in seine anderen Amtssprachen, sofern ein Beteiligter nicht selbst für die Übersetzung zu sorgen hat.

(5) The European Patent Office shall, if necessary, provide at its own expense interpretation into the language of the proceedings, or, where appropriate, into its other official languages, unless such interpretation is the responsibility of one of the parties.

(5) L'Office européen des brevets assure à ses frais, en tant que de besoin, l'interprétation dans la langue de la procédure, ou, le cas échéant, dans ses autres langues officielles, à moins que cette interprétation ne doive être assurée par l'une des parties.

(6) Erklärungen von Bediensteten des Europäischen Patentamts, Beteiligten, Zeugen und Sachverständigen, die in einer Amtssprache des Europäischen Patentamts abgegeben werden, werden in dieser Sprache in die Niederschrift aufgenommen. Erklärungen in einer anderen Sprache werden in der Amtssprache aufgenommen, in die sie übersetzt worden sind. Änderungen einer europäischen Patentanmeldung oder eines europäischen Patents werden in der Verfahrenssprache in die Niederschrift aufgenommen.

(6) Statements by employees of the European Patent Office, parties, witnesses or experts, made in an official language of the European Patent Office, shall be entered in the minutes in that language. Statements made in any other language shall be entered in the official language into which they are translated. Amendments to a European patent application or European patent shall be entered in the minutes in the language of the proceedings.

(6) Les interventions des agents de l'Office européen des brevets, des parties, témoins et experts faites dans une langue officielle de l'Office européen des brevets sont consignées au procès-verbal dans cette langue. Les interventions faites dans une autre langue sont consignées dans la langue officielle dans laquelle elles sont traduites. Les modifications apportées à une demande de brevet européen ou à un brevet européen sont consignées au procès verbal dans la langue de la procédure.

I. European patent 2 861 210 having the title "METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN" is based upon European patent application No. 13 731 230.2 filed on 12-06-2013. It claims priority of US 201261659211 filed on 13-06-2012 and US201361784382 filed on 14-03-2013. The mention of the grant of the patent has been published in the European Patent Bulletin of 03-05-2017.

Proprietor of the patent is:
Ipsen Biopharm Ltd.
Ash Road, Wrexham Industrial Estate, Wrexham, LL13 9UF, GB.

II. A notice of opposition has been filed by:
Teva Pharmaceutical Industries Ltd
5 Basel Street, P.O. Box 3190, 49131 Petah Tiqva, IL
on 05-02-2018.

III. The opponent (further referred to as OI) requests revocation of the patent in its entirety based on Articles 100(a) and (b) EPC because the patent lacks inventive step (Article 56 EPC) and is insufficiently disclosed (Article 83 EPC). The validity of the priority is also challenged by OI. In the notice of opposition OI made reference to documents D1-D16.

As an auxiliary request OI requested oral proceedings (Article 116 EPC).

IV. The opposition is filed in due time, in proper form and is supported by reasoned statement, and is therefore considered to be admissible in that it complies with the requirements of Articles 99(1), 100 EPC and Rules 3(1), 76 EPC.

V. In a reasoned statement received on 24-08-2018 in reply to the notice of opposition, the proprietor (further referred to as P) requested for the patent to be maintained on the basis of a new claim set entitled "Main Request". With his reply, P enclosed documents D15a, D17-D21 and relabelled document D15 cited by OI D15b.

As an auxiliary request P requested oral proceedings (Article 116 EPC).

VI. As requested by both parties oral proceedings will be held. The summons are attached to this communication.

VII. During the oral proceedings it will be discussed whether the opposed patent meets the requirements of Articles 56 and 83 EPC. During the oral proceedings it will be also discussed wether the main claim request complies with the requirements of Rule 80, Articles 84, 123(2) and 123(3) EPC.

Reference will be made to the documents D1-D21 according to the following consolidated list:

D-number	Reference
D1	FDA label (Highlights of Prescribing Information) for FUSILEV (levoleucovorin) (2008)
D2	Gebbia V et al., *Am J Clin Oncol* (2008) 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	Taieb J et al., *Ann Oneal* (2007) 18:498-503
D7	Chen Let al., *J Clin Oneal* (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	Ko AH et al., *J Clin Oneal* (2011) 29(15), 4069
D13	Tsai C-S et al., *J Gastrointest Oneal* (2011) 2(3):185-194
D14	T 1409/06
D15a	"Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer" clinicaltrials.gov posting NCT01494506 as updated on 2011_12_16
D15b	"Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-D15 Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer", Clinical Trials Identifier: NCT01494506 (25 January 2013)
D16	T 1592/12
D17	Commission Implementing Decision and Annexes (Summary of Product Characteristics for Onivyde®)
D18	"FDA approves new treatment for advanced pancreatic cancer" (2015), FDA News Releases
D19	"Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial", *Lancet*, 2016 Feb 6;387(10018):545-57
D20	MHRA Public Assessment Report for 5-FU (2006)
D21	EP 1 210 115 B1 with relevant parts highlighted

VIII. The parties are informed that additional comments, information or requests are to be filed in agreement with the requirements of Rule 116(1) EPC, i.e. not later than the final date indicated on the EPO form 2310 (cf. also the Guidelines E-II.5 and D-VI. 3.2).

IX. Taking into account the submissions made by the parties so far, the opposition division comes to the following preliminary opinion. This opinion is not binding as it is based on the facts and arguments presented until the issuance of this communication and cannot take into account further points which might be made at a later stage of the proceedings.

MAIN REQUEST (MR)

1. Rule 80 EPC

Claim 1 of the MR has been modified by the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)". This amendment is aimed at restoring the validity of the priority, challenged by OI. The validity of the priority claim in turn has an influence on the relevance of certain documents (D4, D8, D10 and D15b) cited by OI in the attack against inventive step. Thus, the OD is of the preliminary opinion that the amendment is occasioned by a ground of opposition (Article 56).

2. Articles 123(2) and (3) EPC

The claims of the MR are identical to the claims as granted with the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)" for the dose of leucovorine. In the preliminary opinion of the OD this deletion of an alternative appears to comply with the requirements of both Articles 123(2) and 123(3) EPC.

3. Priority claim (Article 87 EPC).

3.1. In the notice of opposition OI submitted that the first priority claim of the patent (13.06.2012) is not valid since claim 3 of the priority document US2012/61659211P (PD1) states that "....leucovorin is administered at a dose of 200 mg/m^2" without specifying whether leucovorin is in the l form or in the l+d (racemic) form. According to OI the term leucovorin without any further definition refers to the racemic form, and therefore, PD1 describes a different dose from that specified in claim 1 of patent as granted, which relates to a dose of 200 mg/m^2 of leucovorin in the l form or 400 mg/m^2 of leucovorin in the l+d form.

3.2. In the preliminary view of the OD, this objection has been overcome by the MR, wherein the dose of 400 mg/m^2 has been deleted, and claim 1 has been limited to a dose of 200 mg/m^2 of leucovorin in the l form.

Although PD1 refers to "leucovorin" only, this difference does not appear to be prejudicial to the validity of the priority claim to PD1.

OI expressed the view that at the time of the priority date the term "leucovorin" without any further definition would have been understood to exclusively refer to the racemic form of the drug, based in the fact that D1 reported the term "levoleucovorin" to refer to the levo isomeric form.

The OD does not share this view, since at the time of the priority date it was well known that, being leucovorin an optically active molecule, it could exist in the l-, d- or racemic-form. It was also known that the l-form was the pharmaceutically active form. A number of documents published before the priority date, e.g. D2 (see title; page 461, right column, par. 4; page 462, left-column, par. 4) and D3 (page 1642, right column, par. 1) use the term "leucovorin" to then clearly specify the use of the l-form (or levoleucovorin or levofolinic acid). Thus, the skilled person at the time of PD1 would have considered the generic term "leucovorin" to encompass "l-leucovorin" and its racemic form. Therefore, both forms appear to be implicitly, directly and unambiguously disclosed in PD1. Consequently, the reference in claim 1 of the MR to the l-form of leucovorin is considered to find basis in PD1 as it is a limitation from two possible alternatives, i.e. l-form and racemic-form.

3.3. Based on these considerations, the OD is of the preliminary opinion that claim 1 discloses the same invention as that disclosed in PD1, therefore the MR validly claims the earliest priority date of 13th June 2012. Consequently documents D4, D8, D10 and D15b cited by OI do not appear to represent prior art because they were made available to the public after 13th June 2012.

4. Sufficiency of disclosure (Article 83 EPC)

4.1. In the notice of opposition OI submitted that the patent is devoid of any adequate information demonstrating that the claimed drug dosing regimen is suitable for treating pancreatic cancer in patients who have failed gemcitabine therapy and therefore the requirements of Article 83 EPC are not met. OI also referred to the decision T1592/12 (D16) relating to a herceptin dosing regimen for use in treating breast cancer. The Board held that it was not enough that herceptin itself was known to be useful for the treatment of breast cancer, to meet the requirements of Article 83 EPC it was necessary that the patent described the suitability of the claimed dosage regimen for treating breast cancer.

4.2. In the preliminary opinion of the OD the claims of the MR appear to comply with the requirements of Article 83 EPC since the claimed dosage regimen appears to be sufficiently disclosed and the claimed therapeutic application appeared to be plausible at the relevant date, based on the original disclosure.

In fact, combinations comprising (non-liposomal) irinotecan, 5-FU and leucovorin were already used at the priority date in the treatment of some pancreatic cancers (see par. [0003] of the patent, corresponding to paragraph 4 of page 1 of the application as originally filed). Moreover, Example 6 of the patent (and of the original application) shows that the triple combination of liposomal irinotecan, 5-FU and leucovorin showed promising efficacy and safety data in a Phase 1 trial (PEP0203) which included 5 patients with pancreatic cancer (see Table 2 and paragraph [0083] of the patent). Table 2 indicates that of these 5 pancreatic cancer patients, one patient received a dose of 60 mg/m^2, three patients a dose 80 mg/m^2, and one patient a dose of 120 mg/m^2 of MM-398 (i.e. liposomal irinotecan). Thus, three pancreatic cancer patients where among the 6 patients who received the dose of 80 mg/m^2 in the phase I clinical trial described in example 6 as mentioned in paragraph [0044] of patent. This paragraph specifies that among these 6 patients there were 1 partial response (PR), 4 stable disease (SD) and 1 progressive disease (PD). By consequence, even assuming that one of the three pancreatic cancer patients showed PD, it follows that the other two exhibited either PR or SD. Consequently Example 6 appears to indicate that a positive effect was shown in at least 67% of the pancreatic cancer patients who received liposomal irinotecan at the dose of 80 mg/m^2 in combination with 5-FU and leucovorin.

Although Example 6 does not indicate the doses of 5-FU and leucovorin used in the phase I clinical trial, said doses are consistently indicated in the patent and in the original applications as in the claim of the MR. Therefore, Example 6 appears to sufficiently indicate that at the time of priority date the claimed triple combination was suitable for the treatment of pancreatic cancer.

4.3. In the preliminary opinion of the OD, the decision T1592/12 (D16) does not apply to the facts of the patent in suit. The patent at issue in T1592/12 (EP1 210 115 B1 see D21) related to a new dosage regimen for herceptin. In T1592/12, however, the reason for denying plausibility was the fact that the patent taught weekly administration of herceptin as being most preferred, whereas the claims related to subsequent doses every three weeks. Based on this discrepancy, the Board concluded that there were serious doubts as to the suitability of three-weekly administration for treating breast cancer. This appears to be different from the

situation of the patent in suit, which consistently refers to a very specific combination regimen, which is also claimed in claim 1 of the MR, and which appears to be supported and rendered plausible by the preliminary results of Example 6.

As the plausibility hurdle appears to have been overcome by the application as originally filed, the post-published document D17 can be taken into account as evidence that liposomal irinotecan can effectively treat pancreatic pancreatic cancer when administered according to the specific combination regimen of claim 1 of the MR.

Consequently, it is the OD's preliminary view that the requirements of Article 83 EPC are met.

5. Inventive step

5.1. In the notice of opposition OI raised an objection of lack of inventive step starting from D12 or D13 as the closest prior art using routine methods and/or in combination with the information available in D15b, or in alternative starting from D15b as the closest prior art in combination with the teaching of D12 or D13.

5.2. As indicated in paragraph 3.3. above, as the MR appears to validly claim the earliest priority date of 13th June 2012, document D15b cited by OI does not appear to represent prior art because they were made available to the public on 25.01.2013, i.e after the priority date. Therefore, based on the preliminary opinion of the OD, D15b is not relevant prior art for the issue of inventive step.

5.3. Both D12 and D13 focus on irinotecan monotherapy, but also mention the use of liposomal irinotecan in combination with 5-FU and leucovorin for the treatment of patients with gemcitabine refractory pancreatic cancer. OI submitted that the difference between D12 and D13 with the claimed subject-matter is that neither document describes the doses of the active ingredients or the dosing schedule according to the patent in suit. Thus, OI indicated the technical problem as to provide a suitable dosing regimen for the combination therapy described in the closest prior art. OI further submitted that, based on the decision T1409/06 (D14, paragraph 3.2), finding the optimum dosage is a matter of routine experimentation, thus the mere determination of the dosage which yelds the best effect does not involve an inventive step when the effect (in this case the treatment of gemcitabine refractory pancreatic cancer) is already known.

5.4. During the oral proceeding, in discussing inventive step over the prior art, the problem-solution approach will be used, as set out in the Guidelines GL G-VII,5. The closest prior art should generally be the document which corresponds to a similar use and requires the minimum of modifications to arrive at the claimed invention.

5.5. Both D12 and D13 actually focus on the PEP02 (liposomal irinotecan) monotherapy treatment, and although they mention a PEP02/5-FU/LV combination treatment used in a previous phase I study, they are silent with regard to the respective dosage of the three components of the combination treatment, and do not provide any result specifically relative to said combination treatment either. Results are only provided, both in D12 and D13, for a Phase II clinical trial. D13 reports using PEP02 in monotherapy at a dosage of 120 mg/m^2 every three weeks.

In the OD's preliminary view, D13 appears to represent a better starting point for the discussion of inventive step. In fact, although D12 and D13 contain very similar disclosure, it is noted that D13 cites D12 (see reference D30) and it appears to contain some more information regarding the possible combination treatment. In particular, on page 189, right-hand column, paragraph 2, D13 discloses that in a phase I trial, nanoliposomal CPT-11 (irinotecan) was used in combination with "weekly" 24-hour infusion of high-dose 5-FU/leucovorin (HDFL). Thus, D13 would appear to point to a different schedule regimen for the claimed combination, i.e. to a weekly cycle as opposed to the 2-week cycle according to claim 1 of the MR, as well as according to the schedule of Example 7 of the patent in suit.

5.6. During the oral proceedings the difference(s) between the closest prior art and the MR will have to be identified, the technical problem will have to be formulated. Then, it will have to be discussed whether the dosage regimen (dosage and frequency) for the combination treatment according to claim 1 of the MR meet the requirements of Article 56 EPC in the light of the foregoing discussion.

6. Further remarks

If one of the parties intends to file an additional comment, information or requests on the present case, this should be done in agreement with the requirements of Rule 116(1) EPC, i.e. not later than the final date given on the EPO Form 2310. Reference is made to the Guidelines E-II,5 and D-VI, 3.2.

Submissions not filed in time may be considered as late filed and their admissibility be questioned.

The parties are advised that a decision should be reached by the and of the oral proceedings. According to Article 113(2) EPC the Opposition Division is restricted to the text submitted to it or agreed by the proprietor. As a consequence, the requirements of Article 84 EPC are in general not fulfilled when amended claims are filed without the corresponding adapted description.

The parties are informed that, in the absence of the proprietor at oral proceedings, the lack of an appropriately adapted description may result in the revocation of the patent in accordance with Article 101(3)(b) EPC.

Attention is drawn to OJ EPO 12/2013, 603 concerning handwritten amendments.

Europäisches Patentamt
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Submission in opposition proceedings
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Opponent/representative's reference	X112963EP KJG

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2861210
Application No.	EP13731230.2

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - <other document>	Cover Letter X112963EP.pdf	OTHER-1.pdf
2	Any annexes (other than citation) to an opposition letter - <other document>	R116 Submission X112963EP.pdf	OTHER-2.pdf

Evidence filed subsequently:

D1b	Other evidence	Leucovorin calcium product label, November 2011 original file name: D1b X112963EP.pdf attached as: Other-evidence-1.pdf
D22	Other evidence	L. Chen, et. al., "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid original file name: D22 X112963EP.pdf attached as: Other-evidence-2.pdf

Signatures

Place:	
Date:	**10 May 2019**
Signed by:	**Kirk Gallagher 17437**
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Via Online Filing

9 May 2019

Opposition against EP2861210 (13731230.2) in the name of Ipsen Biopharm Ltd. by Teva Pharmaceutical Industries Limited

Dear Sirs

The enclosed submission is made in advance of the Oral Proceedings scheduled for 10 July 2019.

At the Oral Proceedings the Opponent will be represented by the undersigned and accompanied by one other person. The representative will speak English and require translation if any other language is used.

Yours faithfully
for D Young & Co LLP

Signed and filed electronically

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EUROPEAN PATENT No. 2861210

IPSEN BIOPHARM LTD.

OPPONENT'S REPLY UNDER R116 EPC

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1. INTRODUCTION

1.1. This is the Opponent's response to the preliminary opinion of the Opposition Division (OD) and to the reply of the Patent Proprietor (PP) to the opposition. For the avoidance of doubt all previously raised arguments are maintained.

1.2. In summary, it is the Opponent's position that the claimed subject matter cannot meet the requirements of both Article 83 EPC and Article 56 EPC.

1.3. The only feature of the claimed dosing regimen that is plausibly described in the Patent is the MTD of liposomal irinotecan (80 mg/m^2 of MM-398). The other features of the dosing regimen including the two weekly dosing cycle for all drugs (including MM-398), the dose of 5-FU, the dose of leucovorin and the order of administration of the drugs are not described in the Patent. Similarly, the Patent is silent on the treatment of pancreatic cancer that is recurrent or persistent following primary chemotherapy and resistant to gemcitabine. Therefore, in order to meet the requirements of Article 83 EPC, it is necessary to rely on the common general knowledge to teach the suitability of the claimed dosing regimen for treating the recited form of pancreatic cancer.

1.4. However, should the OD find that the requirements of Article 83 EPC are met, and the above mentioned claim features to be part of the common general knowledge, we note that the dose of liposomal irinotecan (80 mg/m^2) recited in the Patent is also known in the prior art. In particular, D15b already describes this exact same dose of MM-398 in combination with 5-FU and leucovorin for treating gemcitabine-refractory pancreatic cancer, and D22 teaches that the MTD of M-398 is 80mg/m^2 when used in combination with 5-FU and leucovorin.

1.5. Put another way, the Patent provides no more technical information than was already available in the prior art, i.e. that the combination of MM-398, 5-FU and leucovorin is able to treat pancreatic cancer (D13) and that the MTD of MM-398 in this combination is 80 mg/m^2 (D22).

2. NEW EVIDENCE

2.1. The following new documents are introduced into the proceedings:

D1b	Leucovorin calcium product label, November 2011
D22	L. Chen, et. al., "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors." Journal of Clinical Oncology 2010 28:15_suppl, e13024

2.2. D1b is a product label for an injectable formulation of leucovorin available shortly before the relevant date. The label demonstrates that leucovorin in racemic form was authorised for use in the treatment of cancer.

2.3. D22 is an article published in May 2010 i.e. before the relevant date. It describes a clinical study involving MM-398 in combination with 5-FU and leucovorin in the treatment of patients with advanced solid tumours who had failed standard chemotherapy. In the study, MM-398 was administered at doses of 60, 80, 100 and 120 mg/m^2, leucovorin was administered at 200 mg/m^2 and 5-FU was administered at 2000 mg/m^2. Dose limiting toxicities were observed at the higher doses of MM-398 and therefore the MTD for MM-398, when administered in combination with leucovorin and 5-FU, was determined to be 80 mg/m^2.

3. SUFFICIENCY OF DISCLOSURE

3.1. The OD in its preliminary opinion believes the Patent meets the requirements of Article 83 EPC based, primarily, on Example 6. The OD also distinguishes T 1592/12 on the facts.

3.2. However, the OD has impermissibly glossed over the fact that Example 6 does not provide any information on the suitability of the dosing schedule set out in the claims to treat pancreatic cancer that is recurrent or persistent and resistant to gemcitabine. Moreover, with regard to T 1592/12, the OD has failed to apply the clear principles and legal reasoning underlying that decision to the present case. In this regard attention is drawn to the Enlarged Board's comments in, for example, R 11/08, reasons 11, and R 14/11, reasons 2.9.1, which state that differences of fact are normal in cases whereas case law is not confined to similar or identical facts, but lies in the principles or guidance which can be extracted from earlier cases.

3.3. There is no dispute that the established case law of the Boards of Appeal requires that in the case of a medical use claim the patent / application must disclose the suitability of the product for use in the claimed therapeutic application.[1] The relevant disclosure need not be in the form of clinical studies but it is clear that a simple verbal statement is not enough (T 609/02, par. 9).

3.4. Only if this suitability hurdle has been overcome may post-published evidence be taken into account to back-up the findings in the patent application in relation to the claimed medical use.

3.5. This requirement of the established case law follows from the fact that attaining the claimed therapeutic effect is a functional technical feature of the claim. For example, T 609/02, par. 9 states that:

> "Where a therapeutic application is claimed in the form allowed by the Enlarged Board of Appeal in its decision G 5/83 (OJ EPO 1985, 64), i.e. in the form of the use of a substance or composition for the manufacture of a medicament for a defined therapeutic application, attaining the claimed therapeutic effect is a functional technical feature of the claim (see G 2/88 and G 6/88, OJ EPO 1993, 93

[1] Although the originally decided cases concerned claims in the Swiss-style the law has since been applied to EPC 2000-stype medical use claims (T 0895/13, par. 5).

> and 114, Headnote III. And point 9 of the reasons, for non-medical applications, see also T 158/96 of 28 October 1998, point 3.1 of the reasons). **As a consequence**, under Article 83 EPC, unless this is already known to the skilled person at the priority date, the application must disclose the suitability of the product to be manufactured for the claimed therapeutic application." (emphasis added)

3.6. In T 1592/12 the Boards applied this established law to a medical use claim where the new technical feature was not the therapeutic application *per se* but the dosing regimen.

3.7. As the OD will be aware the claims at issue in T 1592/12 concerned Herceptin for use in a method of treating breast cancer according to a particular dosing regimen. The appellant (patentee) argued that in such a case the requirements of Article 83 EPC would be met if the skilled person could carry out the dosing regimen or that it is known that Herceptin is suitable for treating breast cancer. The Board declined to follow either of these arguments, see reasons 17 and 18 of the decision.

3.8. In reasons 20 of its decision (reproduced below) the Board held that from the general principle that that the extent of a monopoly conferred by a patent should correspond to, and be justified by, the technical contribution made to the art, it followed that it is the suitability of the new Herceptin dosing regimen that must be demonstrated.

> "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)." (emphasis added)

3.9. In the present case, the claims relate to a combination of liposomal irinotecan, 5-FU and leucovorin for use in a method of treating recurring or persistent gemcitabine-refractory pancreatic cancer where the drug combination is administered according to a particular dosing schedule.

3.10. The prior art, e.g. D13, already describes the suitability of a combination of liposomal irinotecan, 5-FU and leucovorin for use in a method of treating gemcitabine-refractory pancreatic cancer. Therefore, as in T 1592/12, the alleged technical contribution in this case relates not to the therapeutic application *per se* but to the dosing schedule. Therefore, applying the clear legal principles set down in that decision the present application must demonstrate the suitability of the claimed dosing regimen for treating pancreatic cancer.

3.11. In par. 4.3 of its preliminary opinion, the OD distinguishes T 1592/12 from the present case on the basis of different facts. The OD states that "*In T1592/12, however, the reason for denying plausibility was the fact that the patent taught weekly administration of herceptin as being most preferred, whereas the claims related to subsequent doses every three weeks. Based on this discrepancy, the Board concluded that there were serious doubts as to the*

suitability of three-weekly administration for treating breast cancer. This appears to be different from the situation of the patent in suit, which consistently refers to a very specific combination regimen, which is also claimed in claim 1 of the MR, and which appears to be supported and rendered plausible by the preliminary results of Example 6."

3.12. The implication of these comments by the OD is that the Board doubted the suitability of the Herceptin dosing regimen because of a discrepancy between the claims (dosing every three weeks) and the preferred regimen in the description (weekly dosing) and if only the claims and description had been consistent, as in the present case, the Board would not have reached the same conclusion.

3.13. This is not correct. The Board did not doubt the suitability of the claimed dosing regimen because of a literal discrepancy between the preferred dosing regimen mentioned in the description and the claimed dosing regimen, the Board doubted the suitability of the claimed three weekly dosing regimen because the half-life of Herceptin was known to be only about one-week, see reasons 28. Based on this technical information the Board had reason to doubt that Herceptin was suitable for dosing every three weeks in treating breast cancer.

3.14. It might therefore be argued that T 1592/12 is specific to situations where there are "serious doubts" as to the suitability of the claimed dosing schedule for treating the disease at issue. However, this is not correct either. In the case of a medical use claim it is necessary to positively demonstrate suitability based on the information content of the application as filed together with common general knowledge. The case law concerning the requirement for "serious doubts" to substantiate insufficiency is not relevant to the present situation. This point is made most clearly in T 1045/13 where in relation to a medical use claim the appellant argued that a sufficiency objection could only be raised on the basis of "serious doubts substantiated by verifiable facts" (T 19/90). In reply the Board stated that:

> "The board does not consider T 19/90 to be relevant for the present decision. T 19/90 does not deal with a situation where a therapeutic effect which is a functional technical feature of the claim under consideration has to be established for the first time. This requires (see above) the present application to disclose the suitability of NGF for the claimed therapeutic application. Such suitability has not been convincingly demonstrated." (emphasis added)

3.15. Therefore, according to the approach adopted by the Boards, it is a matter of established legal principle that in the case of a medical use claim, where the contribution to the art lies in a dosing regimen, the application must demonstrate the suitability of the dosing regimen for treating the disease at issue. In this respect the established law relating to the demonstration of serious doubts does not apply until suitability has been convincingly demonstrated.

3.16. In par 4.2 of its preliminary opinion, the OD notes that in the study reported in Example 6 three patients received liposomal irinotecan (80 mg/m^2), 5-FU and leucovorin and in at least two patients a stable disease (SD) response was observed. However, as partly noted by the OD, Example 6 does not disclose the two weekly dosing cycle for any of the drugs (including MM-398), Example 6 does not describe the dose of 5-FU or the dose of leucovorin and Example 6 does not disclose the order of drug administration which is specified in the claim.

Moreover, Example 6 does not describe that the pancreatic cancer is recurrent or persistent following primary chemotherapy, or that the pancreatic cancer is gemcitabine refractory.[2]

3.17. Therefore, the Patent fails to disclose the suitability of the claimed dosing regimen for treating recurrent or persistent gemcitabine-refractory pancreatic cancer.

3.18. In dealing with the lack of any relevant teaching in relation to the dosing regimen, the OD states in its preliminary opinion that "*Although Example 6 does not indicate the doses of 5-FU and leucovorin used in the phase I clinical trial, said doses are consistently indicated in the patent and in the original applications as in the claim of the MR. Therefore, Example 6 appears to sufficiently indicate that at the time of priority date the claimed triple combination was suitable for the treatment of pancreatic cancer.*"

3.19. This explanation from the OD that consistent presentation of the relevant claim features in the application is sufficient perhaps relates to its interpretation of T 1592/12 discussed above. However, if the OD were followed on this point it would mean that a consistent statement in an application that a therapeutic effect is achieved would be enough. However, we know that this is not true. The Boards have consistently stated that a verbal statement of the type given in the present case is not adequate (see, T 609/02, par. 9). Moreover, the OD fails to address the missing information concerning the two week dosing cycle, the order of administration, the nature of the pancreatic cancer as being recurrent or persistent and being gemcitabine resistant, all of which are also technical features of the claims.

3.20. The OD also refers to par. [0003] of the Patent and the summary of the previous attempted treatments for some pancreatic cancers which includes the combination of non-liposomal irinotecan, 5-FU and leucovorin (FOLFIRI). However, there is no indication that any of this background information is common general knowledge. In this regard, attention is drawn to T 2059/13, reasons 4.5, which reiterates that in assessing the sufficiency of disclosure of medical use claims the common general knowledge is represented by basic handbooks and textbooks and does not normally include patent literature and scientific articles. See also, Case Law of the Boards of Appeal of the European Patent Office, 8th ed., ("CLBA") section II.C.3.1, par. 3.

3.21. Finally, we note that the claims relate to a treatment regimen involving "liposomal irinotecan"; yet, to the extent that the Patent contains any technical information corresponding to the scope of the claims, this is limited to a particular liposomal formulation, namely MM-398. There is no evidence in the application as filed (or post-published) that other liposomal formulations of irinotecan, which may have very different pharmacokinetics to MM-398 will be suitable for treating the particular type of pancreatic cancer mentioned in the claims, let alone that they would be suitable at the dose described in the Patent (80 mg/m^2).

3.22. To summarise: the established case law concerning medical uses requires a technical <u>not</u> simply a verbal teaching as to the suitability of the claimed use. The case law applies to the functional technical features of medical use claims, especially dosing regimens, which are relied on as the technical contribution to the art. In the present case no technical teaching is

[2] The Patent also fails to describe that liposomal irinotecan may be administered effectively at a dose of only 60 mg/m^2 dependent on genotype. However, it appears that reducing the dose of irinotecan in patients who are homozygous for the UGT1A1 *28 allele was well-known, see D10.

provided as to the suitability of the claimed dosing regimen for treating recurrent or persistent pancreatic cancer which is also gemcitabine refractory. No technical information is provided concerning other liposomal formulations of irinotecan. These deficiencies have not been addressed with references to the common general knowledge.

3.23. As such, in the present case the claimed therapeutic application has not been plausibly disclosed and the post-published evidence may not be relied on to back-up the findings that are in the Patent. Accordingly, the requirements of Art. 83 EPC are not fulfilled.

4. PRIORITY

4.1. In its preliminary opinion the OD acknowledges the claim to the first priority date (13 June 2012) for the subject matter of the Main request. We disagree.

4.2. According to the Guidelines (F-VI, 1.3 and 2.2) the right to claim priority is to be acknowledged only if the skilled person can derive the subject-matter of the claim directly and unambiguously, using common general knowledge, from the previous application as a whole.

4.3. The Main Request refers to a dose (200 mg/m^2) of leucovorin in the l-form (levo-leucovorin); whereas, the priority document does not mention levo-leucovorin at all. Instead, the priority document refers only to leucovorin (racemic form).

4.4. The OD suggests that at the priority date the skilled person would have understood the term leucovorin to encompass both leucovorin and levo-leucovorin.

4.5. To support this conclusion the OD notes that at the priority date it was well-known that leucovorin is an optically active molecule and that levo-leucovorin is the pharmaceutically active form. This may be correct; however, at the priority date leucovorin (racemic form) was itself an approved medicine marketed for the treatment of cancer. In this regard, attention is drawn to D1b which is the FDA approved product label for leucovorin calcium issued shortly before the earliest priority date. On page 1, col. 2, it describes that leucovorin together with 5-FU are indicated in the treatment of advanced colorectal cancer. Therefore, as the priority document is also concerned with cancer chemotherapy the skilled person would have no reason to doubt that leucovorin (racemic form) was the intended disclosure.

4.6. Further, the approach adopted by the OD goes against well-established principles in the naming of chemical compounds from which the skilled person knows how to describe racemic compounds and enantiomers using recognised naming conventions. In other words, if the skilled person had intended to disclose both the racemic compound (leucovorin) and the pharmaceutically active enantiomer (levo-leucovorin) he would have done so according to accepted practice. To retrospectively decide that the skilled person meant something different from what is literally disclosed in a document, when it would have been very straightforward for the skilled person to disclose that additional subject matter if he had chosen to do so, requires a very high standard of proof that is missing in this case.

4.7. As evidence supporting its preliminary view that the skilled person would have understood the term leucovorin in the priority document to encompass both leucovorin and levo-

leucovorin, the OD refers to D2 (title; page 461, right column, par. 4; page 462, left column, par. 4) and D3 (page 1642, right column, par. 1).

4.8. In one place the authors of D2 do use the term folinic acid (leucovorin) where it appears that the drug actually administered was levo-folinic acid.[3] However, a single disclosure, or even multiple disclosures, in academic papers such as D2 cannot re-write the common general knowledge of the skilled person. Put another way, even if the terms leucovorin and levo-leucovorin are used interchangeably in a number of individual prior art documents, this is insufficient evidence to support the notion that the skilled person would unambiguously consider the disclosure of leucovorin also a disclosure of levo-leucovorin, i.e. that the term leucovorin, as used in the priority document, is a direct and unambiguous disclosure of both leucovorin and levo-leucovorin.

4.9. As regards D3, this document only mentions leucovorin twice (page 1641, right column, par. 1 and page 1642, right column, par. 1) and on both occasions it specifies the l-form. It is unclear how this document supports the approach proposed by the OD.

4.10. In our view it appears that the OD is confusing the issue of whether it would have been obvious to use levo-leucovorin in place of leucovorin with whether levo-leucovorin is directly and unambiguously disclosed in the priority document, which is the correct test.

4.11. Furthermore, it is well-established that the application of EPO law must be consistent and that the concept of disclosure is the same for the purposes of Articles 54, 87 and 123 EPC (G2/03, reasons 2.2.2, par. 4). In other words, the concept of disclosure is the same for both the assessment of novelty and the determination of the right to claim priority. Therefore, an analogy can be drawn between this issue and the established practice of the Boards of Appeal concerning the novelty enantiomers.

4.12. It is now well established that the disclosure of a racemate does not anticipate the novelty of either enantiomer. In this regard, CLBA-I.C.6.2.3 "Novelty of enantiomers" states that:

> "According to decision T 296/87 (OJ 1990, 195), the description of racemates did not anticipate the novelty of the spatial configurations contained in them; racemates were described in the state of the art by means of expert interpretation of the structural formulae and scientific terms; as a result of the asymmetric carbon atom contained in the formula the substances concerned might occur in a plurality of conceivable spatial configurations (D and L enantiomers), but the latter were not by themselves revealed thereby in an individualised form. That methods exist to separate the racemate into enantiomers was something that should only be considered with respect to inventive step.
>
> In T 1048/92 the board observed that the fact that the disclosure of the prior document did not embrace more than two possible steric configurations did not take away the novelty of the specific one which was claimed in the application, because there was no unambiguous technical teaching directed to that configuration. The novelty of such an individual chemical configuration could only

[3] Compare page 461, right column, par. 4 with page 462, left column, par. 4.

> be denied if there was an unambiguous disclosure of this very configuration in the form of a technical teaching. It was thus not sufficient that the configuration in question belonged conceptually to a disclosed class of possible configurations without any pointer to the individual member."

4.13. Thus, the disclosure of a racemate, such as leucovorin, is not considered a direct and unambiguous disclosure of each of the individual enantiomers of leucovorin, e.g. levo-leucovorin. Put another way if the priority document were a prior art disclosure, the dose (200 mg/m^2) of leucovorin in the l-form would still be novel. That being the case, the claimed subject matter which describes a dose of levo-leucovorin cannot be derived directly and unambiguously from the previous application and therefore the priority claim is not valid.

4.14. We note that in its submission of 24 August 2018, the PP argues that its approach (i.e. that the term leucovorin is used to identify both leucovorin in racemic form and leucovorin in its l-form) is consistent with the Board of Appeal's case law on optically active compounds and specifically cites T 658/91. However, this is not correct.

4.15. Firstly, the OD will note that T 658/91 is not mentioned in the relevant section of the CLBA discussed above. This is because the facts of that case were quite specific and different from the present situation. The prior art document at issue in T 658/91 specifically taught that the described compound had an asymmetric centre and that the disclosure concerned both of the single enantiomers as well as the racemic mixture. The Board considered this teaching tantamount to an individualised disclosure of the later claimed enantiomer and therefore the Board upheld the Examining Division's finding of lack of novelty.

4.16. This is confirmed by T 398/01 (reasons 1.1.6, par. 4) which states as follows:

> "Also T 658/91 is not relevant since in that case an enantiomer individually claimed was described in a prior art document by virtue of an individual technical teaching and thus reproducible by the skilled person (reasons no. 2)."

4.17. In the present case; however, there is no corresponding disclosure in the priority document that leucovorin has an asymmetric centre nor is there a teaching that both enantiomers of leucovorin, as well as the racemate, are contemplated. The fact situation of the present case is clearly different from that in T 658/91.

4.18. Further support can also be drawn from T 600/95 (also mentioned by the PP) which states at reasons 3.2, par. 2:

> "In accordance with the above-mentioned consistent jurisprudence of the Boards of Appeal, the novelty of such an individual chemical compound can only be denied if there is an unambiguous disclosure of this very compound in the form of a technical teaching (see in particular T 181/82, OJ EPO 1984, 401, No. 8 of the reasons, and T 296/87, OJ EPO 1990, 195, Nos. 6 and 7 of the reasons). It is thus not sufficient that the compound in question belongs conceptually to a disclosed class of possible compounds, without any pointer to the individual member."

4.19. In the present case, there is no unambiguous disclosure of the very compound (levo-leucovorin) in the form of a technical teaching in the priority document. It might be the case that levo-leucovorin belongs conceptually to the class covered by the term leucovorin but, as made clear by the Board in this case, that is not sufficient.

4.20. Overall, therefore, the claim to the first priority date is not effective.

5. INVENTIVE STEP

5.1. As noted above, the claim to the first priority date is not effective and therefore documents D4, D8, D10 and D15b qualify as prior art.

Selection of the closest prior art

5.2. In its preliminary opinion, although similar, the OD preferred D13 to D12 as a potential closest prior art document. We see no reason to disagree that D13 is preferable to D12; however, as the claim to the first priority date is not valid D15b is also a closest prior art document.

5.3. D15b describes the protocol for a phase III clinical study of liposomal irinotecan (MM-398), optionally in combination with 5-FU and leucovorin for use in treating metastatic pancreatic cancer in patients who have failed gemcitabine based therapy. 5-FU and leucovorin without liposomal irinotecan is the active comparator. Of the two experimental Arms (A and C), Arm A represents a QW3 dosing cycle of MM-398 (120 mg/m^2) and Arm C represents a fortnightly dosing cycle of MM-398 (80 mg/m^2), 5-FU (2400 mg/m^2) and leucovorin (400 mg/m^2).

5.4. The PP argues that because D15b does not disclose the results of the study it cannot be the closest prior art. Instead the PP argues that D13 should be considered as the closest prior art. However, the approach adopted by the PP is incorrect in that (a) the Boards of Appeal have on multiple occasions selected the disclosure of a clinical trial protocol with no results as a closest prior art document, and (b) it is wrong to single out D13 as the closest prior art, both D13 and D15b are eligible to be considered as closest prior art documents.

5.5. In T 239/16 the Board considered the relevance of a number of prior art clinical study protocols to the patentability of claims directed to one of the disclosed regimens. The Board concluded that as no results of the clinical studies were available the claimed subject matter was novel. However, the document describing the clinical study protocols was found to represent the closest prior art.

5.6. Similarly, in T 2506/12 the Board considered the relevance of two prior art documents that described ongoing phase I clinical trials involving a drug combination for treating cancer. Again, the Board decided that as the outcome of the trials was not certain the claims were novel. However, the Board considered the disclosure of the on-going trials to represent the closest prior art.

5.7. As regards T 2154/14, cited by the PP, several points are worth noting. In that case the patent had been revoked by the OD for lack of inventive step over D8 which described ongoing phase II and III clinical studies, but no results. On appeal the patent proprietor urged the Board to select D1 as the closest prior art rather than D8. D1 described earlier clinical

studies relevant to the claimed subject together with results. The Board followed the patent proprietor and still found the claimed subject matter obvious.

5.8. The case law of the Boards of Appeal regularly refers to the concept of 'feasible' or 'suitable' closest prior art documents.[4] In the case in T 2154/14 it appears that both D1, selected by the Board, and D8, selected by the OD, were feasible / suitable starting points. However, as the Board was able to demonstrate a lack of inventive step over D1, the patent proprietor's preferred starting point, there was no need to consider whether the claimed subject matter was also obvious over D8.

5.9. The situation is the same in the present case. The claimed subject matter does not need to be inventive over D15b or D13, the claimed subject matter needs to be inventive over D15b and D13 as both of these documents represent feasible or suitable starting points for assessing the inventive step of the claimed subject matter.

D15b as closest prior art

5.10. The primary difference between the claimed subject matter and the disclosure of D15b (Arm C) is the lack of therapeutic results. However, like the Patent D15b also does not describe the order of administration of the drugs.

5.11. The technical problem may be formulated as the provision of an effective treatment of recurrent or persistent gemcitabine-refractory pancreatic cancer.

5.12. In our view, for the reasons discussed above in relation to sufficiency of disclosure, there is no evidence in the Patent that this problem had been plausibly solved at the relevant date. As there is no plausible disclosure in the Patent, post-published evidence cannot be used as the sole basis for evidencing that the technical problem has been solved (see, CLBA-I.D.4.6). Thus, the technical problem must be formulated less ambitiously along the lines of providing an arbitrary treatment and the claimed subject matter therefore lacks an inventive step.

5.13. However, in the event that the OD considers that the more ambitious problem is solved the claimed subject matter still lacks an inventive step.

5.14. In particular, human clinical trials in general and phase III clinical trials in particular are only authorised once it has been shown that the treatment under investigation has a high level of safety and efficacy in earlier studies. Thus, a skilled person reading D15b knows that the regimen described therein has already demonstrated safety and efficacy in earlier human clinical studies, earlier animal studies and in other preclinical studies. Therefore, starting from D15b the skilled person has a reasonable expectation that the above formulated technical problem would be solved.

5.15. This approach has been followed by the Boards of Appeal in similar situations. Thus in T 239/16, mentioned above, the Board considered the inventiveness of a dosing regimen for treating osteoporosis. The Board started from the disclosure of a clinical trial protocol

[4] See, T 967/97, point 3.2, T 1514/05, point 3.1.6, T 21/08 point 1.2.3, T 561/11, point 1.2.2, T 1289/09, point 4.5.4, T 1921/12, point 7.2 and T 591/04, point 4.1.

describing the regimen but without the results and formulated the technical problem as providing an effective treatment of osteoporosis.

5.16. Having concluded that the technical problem was solved the Board found that the claimed subject matter lacked an inventive step. In particular, the Board concluded that the mere fact that human clinical trials were ongoing was sufficient for the skilled person to a have a reasonable expectation that the formulated technical problem would be solved.

5.17. In reasons 6.5 the Board stated that:

> "The board considers that the mere fact that an active agent selected from the group of bisphosphonates is being tested in a clinical study for the treatment of osteoporosis (as disclosed in document (55)) leads to an expectation of success, due to the fact that clinical studies are based on data obtained by preclinical testing both in vitro and in animals and require authority approval which takes ethical considerations into account. This means in the present case that the skilled person would expect all study arms to treat osteoporosis effectively, unless he was dissuaded from this by the prior art."

5.18. And in reasons 6.6 the Board stated that:

> "Clinical trials in humans are planned scientific investigations. They require authority approval, which is only given after a risk/ benefit evaluation. For ethical (but also economic) reasons it has to be ensured that research risks are minimised and are reasonable in relation to any potential benefits. Ethical and economical considerations require that the "benefit" will arise with reasonable certainty and will not only "be hoped for"." (emphasis added)

5.19. Similar facts were considered by the Board in T 2506/12. That case related to a drug combination ((ET-743) and (PLD)) for treating cancer. Each drug had individually already shown efficacy in the treatment of cancer but the closest prior art was considered to be the disclosure that phase I human studies with the combination were underway, although the results were unavailable. With regard to the phase I study the Board noted that:

> "Document D2 discloses that a clinical phase I study assessing the combination treatment of cancer with Yondelis (ET-743) and Doxil (PLD) was in progress. Thus, at the publication date of D2, the information was available that the envisaged combination treatment was considered by pharmaceutical researchers with an expectation of success sufficient to justify a clinical phase I trial. In this context it is pointed out that drug compounds to be used in a clinical trial with human subjects are not selected based on a general "try-and-see" attitude, but based on existing favourable scientific data, for both ethical and economical reasons. Thus a clinical trial is not a mere screening exercise."

5.20. The facts of the present case exhibit a great deal of similarity to the facts of the above mentioned cases.

5.21. In the present case, the closest prior art (D15b) describes a phase III study involving a combination of liposomal irinotecan, 5-FU and leucovorin according to a particular dosing schedule for treating metastatic pancreatic cancer in patients who have failed gemcitabine based therapy. Following the logic of the Boards in T 239/16 and T 2506/12, the fact that the study is a human clinical study (phase III) is, in itself, sufficient for the skilled person to have a reasonable expectation that the clinical study will be successful. However, in the present case the skilled person also knows explicitly from D13 that the previous phase I clinical studies using a combination of liposomal irinotecan, 5-FU and leucovorin were successful in treating gemcitabine-refractory pancreatic cancer.

5.22. Thus, the skilled person starting from D15b would have a reasonable expectation that the above mentioned technical problem would be solved.

5.23. In its reply to the notice of opposition, the PP argues that "*It has been acknowledged by the Boards of Appeal that the disclosure that a particular treatment is undergoing clinical trials is merely "speculative" (T 715/03) of the treatment having any therapeutic efficacy and safety.*"

5.24. However, reliance on T 715/03 is misplaced. That decision concerned a patent directed to the use of ziprasidone in treating Tourette's syndrome (TS). The closest prior art did describe an ongoing phase II clinical study; however, in that case it was recognised that no earlier clinical studies on efficacy in TS had been concluded and that there were no animal models or other preclinical studies for TS that could have given a prior indication of efficacy. This interpretation of the decision is supported by the Board's comments in T 239/16:

> "The present situation furthermore differs from the one underlying decision T 715/03, also cited by the appellant-proprietors, in that, unlike the situation in T 715/03, animal models for osteoporosis exist and in that zoledronic acid has been successfully tested in those animal models (see document (20))." (reasons 6.6)

5.25. In contrast to the facts in T 715/03, in the present case the closest prior art describes a phase III study which would have necessarily been preceded by successful phase II efficacy studies. Moreover, D13 describes successful phase I efficacy studies with the same combination of drugs. Further, as evidenced by D13 itself (page 189), unlike the situation for TS, animal models do exist for pancreatic cancer.

5.26. Overall, therefore, T 715/03 is not relevant to the present case.

5.27. The PP also argues that the skilled person would prefer Arm A of D15b (monotherapy) over Arm C (combination therapy) because of the earlier published reports concerning monotherapy. However this is not correct because, as described above, the skilled person would know that the phase III study into the combination would only have received the requisite approval if earlier studies had been successfully completed with the combination. Moreover, a similar situation existed in T239/16 in which the clinical study described in the closest prior art document contained five study arms. The Board held that as each study was presented in the same manner (as in this case) each could be seen as a suitable starting point. However, the Board started its inventive step analysis from the study arm most closely resembling the claimed subject matter (in this case Arm C).

5.28. As noted above, like the Patent, D15b fails to describe the order of administration "wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU." However, to the extent that the Patent is considered to meet the requirements of Article 83 EPC then this feature must exist in the common general knowledge. Further, no specific advantage has been demonstrated for the order of addition but in any event it appears to be routine to administer the combination in the order irinotecan followed by leucovorin followed by 5-FU, see for example, D2, D4, D6 and D10 (par. 2.1).

5.29. As such the claimed subject matter lacks an inventive step starting from D15b as closest prior art.

D13 as closest prior art – priority claim not valid

5.30. D13 discloses that the combination of liposomal irinotecan (MM-398), leucovorin and 5-FU is efficacious in treating gemcitabine-refractory pancreatic cancer.

5.31. Although, as pointed out by the PP, neither D12 nor D13 separate the results of the phase I monotherapy trial and the phase I combination therapy trial, it is inconceivable that the results would have been presented in these documents in the way they have, had the combination trial failed to provide efficacious results.

5.32. Both the OD and the PP point out that D12 and D13 focus more on the phase II study concerning liposomal irinotecan monotherapy than the phase I combination study. Whilst this is true it seems irrelevant to the question of whether or not the skilled person would have developed the combination therapy (as well). The test is not what would the skilled person have developed first or next, the test is simply whether the claimed subject matter is obvious in view of the technical information in D12/D13. These documents clearly describe that liposomal irinotecan in combination with 5-FU and leucovorin had demonstrated efficacy in humans with gemcitabine resistant pancreatic cancer. As already discussed in connection with T 2506/12, such human clinical studies are only commenced when there is already a reasonable expectation of success. In this case the skilled person has more than a reasonable expectation, he knows from D12/D13 that the combination is able to treat gemcitabine-refractory pancreatic cancer and therefore he would have taken this combination regimen forward for further development.

5.33. The PP also argues that as D13 does not describe taking the combination therapy forward to phase II, it would have been reasonable for the skilled person to conclude that the combination regimen showed unacceptable levels of adverse events without sufficient signs of activity.

5.34. This analysis is both speculative and wrong. For the reasons given above, D15b demonstrates that the combination therapy showed sufficiently promising results in earlier studies to have been approved for human phase III studies. Moreover, D22 demonstrates that the combination (including 80 mg/m^2 of MM-398) demonstrated efficacy and acceptable safety in patients with refractory solid tumours.

5.35. A more accurate explanation of why D13 describes taking monotherapy into phase II and not the combination therapy is simply that the combination therapy was behind monotherapy in terms of development timelines. This is apparent from page 198, col. 2 which describes that "*The observation [efficacy with monotherapy] was further extended in a phase I trial for nanoliposomal CPT-11in combination with weekly 24-hour infusion of high-dose 5-FU/LV (HDFL).*", i.e. that the monotherapy trial was completed before the combination therapy trial started. Similarly, in December 2011 phase III studies comparing liposomal irinotecan monotherapy against leucovorin and 5-FU were announced (D15) and subsequently in January 2013 the combination treatment Arm was added to the phase III study (D15b).

5.36. Studies with liposomal irinotecan monotherapy being conducted before studies with liposomal irinotecan in combination with 5-FU and leucovorin also reflect what happened previously with non-liposomal irinotecan. Thus, D4 reports on preclinical studies with irinotecan in pancreatic cancer models and subsequent clinical studies with irinotecan monotherapy in patients with pancreatic cancer occurring ahead of similar studies with irinotecan and 5-FU and irinotecan, 5-FU and leucovorin, see page 4534, col. 2, lines 20-32.

5.37. Therefore, the disclosure in D13 that the combination of liposomal irinotecan (MM-398), leucovorin and 5-FU is efficacious in treating gemcitabine-refractory pancreatic cancer represents a feasible starting point for the inventive step analysis. It certainly fulfils the criteria of being "*that [disclosure] which corresponds to a similar use and requires the minimum of structural and functional modifications to arrive at the claimed invention (see T 606/89)*", GL (G-VII, 5.1).

5.38. Starting from this disclosure in D13 the technical problem may be formulated as the provision of an effective dosage regimen for the combination of liposomal irinotecan, leucovorin and 5-FU in patients with recurring or persistent gemcitabine-refractory pancreatic cancer.

5.39. As discussed above under the heading of sufficiency of disclosure, there is no evidence in the Patent that this problem had been plausibly solved at the relevant date. The only relevant information provided by the Patent is the dose of one type of liposomal irinotecan (80 mg/m^2 of MM-398) and even then this is not in connection with the treatment of the particular type of pancreatic cancer recited in the claims (recurrent or persistent and gemcitabine-refractory). Moreover, there is no information in the Patent concerning other liposomal formulations of irinotecan and no information on the doses of leucovorin or 5-FU and no indication of the dosing cycle.

5.40. As there is no plausible disclosure in the Patent that the technical problem has been solved, post-published evidence cannot be used as the sole basis for evidencing that the technical problem has been solved (see, CLBA-I.D.4.6). Thus, the technical problem must be formulated less ambitiously along the lines of providing an alternative dosing regimen and therefore the claimed subject matter lacks an inventive step.

5.41. However, in the event that the OD considers that the more ambitious problem is solved the claimed subject matter still lacks an inventive step.

5.42. As described above, D15b discloses almost all of the features of the claimed dosing regimen. Moreover, being a phase III clinical trial protocol the skilled person would have a reasonable expectation that the described dosing regimen would be successful.

5.43. Like the Patent, D15b fails to describe the order of administration wherein "*the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.*" However, to the extent that the Patent is considered to meet the requirements of Article 83 EPC then this feature must exist in the common general knowledge. Further, no specific advantage has been demonstrated for the order of addition but in any event it appears to be routine to administer the combination in the order irinotecan followed by leucovorin followed by 5-FU, see for example, D2, D4, D6 and D10 (par 2.1).

5.44. As such, the claimed subject matter lacks an inventive step over D13 in view of D15b.

D13 as closest prior art – priority claim valid

5.45. If the priority claim is found to be valid, and D15b is not prior art, the claimed subject matter still lacks an inventive step.

5.46. As discussed above, it is not correct to dismiss the teaching in D13 that the combination of MM-398, 5-FU and leucovorin had shown efficacy in human clinical trials against gemcitabine-resistant pancreatic cancer. Efficacy in human studies is clearly significant, particularly when shown against such a deadly and difficult to treat disease. In T 2506/12 the Boards acknowledged that human clinical studies of the type reported in D12/D13 are only commenced when there is already a reasonable expectation of success. In this case the skilled person has more than a reasonable expectation, he knows that the regimen is able to treat gemcitabine-refractory pancreatic cancer.

5.47. For the reasons given above, in terms of framing the technical problem, the only technical problem plausibly solved across its scope at the relevant date is one formulated along the lines of providing an alternative dosing regimen for the combination described in D13. As such, the claimed subject matter lacks an inventive step.

5.48. However, even if a more ambitious problem is considered solved the claimed subject matter still lacks an inventive step.

5.49. The contribution to the art starting from D13 is the claimed dosing regimen and therefore the technical problem may be framed as providing an effective dosing regimen for the combination of liposomal irinotecan, leucovorin and 5-FU in treating gemcitabine-refractory pancreatic cancer.

5.50. However, for the reasons discussed above in relation to sufficiency of disclosure, there is no evidence in the Patent of this contribution to the art at the relevant date. Therefore, in order to meet the requirements of Article 83 EPC, it is necessary to rely on the common general knowledge to teach the suitability of the claimed dosing regimen for treating the recited form of pancreatic cancer. However, the same common general knowledge must also be citable for inventive step against the granted claims in which case all the features of the dosing

regimen, aside from the dose of MM-398, should be considered part of the common general knowledge.

5.51. As such, it is only really necessary to determine whether the dose of MM-398 is obvious over the cited prior art as all the other features of the claim are common general knowledge.

5.52. In this regard, as mentioned in the opposition statement, the position of the Boards of Appeal is that although an invention may 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 the treatment of gemcitabine-refractory pancreatic cancer) is already known. 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. The Boards have also addressed the routine nature of optimizing combination dosing regimens, see T 2506/12 and the discussion of inventive step in relation to the auxiliary requests.

5.53. Therefore, following this logic of the Boards, the dose of MM-398 would have been arrived at using only routine skill. As all the other features of the claimed dosing regimen must be common general knowledge the claimed subject matter must be obvious over D13 in light of the common general knowledge.

5.54. However, even if the OD does not accept that the dose of MM-398 would be arrived at using only routine skill or that all the other features of the claimed dosing regimen are part of the common general knowledge, there are sufficient pointers in the prior art that would lead the skilled person to identify the claimed dosing regimen as an effective dosing regimen without inventive effort.

5.55. In this regard, whilst we do not accept that the following information is common general knowledge and therefore it is not suited to meeting the requirements of Article 83 EPC, the doses of MM-398, 5-FU and leucovorin recited in the Patent is the same or similar to the doses of these drugs already used in combination to treat refractory solid tumours generally.

5.56. Thus, D22 describes a clinical study involving MM-398 in combination with 5-FU and leucovorin in the treatment of patients with advanced solid tumours who had failed standard chemotherapy. MM-398 was administered at doses of 60, 80 100 and 120 mg/m^2, leucovorin was administered at 200 mg/m^2 and 5-FU was administered at 2000 mg/m^2. Dose limiting toxicities were observed at the higher doses of MM-398 when administered in combination with leucovorin and 5-FU and therefore the MTD for MM-398 in the combination regimen was determined to be 80 mg/m^2.

5.57. Although pancreatic cancer is not specifically mentioned in D22, this maximum tolerated dose of MM-398 is not dependent on the type of cancer being treated. Moreover, the fact that several different solid tumour types were treated in D22 with the same regimen (at least gastric cancer and breast cancer) is itself evidence that such regimens are used across different solid tumour types. Further evidence is seen in D2 (page 461, col. 2, final complete paragraph), which describes using the irinotecan / 5-FU / leucovorin dosing regimen developed in gastric cancer for use in treating pancreatic cancer.

5.58. Thus, the only feature of the claimed dosing regimen that is plausibly described in the Patent (the MTD of MM-398) had already been identified at the priority date. In fact, whilst the level of detail is different the study described in Example 6 of the Patent is the same study reported in D22 - same number of patients enrolled (16), same number of evaluable patients (15), same number of patients receiving 80 mg/m^2 of MM-398 (6), same number of responses (PR-2 (in gastric cancer and breast cancer), SD-9 and PD-4).

5.59. Further, the doses and frequency of administration of 5-FU and leucovorin recited in the Patent is the same or similar to the doses and frequency of administration of these drugs used in combination regimens with non-liposomal irinotecan when treating gemcitabine-resistant pancreatic cancer.

5.60. Thus, documents D3 and D5 describe positive studies with a combination of irinotecan, leucovorin and 5-FU in treating advanced pancreatic gemcitabine-refractory cancer. The treatment schedule described in these documents is summarised in the Table below:

	Irinotecan	LV	Levo-LV	5-FU	Frequency
D3	180 mg/m^2		200 mg/m^2	400 mg/m^2 600 mg/m^2	every 2 weeks
D5	70 mg/m^2 70 mg/m^2	400 mg/m^2		20000 mg/m^2	every 2 weeks

5.61. Thus, at least the twice weekly dosing cycle and the leucovorin dose recited in the Patent appear to be routinely used with irinotecan in treating gemcitabine-refractory pancreatic cancer.

5.62. Overall, starting from D13, the skilled person would arrive at the claimed dosing regimen applying only routine techniques in light of the regimens already known from the prior art.

BEDFORD LABORATORIES

LEUCOVORIN CALCIUM INJECTION USP
LEUCOVORIN CALCIUM FOR INJECTION

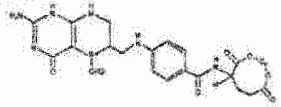
LCV-PAS-P03

Rx ONLY

DESCRIPTION

Leucovorin is one of several active, chemically reduced derivatives of folic acid. It is useful as an antidote to drugs which act as folic acid antagonists.

Also known as folinic acid, Citrovorum factor, or 5-formyl-5,6,7,8-tetrahydrofolic acid, this compound has the chemical designation of Calcium *N*-[*p*-[[(6*S*)-2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl]methyl]amino]benzoyl]-L-glutamate (1:1). The structural formula of leucovorin calcium is:



$C_{20}H_{21}CaN_7O_7$ M.W.=511.51

Leucovorin Calcium Injection USP is a sterile, preservative-free solution indicated for intramuscular (IM) or intravenous (IV) administration as a 50 mL single-dose vial. Each mL contains leucovorin calcium equivalent to 10 mg Leucovorin, USP; 8 mg sodium chloride; sodium hydroxide and/or hydrochloric acid for pH adjustment pH 7.8 (6.5 to 9.5).

There is 0.004 mEq of calcium per mg of leucovorin. Solution contains no bacteriostat or antimicrobial agents.

Leucovorin Calcium for Injection is a sterile product indicated for intramuscular (IM) or intravenous (IV) administration and is supplied in 50 mg, 100 mg, 200 mg, and 350 mg vials.

Each 50 mg vial of Leucovorin Calcium for Injection, when reconstituted with 5 mL of sterile diluent, contains leucovorin (as the calcium salt) 10 mg/mL.

Each 100 mg vial of Leucovorin Calcium for Injection, when reconstituted with 10 mL of sterile diluent, contains leucovorin (as the calcium salt) 10 mg/mL.

Each 200 mg vial of Leucovorin Calcium for Injection, when reconstituted with 20 mL of sterile diluent, contains leucovorin (as the calcium salt) 10 mg/mL.

Each 350 mg vial of Leucovorin Calcium for Injection, when reconstituted with 17.5 mL of sterile diluent, contains leucovorin (as the calcium salt) 20 mg/mL.

In each dosage form, one milligram of leucovorin calcium contains 0.002 mmol of leucovorin and 0.002 mmol of calcium.

These lyophilized products contain no preservative. The inactive ingredient is Sodium Chloride, USP added to adjust tonicity. Reconstitute with Bacteriostatic Water for Injection, USP, which contains benzyl alcohol (see **WARNINGS** section), or with Sterile Water for Injection, USP.

CLINICAL PHARMACOLOGY

Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active component of the mixture is the (-)-*d*-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of "one-carbon" moieties. *l*-Leucovorin (5-formyltetrahydrofolate) is rapidly metabolized (via 5, 10-methenyltetrahydrofolate then 5, 10-methylenetetrahydrofolate) to 5-methyltetrahydrofolate. 5-Methyltetrahydrofolate can in turn be metabolized via other pathways back to 5,10-methylenetetrahydrofolate, which is converted to 5-methyltetrahydrofolate by an irreversible, enzyme catalyzed reduction using the cofactors $FADH_2$ and NADPH.

Administration of leucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase.

In contrast, leucovorin can enhance the therapeutic and toxic effects of fluoropyrimidines used in cancer therapy, such as 5-fluorouracil. Concurrent administration of leucovorin does not appear to alter the plasma pharmacokinetics of 5-fluorouracil. 5-Fluorouracil is metabolized to fluorodeoxyuridylic acid, which binds to and inhibits the enzyme thymidylate synthase (an enzyme important in DNA repair and replication).

Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid to thymidylate synthase and thereby enhances the inhibition of this enzyme.

The pharmacokinetics after intravenous, intramuscular and oral administration of a 25 mg dose of leucovorin were studied in male volunteers. After intravenous administration, serum total reduced folates (as measured by *Lactobacillus casei* assay) reached a mean peak of 1259 ng/mL (range 897 to 1625). The mean time to peak was 10 minutes. This initial rise in total reduced folates was primarily due to the parent compound 5-formyl-THF (measured by *Streptococcus faecalis* assay) which rose to 1209 ng/mL at 10 minutes. A sharp drop in paired compound followed and coincided with the appearance of the active metabolite 5-methyl-THF which became the predominant circulating form of the drug.

The mean peak of 5-methyl-THF was 259 ng/mL and occurred at 1.3 hours. The terminal half-life for total reduced folates was 6.2 hours. The area under the concentration versus time curves (AUCs) for *l*-leucovorin, *d*-leucovorin and 5-methyltetrahydrofolate were 26.4 ± 3.6, 956 ± 97 and 129 ± 12 (mg/mL/h ± S.E.). When a higher dose of *d,l*-leucovorin (200 mg/m²) was used, similar results were obtained. The *d*-isomer persisted in plasma at concentrations greatly exceeding those of the *l*-isomer.

After intramuscular injection, the mean peak of serum total reduced folates was 435 ng/mL (range 240 to 725) and occurred at 32 minutes. Similar to IV administration, the initial sharp rise was due to the parent compound. The mean peak of 5-formyl-THF was 360 ng/mL and occurred at 26 minutes. The level of the metabolite 5-methyl-THF increased subsequently over time until at 1.5 hours it represented 50% of the circulating total folates. The mean peak of 5-methyl-THF was 226 ng/mL at 2.8 hours. The terminal half-life of total reduced folates was 6.2 hours. There was no difference of statistical significance between IM and IV administration in the AUC for total reduced folates, 5-formyl-THF, or 5-methyl-THF.

After oral administration of leucovorin reconstituted with aromatic elixir, the mean peak concentration of serum total reduced folates was 358 ng/mL (range 160 to 550). The mean time to peak was 2.3 hours and the terminal half-life was 5.7 hours. The major component was the metabolite 5-methyltetrahydrofolate to which leucovorin is primarily converted in the intestinal mucosa. The mean peak of 5-methyl-THF was 357 ng/mL at 2.4 hours. The peak level of the parent compound was 51 ng/mL at 1.2 hours. The AUC of total reduced folates after oral administration of the 25 mg dose was 92% of the AUC after intravenous administration.

Following oral administration, leucovorin is rapidly absorbed and expands the serum pool of reduced folates. At a dose of 25 mg, almost 100% of the *l*-isomer but only 20% of the *d*-isomer is absorbed. Oral absorption of leucovorin is saturable at doses above 25 mg. The apparent bioavailability of leucovorin was 97% for 25 mg, 75% for 50 mg, and 37% for 100 mg.

In a randomized clinical study conducted by the Mayo Clinic and the North Central Cancer Treatment Group (Mayo/NCCTG) in patients with advanced metastatic colorectal cancer three treatment regimens were compared: Leucovorin (LV) 200 mg/m² and 5-fluorouracil (5-FU) 370 mg/m² versus LV 20 mg/m² and 5-FU 425 mg/m² versus 5-FU 500 mg/m². All drugs were administered by slow intravenous infusion daily for 5 days repeated every 28 to 35 days. Response rates were 26% ($p=0.04$ versus 5-FU alone), 42% ($p<0.001$ versus 5-FU alone) and 10% for the high dose leucovorin, low dose leucovorin and 5-FU alone groups respectively. Respective median survival times were 12.2 months ($p=0.027$), 12 months ($p=0.050$), and 7.7 months. The low dose LV regimen gave a statistically significant improvement in weight gain of more than 5%, relief of symptoms, and improvement in performance status. The high dose LV regimen gave a statistically significant improvement in performance status and trended toward improvement in weight gain and in relief of symptoms but these were not statistically significant.

In a second Mayo/NCCTG randomized clinical study the 5-FU alone arm was replaced by a regimen of sequentially administered methotrexate (MTX), 5-FU, and LV. Response rates with LV 200 mg/m² and 5-FU 370 mg/m² versus LV 20 mg/m² and 5-FU 425 mg/m² versus sequential MTX and 5-FU and LV were respectively 31% ($p<0.01$), 42% ($p<0.01$), and 14%. Respective median survival times were 12.7 months ($p<0.04$), 12.7 months ($p<0.01$), and 8.4 months. No statistically significant difference in weight gain of more than 5% or in improvement in performance status was seen between the treatment arms.

The pharmacokinetics of 200 mg doses of leucovorin administered intravenously and orally (reconstituted powder, not tablets) have been evaluated in healthy male subjects. The serum clearance corrected for bioavailability, terminal half-life, and apparent volume of distribution of total folate were not significantly different between routes of administration. The oral bioavailability of the 200 mg dose was 31%. Eighty-three percent of the biologically active IV dose was recovered in the urine within 24 hours, 31% as 5-methyltetrahydrofolate. Twenty percent of the oral dose was excreted in 24 hours, 16% as 5-methyltetrahydrofolate.

INDICATIONS AND USAGE

Leucovorin calcium rescue is indicated after high dose methotrexate therapy in osteosarcoma. Leucovorin calcium is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosages of folic acid antagonists.

Leucovorin calcium is indicated in the treatment of megaloblastic anemias due to folic acid deficiency when oral therapy is not feasible.

Leucovorin is also indicated for use in combination with 5-fluorouracil to prolong survival in the palliative treatment of patients with advanced colorectal cancer. Leucovorin should not be mixed in the same infusion as 5-fluorouracil because a precipitate may form.

CONTRAINDICATIONS

Leucovorin is improper therapy for pernicious anemia and other megaloblastic anemias secondary to the lack of vitamin B_{12}. A hematologic remission may occur while neurologic manifestations continue to progress.

WARNINGS

In the treatment of accidental overdosages of folic acid antagonists, intravenous leucovorin should be administered as promptly as possible. As the time interval between antidote administration (e.g., methotrexate) and leucovorin rescue increases, leucovorin's effectiveness in counteracting toxicity decreases. In the treatment of accidental overdosages of intrathecally administered folic acid antagonists, do not administer leucovorin intrathecally. **LEUCOVORIN MAY BE HARMFUL OR FATAL IF GIVEN INTRATHECALLY.**

Monitoring of the serum methotrexate concentration is essential in determining the optimal dose and duration of treatment with leucovorin.

Delayed methotrexate excretion may be caused by a third space fluid accumulation (i.e., ascites, pleural effusion), renal insufficiency, or inadequate hydration. Under such circumstances, higher doses of leucovorin or prolonged administration may be indicated. Doses higher than those recommended for oral use must be given intravenously.

Because of the benzyl alcohol contained in certain diluents used for reconstituting Leucovorin Calcium for Injection, when doses greater than 10 mg/m² are administered, Leucovorin Calcium for Injection should be reconstituted with Sterile Water for Injection, USP, and used immediately (see **DOSAGE AND ADMINISTRATION**).

Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL, or 8 mL of a 20 mg/mL solution per minute).

Leucovorin enhances the toxicity of 5-fluorouracil. When these drugs are administered concurrently in the palliative therapy of advanced colorectal cancer, the dosage of 5-fluorouracil must be lower than usually administered. Although the toxicities observed in patients treated with the combination of leucovorin plus 5-fluorouracil are qualitatively similar to those observed in patients treated with 5-fluorouracil alone, gastrointestinal toxicities (particularly stomatitis and diarrhea) are observed more commonly and may be more severe and of prolonged duration in patients treated with the combination.

In the first Mayo/NCCTG controlled trial, toxicity, primarily gastrointestinal, resulted in 7% of patients requiring hospitalization when treated with 5-fluorouracil alone or 5-fluorouracil in combination with 200 mg/m² of leucovorin and 20% when treated with 5-fluorouracil in combination with 20 mg/m² of leucovorin. In the second Mayo/NCCTG trial, hospitalizations related to treatment toxicity also appeared to occur more often in patients treated with the low dose leucovorin/5-fluorouracil combination than in patients treated with the high dose combination — 11% versus 3%. Therapy with leucovorin and 5-fluorouracil must not be initiated or continued in patients who have symptoms of gastrointestinal toxicity of any severity, until those symptoms have completely resolved. Patients with diarrhea must be monitored with particular care until the diarrhea has resolved, as rapid clinical deterioration leading to death can occur. In an additional study utilizing higher weekly doses of 5-fluorouracil and leucovorin, elderly and/or debilitated patients were found to be at greater risk for severe gastrointestinal toxicity.

Seizures and/or syncope have been reported rarely in cancer patients receiving leucovorin, usually in association with fluoropyrimidine administration, and most commonly in those with CNS metastases or other predisposing factors, however, a causal relationship has not been established.

The concomitant use of leucovorin with trimethoprim-sulfamethoxazole for the orally treatment of Pneumocystis carinii pneumonitis in patients with HIV infection was associated with increased rates of treatment failure and morbidity in a placebo-controlled study.

PRECAUTIONS

General: Parenteral administration is preferable to oral dosing if there is a possibility that the patient may vomit and not absorb the leucovorin. Leucovorin has no effect on non-hematologic toxicities of methotrexate such as the nephrotoxicity resulting from drug and/or metabolite precipitation in the kidney.

Since leucovorin enhances the toxicity of fluorouracil, levamisole/5-fluorouracil combination therapy for advanced colorectal cancer should be administered under the supervision of a physician experienced in the use of antimetabolite cancer chemotherapy. Particular care should be taken in the treatment of elderly or debilitated colorectal cancer patients, as these patients may be at increased risk of severe toxicity.

Laboratory Tests

Patients being treated with the leucovorin/5-fluorouracil combination should have a CBC with differential and platelets prior to each treatment. During the first two courses a CBC with differential and platelets has to be repeated weekly and thereafter once each cycle at the time of anticipated WBC nadir. Electrolytes and liver function tests should be performed prior to each treatment for the first three cycles then prior to every other cycle. Dosage modifications of 5-fluorouracil should be instituted as follows, based on the most severe toxicities:

Diarrhea and/or Stomatitis	WBC/mm³ Nadir	Platelets/mm³ Nadir	5-FU Dose
Moderate	1,000 - 1,900	25-75,000	decrease 20%
Severe	<1,000	<25,000	decrease 30%

If no toxicity occurs, the 5-fluorouracil dose may increase 10%. Treatment should be delayed until WBCs are 4,000/mm³ and platelets 100,000/mm³. If blood counts do not reach these levels within two weeks, treatment should be discontinued. Patients should be followed up with physical examination prior to each treatment course and appropriate radiological examinations as needed. Treatment should be discontinued when there is clear evidence of tumor progression.

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 pediatric patients.

Preliminary animal and human studies have shown that small quantities of systemically administered leucovorin enter the CSF primarily as 5-methyltetrahydrofolate and, in humans, namely 1 to 3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration. However, high doses of leucovorin may reduce the efficacy of intrathecally administered methotrexate.

Leucovorin may enhance the toxicity of 5-fluorouracil (see **WARNINGS**).

Pregnancy: Teratogenic Effects: *Pregnancy Category C.* Adequate animal reproduction studies have not been conducted with leucovorin. It is also not known whether leucovorin can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Leucovorin should be given to a pregnant woman only if clearly needed.

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 leucovorin is administered to a nursing mother.

Pediatric Use: See **PRECAUTIONS, Drug Interactions.**

Geriatric Use: Clinical studies of leucovorin calcium did not show differences in safety or effectiveness between subjects over 65 and younger subjects. Other clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older patients cannot be ruled out. This drug is known to be excreted by the kidney and the risk of toxic reactions to the drug may be greater in patients with impaired renal function. Because older patients are more likely to have decreased renal function, care should be taken in dose selection, and it may be useful to monitor renal function.

ADVERSE REACTIONS

Allergic sensitization, including anaphylactoid reactions and urticaria, has been reported following the administration of both oral and parenteral leucovorin. No other adverse reactions have been attributed to the use of leucovorin *per se*.

The following table summarizes significant adverse events occurring in 316 patients treated with the leucovorin/5-fluorouracil combinations compared against 70 patients treated with 5-fluorouracil alone for advanced colorectal carcinoma. These data are taken from the Mayo/NCCTG large multicenter prospective trial evaluating the efficacy and safety of the combination regimen.

PERCENTAGE OF PATIENTS TREATED WITH LEUCOVORIN/FLUOROURACIL FOR ADVANCED COLORECTAL CARCINOMA REPORTING ADVERSE EXPERIENCES OR HOSPITALIZED FOR TOXICITY

	(High LV) /5-FU (N=165)		(Low LV) /5-FU (N=161)		5-FU Alone (N=70)	
	Any (%)	Grade 3+ (%)	Any (%)	Grade 3+ (%)	Any (%)	Grade 3+ (%)
Leukopenia	69	14	69	23	93	48
Thrombocytopenia	8	2	6	1	18	3
Infection	8	1	3	1	7	2
Nausea	74	10	60	9	60	6
Vomiting	46	8	44	9	40	7
Diarrhea	66	18	67	14	43	11
Stomatitis	75	27	84	29	59	16
Constipation	3	0	4	0	1	-
Lethargy/Malaise/Fatigue	13	3	12	2	6	3
Alopecia	42	5	43	6	37	7
Dermatitis	21	2	25	1	13	-
Anorexia	14	1	22	4	14	-
Hospitalization for Toxicity	9%		15%		7%	

High LV = Leucovorin 200 mg/m². Low LV = Leucovorin 20 mg/m²
Any = percentage of patients reporting toxicity of any severity
Grade 3+ = percentage of patients reporting toxicity of Grade 3 or higher

OVERDOSAGE

Excessive amounts of leucovorin may nullify the chemotherapeutic effect of folic acid antagonists.

DOSAGE AND ADMINISTRATION

Advanced Colorectal Cancer: Either of the following two regimens is recommended:

1. Leucovorin is administered at 200 mg/m² by slow intravenous injection over a minimum of 3 minutes, followed by 5-fluorouracil at 370 mg/m² by intravenous injection.
2. Leucovorin is administered at 20 mg/m² by intravenous injection followed by 5-fluorouracil at 425 mg/m² by intravenous injection.

5-fluorouracil and leucovorin should be administered separately to avoid the formation of a precipitate.

Treatment is repeated daily for five days. This five-day treatment course may be repeated at 4 week (28-day) intervals, for 2 courses and then repeated at 4 to 5 week (28 to 35 day) intervals provided that the patient has completely recovered from the toxic effects of the prior treatment course.

In subsequent treatment course, the dosage of 5-fluorouracil should be adjusted based on patient tolerance of the prior treatment course. The daily dosage of 5-fluorouracil should be reduced by 30% for patients who experienced moderate hematologic or gastrointestinal toxicity in the prior treatment course, and by 50% for patients who experienced severe toxicity (see **PRECAUTIONS; Laboratory Tests**). For patients who experienced no toxicity in the prior treatment course, 5-fluorouracil dosages may be increased by 10%. Leucovorin dosages are not adjusted for toxicity.

Leucovorin Rescue After High-Dose Methotrexate Therapy: The recommendations for leucovorin rescue are based on a methotrexate dose of 12 to 15 grams/m² administered by intravenous infusion over 4 hours (see methotrexate package insert for full prescribing information).

Leucovorin rescue at a dose of 15 mg (approximately 10 mg/m²) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion. In the presence of gastrointestinal toxicity, nausea or vomiting, leucovorin should be administered parenterally. Do not administer leucovorin intrathecally.

Serum creatinine and methotrexate levels should be determined at least once daily. Leucovorin administration, hydration, and urinary alkalization (pH of 7.0 or greater) should be continued until the methotrexate level is below 5×10^{-8} M (0.05 micromolar). The leucovorin dose should be adjusted or leucovorin rescue extended based on the following guidelines:

GUIDELINES FOR LEUCOVORIN DOSAGE AND ADMINISTRATION

DO NOT ADMINISTER LEUCOVORIN INTRATHECALLY

Clinical Situation	Laboratory Findings	Leucovorin 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.	15 mg PO, IM, or 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 15 mg PO, IM, or 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 150% 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).	150 mg IV q 3 hours, until methotrexate level is less than 1 micromolar; then 15 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 leucovorin therapy, these patients require continuing hydration and urinary alkalization, 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 abnormalities described in the table above. These abnormalities may or may not be associated with significant clinical toxicity. If significant clinical toxicity is observed, leucovorin 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.

Impaired Methotrexate Elimination or Inadvertent Overdosage: Leucovorin rescue should begin as soon as possible after an inadvertent overdosage and within 24 hours of methotrexate administration when there is delayed excretion (see **WARNINGS**). Leucovorin 10 mg/m² should be administered IV, IM, or PO every 6 hours until the serum methotrexate level is less than 10^{-6} M. In the presence of gastrointestinal toxicity, nausea, or vomiting, leucovorin should be administered parenterally. Do not administer leucovorin intrathecally.

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 leucovorin should be increased to 100 mg/m² IV every 3 hours until the methotrexate level is less than 10^{-6} M.

Hydration (3 L/d) and urinary alkalinization with sodium bicarbonate solution should be employed concomitantly. The bicarbonate dose should be adjusted to maintain the urine pH at 7.0 or greater.

Megaloblastic Anemia Due to Folic Acid Deficiency: Up to 1 mg daily. There is no evidence that doses greater than 1 mg/day have greater efficacy than those of 1 mg; additionally, loss of folate in urine becomes roughly logarithmic as the amount administered exceeds 1 mg.

Each 50, 100, and 200 mg vial of Leucovorin Calcium for Injection when reconstituted with 5, 10, and 20 mL, respectively, of sterile diluent yields a leucovorin concentration of 10 mg per mL. Each 350 mg vial of Leucovorin Calcium for Injection when reconstituted with 17.5 mL of sterile diluent yields a leucovorin concentration of 20 mg per mL. Leucovorin Calcium for Injection contains no preservative. Reconstitute the lyophilized vial products with Bacteriostatic Water for Injection, USP (benzyl alcohol preserved), or Sterile Water for Injection, USP. When reconstituted with Bacteriostatic Water for Injection, USP, the resulting solution must be used within 7 days. If the product is reconstituted with Sterile Water for Injection, USP, use immediately and discard any unused portion.

Because of the benzyl alcohol contained in Bacteriostatic Water for Injection, USP, when doses greater than 10 mg/m² are administered, Leucovorin Calcium for Injection should be reconstituted with Sterile Water for Injection, USP, and used immediately. (See **WARNINGS**.) Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL, or 8 mL of a 20 mg/mL solution per minute).

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

Leucovorin should not be mixed in the same infusion as 5-fluorouracil, since this may lead to the formation of a precipitate.

HOW SUPPLIED

Leucovorin Calcium Injection USP, 10 mg/mL, is supplied in sterile, single use vials as follows:
NDC 55390-009-01 500 mg individually boxed.
Store in refrigerator 2° to 8°C (36° to 46°F). **Protect from light.** Discard unused portion. Retain in carton until time of use.

Leucovorin Calcium for Injection is supplied in sterile, single use vials as follows:
NDC 55390-051-10 50 mg boxed vial, packs of 10.
NDC 55390-052-10 100 mg boxed vial, packs of 10.
NDC 55390-053-01 200 mg boxed vial.
NDC 55390-054-01 350 mg boxed vial.
Store at 20°C to 25°C (68° to 77° F). [See USP Controlled Room Temperature.] **Protect from light.** Retain in carton until time of use.

MANUFACTURED BY:
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November 2011

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LCV-PAS-P03

DEVELOPMENTAL THERAPEUTICS-CLINICAL PHARMACOLOGY AND IMMUNOTHERAPY

Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumors.

L. Chen, H. Shiah, T. Chao, R. K. Hsieh, G. Chen, J. Chang...

Show More

Abstract

e13024

Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved PK and tumor biodistribution of CPT-11 and its active metabolite-SN38 with encouraging safety and tumor response in preclinical studies and a single-agent phase I study. The study is to define the DLT, MTD, and PK of PEP02 when in combination with high-dose fluorouracil/leucovorin (HDFL) in patients (pt) with advanced solid tumors.

Methods: Pts who had failed to standard chemotherapy, ECOG PS 0-1 and adequate organ functions, no prior CPT-11, were eligible. PEP02 was given as 90 mins i.v. infusion on D1 in combination with 24-hr infusion of 5FU (2,000 mg/m^2)/ LV (200 mg/m^2) on D1 and D8, every 3 weeks. Cohorts of 3-6 pts were treated at 60, 80, 100, and 120 mg/m^2. PK and PGx samples were collected.

Results: A total of 16 pts were enrolled, with 3, 6, 5, and 2 at 60, 80, 100, and 120 mg/m^2. DLTs were observed in 4 pts, including 2 each at 100 and 120 mg/m^2 dose levels. DLTs were mainly G3 diarrhea and G4 hematologic toxicities. MTD was determined as 80 mg/m^2. Grade 3 or above adverse events at the MTD dose and all dose levels were 10.6% and 18.4%, respectively. The PK of total CPT-11 after PEP02 (at 80 mg/m^2) in combination with HDFL was characterized by low clearance (mean = 116.4 mL/m^2/hr) and small volume of distribution (mean = 2.93 L/m^2, similar to plasma volume) as did of PEP02 monotherapy study. Compared to the PK of SN-38 after 250 mg/m^2 of CPT-11 (in combination with capecitabine, Ann Oncol 2005; 16: 1123-32), the C_{max} after 80 mg/m^2 of PEP02 was lower (7.98 ± 4.39 *vs* 62.0 ± 37.4 ng/mL), but the $AUC_{0\rightarrow t}$was similar (354.77 ± 145.35 *vs* 396 ± 247 ng×h/mL). The correlation of UGT1A family with PK and toxicity was not observed. However, the only subject with the coexistence of two variants of *UGT1A1*6* and **28* had higher dose-normalized $AUC_{SN-38}$and experienced DLT. The best response of 15 evaluable pts was PR in 2 (gastric cancer and breast cancer) and SD in 9.

Conclusions: The MTD of PEP02 in combination with HDFL given every-3-week is 80 mg/m^2. The observation of tumor response in two heavily pre-treated patients suggests the combination deserves further exploration in advanced solid tumor patients who are refractory to standard therapy.

Author Disclosure

Employment or Leadership Position	Consultant or Advisory Role	Stock Ownership	Honoraria	Research Funding	Expert Testimony	Other Remuneration
PharmaEngine	PharmaEngine	PharmaEngine	PharmaEngine			

Europäisches Patentamt
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Office européen des brevets

Submission in opposition proceedings
made following summons to attend oral proceedings

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Proprietor/representative's reference	O008029EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2861210
Application No.	EP13731230.2

Documents attached:

	Description of document	Original file name	Assigned file name
1	Auxiliary request in opposition	AR1 (clean).pdf	AUXREQ-1.pdf
2	Auxiliary request in opposition	AR1 (marked-up).pdf	AUXREQ-2.pdf
3	Auxiliary request in opposition	AR2 - (clean).pdf	AUXREQ-3.pdf
4	Auxiliary request in opposition	AR2 (marked-up).pdf	AUXREQ-4.pdf
5	Auxiliary request in opposition	AR3 - (clean).pdf	AUXREQ-5.pdf
6	Auxiliary request in opposition	AR3 - (marked-up).pdf	AUXREQ-6.pdf
7	Any annexes (other than citation) to an opposition letter - Cover letter to EPO	O008029EP O003 (signed).pdf	OTHER-1.pdf

Signatures

Place:	London
Date:	28 June 2019
Signed by:	/OATES, Edward Christopher/
Representative name:	Edward Christopher OATES
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Your Ref 13731230.2 - 1109 / 2861210
Our Ref O008029EP:ECO/SJD/FJT
Date 28th June 2019

Oral proceedings scheduled for 10th July 2019 - urgent

Dear Sirs,

Re: European Patent No. 2861210
In the name of IPSEN BIOPHARM LTD.
Opposed by TEVA PHARMACEUTICAL INDUSTRIES LTD.

The "Facts and Arguments" document supporting the notice of opposition had seven pages of text. The opponent filed new submissions on 10th May 2019 (dated 9th May 2019) containing, in addition to two new citations, seventeen pages of text, i.e. over twice the length of its original notice of opposition. In response to these new and lengthy submissions, I am now filing Auxiliary Requests 1-3 to supplement the Main Request filed in August 2018.

Auxiliary Request 1 ("AR1")

The preliminary opinion of the Opposition Division correctly states that the Main Request validly claims the earliest priority date of 13th June 2012. The opponent is wrong to argue otherwise. In particular, the most recent submissions from the opponent incorrectly apply the case law relating to the novelty of enantiomers to the facts of the present case. Nonetheless, AR1 is submitted in case the opponent's arguments on priority succeed and the subject-matter of the main request is found to lack an inventive step over D15b, which is alleged to have been published during the convention year. AR1 addresses the inventive step objection based on D15b.

In AR1, claim 1 of the Main Request has been amended to specify that "*the patient achieves a response which is at least stable disease*".

This amendment finds basis on pages 16 and 17 of the application as filed. Page 16, towards the bottom of the page, and continuing onto page 17, lists the various categories of treatment response. The middle of page 17, in the fourth complete paragraph on that page, specifically discloses an embodiment in which the patient exhibits stable disease, partial response, complete response, or pathologic complete response ("pCR, CR, PR, or SD"). Thus the amended claim finds basis in the original application.

The skilled person seeking to provide an improved therapy for pancreatic cancer such that the patient achieves a response which is at least stable disease (SD) would not have been led to the claimed subject-matter by Arm C of D15b with a reasonable expectation of success because D15b gives no information about clinical outcomes. Therefore, the subject-matter of AR1 involves an inventive step even if D15b is taken into account.

Auxiliary Request 2 ("AR2")

In paragraph 3.21 of its submissions of 10th May 2019, the opponent raises a new attack under the provisions of Article 83 EPC. The opponent's allegation appears to be that the disclosure within the patent is insufficient having regard to the scope of claim 1 of the proprietor's Main Request. The opponent's objection should fail because it is not based on serious doubts substantiated by verifiable facts (T19/09 etc.). Nonetheless, in order to address this objection through an amendment, I am filing Auxiliary Request 2 ("AR2").

In AR2, claim 1 of the Main Request has been amended to include the feature of granted dependent claim 4, i.e. that the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection. Granted dependent claim 4 was not attacked for added matter. Indeed, the feature is disclosed in numerous places in the original application, e.g. page 6, first full paragraph. The dependent claims have been amended accordingly, and granted claim 4 has been deleted.

Auxiliary Request 3 ("AR3")

Should the amendments of both AR1 and AR2 be simultaneously necessary, I am filing AR3 which combines AR1 and AR2. The above comments apply in tandem.

The filing of Auxiliary Requests 1-3 is not the patent proprietor abandoning any subject matter. The proprietor reserves the right to reinstate any deleted subject matter. It also requests that it be permitted to make further claim requests and amendments in response to new points from the opponent or the Opposition Division.

I look forward to discussing this case with the Opposition Division on 10th July 2019. A copy of this letter and its enclosures will today be sent to the opponent's representative by email.

Yours faithfully,

// ELECTRONICALLY SIGNED AND SUBMITTED //

OATES, Edward Christopher
Carpmaels & Ransford LLP Professional Association No. 182

Encl. Auxiliary Requests 1-3

cc: Kirk Gallagher (kjg@dyoung.com)

Auxiliary Request 1

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

5. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 1

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

5. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 2

1. 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

~~4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.~~

5~~4~~. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 2

1. 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:
(a) either of distant metastasis or peripancreatic extension of the tumor; or
(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 33

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

~~4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.~~

54. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

Auxiliary Request 3

1. Liposomal irinotecan for use in a method of treating pancreatic cancer in a human patient such that the patient achieves a response which is at least stable disease, 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 co-administration 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 UGT1A 1 *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);

and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU;

and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

2. The liposomal irinotecan for use according to claim 1 wherein:

(a) after cycle 1 the dose of liposomal irinotecan administered to the patient homozygous for the UGT1A1 *28 allele is increased to 80 mg/m^2; and/or

(b) the 5-FU is administered intravenously over 46 hours; and/or

(c) the leucovorin is administered intravenously over 30 minutes.

3. The liposomal irinotecan for use according to claim 1 or claim 2 wherein:

(a) the liposomal irinotecan is administered intravenously over 90 minutes; and/or

(b) prior to each administration of liposomal irinotecan, the patient is pre- medicated with dexamethasone and/or a 5-HT3 antagonist or another anti-emetic; and/or

(c) the pancreatic cancer is an exocrine pancreatic cancer selected from the group consisting of acinar cell carcinoma, adenocarcinoma, adenosquamous carcinoma, giant cell tumor, intraductal papillary-mucinous neoplasm (IPMN), mucinous cystadenocarcinoma, pancreatoblastoma, serous cystadenocarcinoma, and solid and pseudopapillary tumors.

4. The liposomal irinotecan for use according to any one of the preceding claims, wherein the cancer is advanced pancreatic cancer, which is a pancreatic tumor that exhibits:

(a) either of distant metastasis or peripancreatic extension of the tumor; or

(b) both distant metastasis and peripancreatic extension of the tumor.

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Application No. / Patent No.	Ref.	Date
13 731 230.2 - 1109 / 2 861 210 /	P067376EP:ECO	28.08.2019
Proprietor Ipsen Biopharm Ltd.		

Provision of a copy of the minutes in accordance with Rule 124(4) EPC

The attached copy of the minutes of the oral proceedings is sent to you in accordance with Rule 124(4) EPC.

Kobylkova Fingerova
Formalities Officer
Tel. No.: +31 70 340 - 3260 **Branch at The Hague**

Enclosure(s): Copy of the minutes (Form 2309)

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Application No.: 13 731 230.2

Patent No.: EP-B-2 861 210

Minutes of the oral proceedings before the OPPOSITION DIVISION

The proceedings were public.

Proceedings opened on 10.07.2019 at 09:00 hours

Present as members of the opposition division:

Chairman:	Hoff, Philippe
1st member:	Bazzanini, Rita
2nd member:	Gradassi, Giulia
Minute writer:	Gradassi, Giulia

Present as or for the party or parties:

- For the Proprietor(s): Ipsen Biopharm Ltd.

  E. OATES, as representative
  H. ADAMS, as representative
  S. DUFFIELD, as representative
  F. TYRRELL, as representative
  E. ENNIS, as accompagnying person
  B. BELANGER, as accompagnying person
  S. JAGUELIN, as accompagnying person

- For the Opponent 1: Teva Pharmaceutical Industries Ltd

  K. Gallagher as representative

The identity of the person/s (as well as, if applicable, that of the witness or witnesses) and, where necessary, the authorisation to represent/authority to act were checked.

Essentials of the oral proceedings and relevant statements of the parties:

The chairperson (C) summarized the documents and the requests on file.

The opponent (O) confirmed all the requests on file. The patent proprietor (P) confirmed the requests on file and asked to replace the Main Request on file with Auxiliary Request 2. Consequently, Auxiliary Request 2 submitted on 28.06.2019 became the Main Request (MR) and the Main Request submitted on 24.08.2018 was renamed Auxiliary Request 2 (AR2).

MAIN REQUEST (MR)

Article 123(2) and Rule 80 EPC

O confirmed that he had no objection under Article 123(2) and Rule 80 EPC.

C declared that the opposition Division (OD) was of the opinion that the MR satisfied the requirements of Article 123(2) EPC and Rule 80 EPC.

Article 83 EPC

Article 83 EPC was discussed. Each party was given at least three opportunities to speak.

O reiterated the arguments put forward during the written proceedings. O added that the information with regard to the dosage regimen of the claims was not consistent throughout the patent and cited several passages of the description as examples. According to O, example 6 did not reflect the claimed dosage regimen. Said example was discussed in detail. O further cited D22 to support said argument.

P counter-argued that the patent consistently made reference to the same dosage regimen and that example 6 rendered the dosage regimen of the claims plausible even if the complete dosage regimen and the type of patients was not specified in said example. Reference was made to several passages of the patent as well as the first sentence of example 7 mentioning example 6. P further referred to documents D3, D5 and D6 and to decision T108/09, to substantiate that it was plausible at the date of filing of the application that the claimed therapy would work in the claimed type of patients. Arguments against T1592/12 were also provided.

Both parties questioned the pertinence of the documents cited by the other party with regard to Article 83 EPC. In particular, O contested that D3, D5 and D6 represented common general knowledge and referred to T2059/13. Said decision was then discussed by both parties.

The oral proceedings were interrupted at 10.30 hrs, to reach a conclusion on Article 83 EPC.

The oral proceedings were resumed at 10.55 hrs.

C declared that OD was of the opinion that the MR complied with the requirements of Article 83 EPC.

Priority

The validity of the priority claim with regard to document PCT/US2013/045495 was discussed. Each party was given at least three opportunities to speak.

Both O and P reiterated the arguments along the line of the written submissions.

P argued that in the context of the priority document L-leucovorin was intended when "leucovorin" was mentioned and that only one interpretation was possible. According to P, said information could be derived from the background section of the priority document wherein reference was made to the FOLFIRI trial, as well as from two passages of the description which mentioned that "leucovorin acts as a biochemical cofactor". P further cited D1-D5 to substantiate said reasoning.

O disputed said arguments and emphasized that there was not direct and unambiguous disclosure of L-leucovorin in the priority document.

C inquired why the racemic form of leucovorin could not be considered a biochemical cofactor. Said point was further discussed by both parties.

The oral proceedings were interrupted at 11.20 hrs, to reach a conclusion on the validity of the priority.

The oral proceedings were resumed at 11.45 hrs.

C declared that OD was of the opinion that the MR did not validly claim the priority date. The L-form of leucovorin was not directly and unambiguously disclosed in the priority document. Consequently, D4, D8, D10 and D15b represented prior art under Article 54(2) EPC.

Article 56 EPC

Closest prior art

C invited the parties to select the closest prior art. Each party was given at least three opportunities to speak.

O indicated D15b as closest prior art, whereas P selected D13. Both parties provided arguments to substantiate their choice. In particular, P referred to 2154/14 to defend why D13 represented the more suitable starting point. P further provided arguments against selecting D15b as closest prior art, including the fact that D15 did not mention what was MM-318.

C mentioned that what was MM-318 seemed to be known at the date of publication of D15b and referred to D13. P did not contest said point.

C further asked P whether a clinical trial phase III was not indicative that a certain efficacy had already been demonstrated before starting the trial. P provided arguments to explain why, according to him, said information was not implicit to every clinical trial phase III and thus to D15b. According to P, the clinical trial of D15b was not a regular clinical trial in view of the type of patients who were enrolled.

O cited T2506/12 against P's arguments.

P referred to T239/16 and argued that it was not enough to have a protocol of a clinical trial to deduce that there was an expectation of success. The whole context of the clinical trial, i.e. the compound, the disease and the prior art, had to be taken into consideration.

The oral proceedings were interrupted at 12.30 hrs, to reach a conclusion on the closest prior art.

The oral proceedings were resumed at 13.40 hrs.

C declared that OD was of the opinion that D15b was the closest prior art, since it had the same purpose of the claims and since less modifications had to be carried out to arrive at the subject-matter of the MR.

The parties were then invited to develop their inventive step reasoning starting from D15b as closest prior art.

Further steps of the problem-solution approach

Each party applied the problem-solution approach and provided arguments starting from D15b as closest prior document. Each party was given at least three opportunities to speak.

O reiterated the same reasoning as put forward in writing with regard to the difference, the technical effect, the problem to be solved as well as the obviousness of the solution and concluded that the MR did not meet the requirements of inventive step.

P identified the lack of data and the lack of information about MM-3198 in D15b as the main differences and defined the problem to be solved as "the provision of a safe and effective treatment of gemcitabine-resistant pancreatic cancer which is an improvement as to compared to the monotherapy arm". P further cited D17, D18 and D19 and referred to his letter of 24.08.2018 as evidence that said problem had been solved. P then discussed the non-obviousness of the solution. Several lines of arguments were provided:

- Lack of pointer to the selection of irinotecan sucrose octasulfate salt liposome, since said feature was not derivable from any document and since it was not known if it was inherent to MM-3198.

- No expectation of improved therapy with regard to the monotherapy.

- The situation of D15b was different from the situation in T239/16 or other decisions, since there was no evidence of efficacy and safety for 80 mg/m^2 liposomal irinotecan against pancreatic cancer neither in monotherapy nor in combination. P further referred to D1a, D7, D8, D10, D12, D13, D20 and D22 to substantiate said argument. In addition, according to P, D13 and D22 tought away from using the triple combination in the claimed dosage regimen. Thus, the overall teaching from prior art was dissuading the skilled person from thinking that a safe and effective treatment was to be expected from the trial of D15b.

C inquired whether there was a technical effect associated with the specific liposomal irinotecan of the claims. P did not mentioned any effect, and repeated that there was, however, no teaching in the prior art to use that specific form of liposomal irinotecan.

O objected that a new issue was raised during the oral proceedings with regard to MM-3198 which was not raised in the written proceedings and he therefore asked for an adjournment of the oral proceedings should said point become relevant for the assessment of inventive step.

C agreed to grant O's request if said feature would indeed become relevant.

The arguments and documents provided by each party was further discussed at length by the opposing party.

The oral proceedings were interrupted at 16.05 hrs, to reach a conclusion on the inventive step.

The oral proceedings were resumed at 17.10 hrs.

C declared that OD was of the opinion that the MR did not involve an inventive step, since D15b lead to an expectation of success.

C further declared that the same conclusion seemed to apply to AR1, AR2 and AR3.

The oral proceedings were interrupted at 17.12 hrs to give P time to consider any further request.

The oral proceedings were resumed at 17.20 hrs.

P declared that he wanted to proceed with AR3.

AUXILIARY REQUEST 3 (AR3)

Neither O, nor OD had any objection with regard to Article 123(2) EPC.

C declared that AR3 complied with Article 123(2) EPC as well as with Article 83 EPC. C then invited the parties to discuss inventive step starting from D15b which was still considered the closest prior art document to the subject-matter of the claims by O as well as by OD.

Article 56 EPC

Each party was given at least three opportunities to speak.

P argued that the problem to be solved underlying AR3 was to achieve at least a stable disease in a patient. Thus, a particular level of efficacy of the treatment should be achieved. P provided arguments to substantiate why said problem to be solved could be derived from the application as filed and to explain why it was not obvious to expect said specific and higher level of efficacy from D15b.

O disputed the problem to be solved put forward by P and argued that the same problem as for the MR applied. O added that the difference with the MR was that only failures were excluded from the subject-matter of the claims of AR3. O further argued that it was reasonable to expect to achieve stable disease at least in some patients.

Both parties gave further arguments in favor or against inventive step of AR3, in particular with regard to the expectation to achieve a stable disease. D2, D3 and D22 were cited and discussed by both parties.

The oral proceedings were interrupted at 18.10 hrs to reach a conclusion on inventive step.

The oral proceedings were resumed at 18.35 hrs.

C declared that OD was of the opinion that AR3 did not involve an inventive step and that the same conclusion applied to AR1 and AR2.

P declared that he had no further requests.

C announced the decision of OD and closed the proceedings.

After deliberation of the opposition division,

- the chairman announced the following **decision** :

"The European patent is revoked."

Regarding the reasons for the decision, the chairman referred to:

The division's opinion is that, even taking into consideration the amendments made by the proprietor of the patent during the opposition proceedings, the patent does **not** meet the requirements of the Convention (Article 101(3)(b)EPC)

The opposition division is of the opinion that the Main Request and Auxiliary Requests 1-3 do not meet the requirements of Article 56 EPC.

The party/parties was/were informed that the minutes of the oral proceedings and a written reasoned decision (including an indication of the possibility of appeal) will be notified to him/them as soon as possible.

The chairman **closed the oral proceedings** on 10.07.2019 at 18:38 hours.

signed:

Hoff, Philippe

. . . . . .

Chairman

signed:

Gradassi, Giulia

. . . . . .

Minute Writer

Enclosure(s):

Europäisches Patentamt
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Application No. / Patent No.	Ref.	Date
13 731 230.2 - 1109 / 2 861 210 /	P067376EP:ECO	28.08.2019
Proprietor Ipsen Biopharm Ltd.		

Decision revoking the European Patent (Art. 101(3)(b) EPC)

The Opposition Division - at the oral proceedings dated 10.07.2019 - has decided:

European Patent No. EP-B- 2 861 210 is revoked.

The reasons for the decision are enclosed.

Possibility of appeal
This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 and Rules 97 to 98 EPC.

Opposition Division:

Chairman: Hoff, Philippe
2nd Examiner: Gradassi, Giulia
1st Examiner: Bazzanini, Rita

Kobylkova Fingerova
Formalities Officer
Tel. No.: +31 70 340-3260 **Branch at The Hague**

Enclosure(s): 22 page(s) reasons for the decision (Form 2916)
Wording of Articles 106 - 108 and Rules 97-98 EPC (Form 2019)
Minutes of oral proceedings

to EPO postal service: 22.08.19

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Application No.: 13 731 230.2

Patent No.: EP-B-2 861 210

Direct Decision:
☑ **yes** ☐ **no**

Revocation of the European Patent (Art. 101(3)(b) EPC)

The Opposition Division - at the oral proceedings dated 10.07.2019 - has decided:

European Patent No. EP-B- 2 861 210 is revoked.

The Grounds for the decision (Form 2916) are enclosed.

22/7/2019
Date

Chairman	1st Examiner	2nd Examiner	Legally qualified member
Hoff, Philippe	Bazzanini, Rita	Gradassi, Giulia	

DECISION

Facts and Submissions

I. European patent 2 861 210 having the title "METHODS FOR TREATING PANCREATIC CANCER USING COMBINATION THERAPIES COMPRISING LIPOSOMAL IRINOTECAN" is based upon European patent application No. 13 731 230.2 filed on 12-06-2013. It claims priority of US 201261659211 filed on 13-06-2012 and US201361784382 filed on 14-03-2013. The mention of the grant of the patent has been published in the European Patent Bulletin of 03-05-2017.

Proprietor of the patent is:
Ipsen Biopharm Ltd.
Ash Road, Wrexham Industrial Estate, Wrexham, LL13 9UF, GB.

II. A notice of opposition has been filed by:
Teva Pharmaceutical Industries Ltd
5 Basel Street, P.O. Box 3190, 49131 Petah Tiqva, IL
on 05-02-2018.

III. The opponent (further referred to as OI) requests revocation of the patent in its entirety based on Articles 100(a) and (b) EPC because the patent lacks inventive step (Article 56 EPC) and is insufficiently disclosed (Article 83 EPC). The validity of the priority is also challenged by OI. In the notice of opposition OI made reference to documents D1-D16.

As an auxiliary request OI requested oral proceedings (Article 116 EPC).

IV. The opposition is filed in due time, in proper form and is supported by reasoned statement, and is therefore considered to be admissible in that it complies with the requirements of Articles 99(1), 100 EPC and Rules 3(1), 76 EPC.

V. In a reasoned statement received on 24-08-2018 in reply to the notice of opposition, the proprietor (further referred to as P) requested for the patent to be maintained on the basis of a new claim set entitled "Main Request". With his reply, P enclosed documents D15a, D17-D21 and relabelled document D15 cited by OI D15b.

As an auxiliary request P requested oral proceedings (Article 116 EPC).

VI. With official communication of 30.01.2019 the parties were summoned to Oral proceedings, to be held on 10.07.2019. In the annex to the summons the OD expressed a preliminary positive opinion with regard to Article 83, 87 and 56.

VII. With letter of 10.05.2019, within the limits under Rule 116(1) EPC, OI filed documents D1b and D22 and submitted further arguments in support of his objections under Articles 83, 87 and 56 EPC.

VIII. With letter of 28.06.2019, in reply to OI's submissions, P filed Auxiliary Requests 1-3.

IX. Reference will be made to the documents D1-D22 according to the following consolidated list:

D-number	Reference
D1	FDA label (Highlights of Prescribing Information) for FUSILEV (levoleucovorin) (2008)
D2	Gebbia V et al., *Am J Clin Oncol* (2008) 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	Taieb J et al., *Ann Oneal* (2007) 18:498-503
D7	Chen Let al., *J Clin Oneal* (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	Ko AH et al., *J Clin Oneal* (2011) 29(15), 4069
D13	Tsai C-S et al., *J Gastrointest Oneal* (2011) 2(3):185-194
D14	T 1409/06
D15a	"Study of MM-398 Versus 5-Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer" clinicaltrials.gov posting NCT01494506 as updated on 2011_12_16
D15b	"Study of MM-398 With or Without 5-Fluorouracil and Leucovorin, Versus 5-D15 Fluorouracil and Leucovorin in Patients With Metastatic Pancreatic Cancer", Clinical Trials Identifier: NCT01494506 (25 January 2013)
D16	T 1592/12
D17	Commission Implementing Decision and Annexes (Summary of Product Characteristics for Onivyde®)
D18	"FDA approves new treatment for advanced pancreatic cancer" (2015), FDA News Releases
D19	"Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial", *Lancet*, 2016 Feb 6;387(10018):545-57

D20	MHRA Public Assessment Report for 5-FU (2006)
D21	EP 1 210 115 B1 with relevant parts highlighted
D1b	Leucovorin calcium product label, November 2011
D22	L.Chen, et al., "Phase I study of liposome irinotecan (PEP02) in combination with weekly infusion of 5-FU/LV in advanced solid tumours", Journal of Clinical Oncology, 2010, 28:15 suppl, e13024

X. During the oral proceedings, held on 10.07.2019, OI confirmed his previous requests. P requested to promote AR2 filed on 28.06.2019 to Main Request. The previous Main Request filed on 24-08-2018 was designated as AR2. After the OD came to the conclusion that the main request did not comply with Article 56 EPC, P requested to discuss AR3.

XI. Following the discussion between the parties, the OD came to the conclusion that the requirements of Rule 80, 123(2) and 123(3) EPC were met. However, none of the requests on file complied with the requirements of Article 56 EPC. Therefore, the patent was revoked under Articles 101(3)(b).

Reasons for the decision

MAIN REQUEST (MR)

Claim 1 of the MR recites as follows:

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 co-administration 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 UGT1A 1 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); and wherein in each cycle, the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU; and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection.

1. Rule 80 EPC

1.1. Claim 1 of the MR has been modified by the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)". This amendment was made in an attempt to restore the validity of the priority, challenged by OI. The validity of the priority claim in turn has an influence on the relevance of certain documents (D4, D8, D10 and D15b) cited by OI in the attack against inventive step.

1.2. Claim 1 of the MR has been further modified by incorporation of the feature "*and wherein the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*". This amendment was made in response to the attack under Article 83 EPC raised by OI in the submission of 10.05.2019.

1.3. OI did not raise objections under Rule 80 EPC.

1.4. Thus, the OD is of the opinion that the amendments are occasioned by grounds of opposition (Article 56 and 83 EPC) and therefore are admissible under Article 80 EPC.

2. Articles 123(2) and (3) EPC

2.1. The claims of the MR are identical to the claims as granted with the deletion of the alternative "or 400 mg/m^2 (l + d racemic form)" for the dose of leucovorine, and with the addition of the limitation of liposomal irinotecan to its sucrose octasulfate salt liposome injection with basis in several parts of the original application (e.g. original claims 11 and 20 as well as in the examples) and in claim 4 of the patents as granted.

2.1. OI did not raise objections under Article 123(2) or (3) EPC.

2.2. In the opinion of the OD, the deletion of an alternative appears as well as the limitation to the specific preferred salt comply with the requirements of both Articles 123(2) and 123(3) EPC.

3. Sufficiency of disclosure (Article 83 EPC)

The OD is of the opinion that the MR complies with the requirements of Article 83 EPC.

3.1. In the notice of opposition OI submitted that the patent is devoid of any adequate information demonstrating that the claimed drug dosing regimen is suitable for treating pancreatic cancer in patients who have failed gemcitabine therapy and therefore the requirements of Article 83 EPC are not met.

3.1.1. OI referred to the decision T1592/12 (D16) relating to a herceptin dosing regimen for use in treating breast cancer. The Board held that it was not enough that herceptin itself was known to be useful for the treatment of breast cancer, to meet the requirements of Article 83 EPC it was necessary that the patent described the suitability of the claimed dosage regimen for treating breast cancer.

During OP, O1 further argued that in the framework of suitability, in accordance with T1592/12, the application must provide the required support for both the therapeutic indication, i.e. gemcitabine (GEM)-resistant pancreatic cancer (PC), and the claimed dosage regimen. However, according to OI Example 6 fails to do so since it does not indicate that GEM-resistant PC patients were treated, does not provide the doses for 5-fluorouracil (5-FU) or leucovorin (LV), does not mention the 2-week cycle, and does not indicate the order of administration of the drugs of the triple combination. Therefore, Example 6 is very different from original claim 3, which forms the basis of claim 1 of the MR.

3.1.2. With regard to the clinical trial described in Example 7, OI argued that it is only prophetic, and according to T609/02 a simple verbal statement of success is not enough to ensure sufficiency of disclosure in relation to a claim to a pharmaceutical use.

3.1.3. OI added that the original application did not provide consistent disclosure about the dosage regimen, with several variations of doses, order of administration, cycle duration and patients to be treated: e.g. page 14, lines 12-20 disclose a dose of 120 mg/m^2 with a cycle of 3 weeks; page 13, lines 5-18 indicate that simultaneous administration is also possible; page 12, line 15-34 disclose the treatment of all pancreatic cancer, including those that are refractory or resistant to other anti-cancer treatments (carboplatin, cisplatin), not only gemcitabine.

3.1.4. OI also alleged that, based on the number of patients and general outcome, the phase I clinical study described in document D22 was obviously the same study of Example 6. Thus it was possible to extract the information contained in D22 on the dosage regimen of this study to know that in the study of Example 6 the dose of 5-FU was 2000 mg/mg^2 (not 2400 mg/mg^2 according to the claims of the MR); racemic LV was used instead of its levo form; and a 3-week dosing cycle was used instead of a 2-week cycle. Thus it is not credible that study of Example 6 relates to the claimed regimen.

3.1.5. OI concluded that as the credibility test failed, P could not rely on post-published evidence.

3.2. P argued that the claims of the MR met the requirements of sufficiency.

3.2.1. P submitted that absolute proof is not required under Article 83 EPC. Nevertheless, the application provides lots of preclinical and clinical data, relative to pancreatic cancer, and using a regimen combining 5-FU, leucovorin and MM-398. In particular, the specific claimed dosage regimen is rendered plausible by the combined teaching of Example 6 and 7.

3.2.2. According to P the absence of specific indication in Example 6 that GEM-resistance PC patients have been treated does not undermine the plausibility, because at the time of the application it was known from e.g. D3, D5 and D6 that there is no cross-resistance between gemcitabine and irinotecan.

3.2.3. P also invoked decision T108/09, where although no data was provided for the claimed third line therapy, the patent was still considered sufficiently disclosed based on the results provided for the second line therapy.

3.3. The OD is of the opinion that the claims of the MR comply with the requirements of Article 83 EPC since the claimed dosage regimen is sufficiently disclosed and the claimed therapeutic application appears to be plausible at the relevant date, based on the original disclosure in combination with the common general knowledge.

3.3.1. Example 7 of the patent in suit, describes a prophetic Phase 3 clinical trial protocol for which no results are provided. The patients of the Phase 3 clinical trial of Example 7 are the same of claim 1 of the MR, i.e. patients with metastatic pancreatic cancer that have progressed on gemcitabine based therapy (see page 25, lines 15-16; page 27, lines 25-26 of the original application). The dosage regimen of the triple combination according to Arm C (see paragraph bridging page 25 and 26) is identical the the claimed dosage regimen.

Example 7 of the patent in suit, concerning a protocol for a phase 3 clinical trial which may or may not be carried out in the future, does not put into practice the invention defined in the claims as granted and can therefore, if taken alone, cannot serve as a basis for sufficient disclosure. However, the evaluation of sufficiency of disclosure takes account of the entire information to be found in the patent, including claims, description, examples and figures. The OD notes that the heading of Example 7, on page 24, below table 2, reads as follows: "*The promising efficacy and safety data from the Phase 1 trial (described above) warrant the MM-398 and 5-FU plus leucovorin combination to expand further in a phase 3 study*". This clearly indicates that Example 7 is not to be read in isolation, but in combination with the "promising" results of the previous Phase I trial, i.e. the study of Example 6.

3.3.2. Example 6 of the patent (and of the original application) shows that the triple combination of liposomal irinotecan, 5-FU and leucovorin showed promising efficacy and safety data in a Phase 1 trial (PEP0203) which included 5 patients with pancreatic cancer (see Table 2 and paragraph [0083] of the patent). Table 2 indicates that of these 5 pancreatic cancer patients, one patient received a dose of 60 mg/m^2, three patients a dose 80 mg/m^2, and one patient a dose of 120 mg/m^2 of MM-398 (i.e. liposomal irinotecan). Thus, three pancreatic cancer patients where among the 6 patients who received the dose of 80 mg/m^2 in the phase I clinical trial described in example 6 as mentioned in paragraph [0044] of the patent. This paragraph specifies that among these 6 patients there were 1 partial response (PR), 4 stable disease (SD) and 1 progressive disease (PD). By consequence, even assuming that one of the three pancreatic cancer patients showed PD, it follows that the other two exhibited either PR or SD. Consequently Example 6 appears to indicate that a positive effect was shown in at least 67% of the pancreatic cancer patients who received liposomal irinotecan at the dose of 80 mg/m^2 in combination with 5-FU and leucovorin. Although Example 6 does not indicate the doses of 5-FU and leucovorin used in the phase I clinical trial, said doses are consistently indicated in the patent and in the original applications as in the claim of the MR. Therefore, Example 6 appears to sufficiently indicate that at the time of priority date the claimed triple combination was suitable for the treatment of pancreatic cancer.

3.3.3. OI relied on document D22 in an attempt to extract information on the possible dosage regimen used in Example 6 of the application. However, the OD is the opinion that D22 cannot be used to interpret Example 6, since this isolated abstract publication cannot be considered to represent the common general knowledge at the time of the application and does not relate to the treatment of PC.

3.3.4. The OD agrees with OI that the application as filed also mentions different dosages for liposomal irinotecan and a 3-week cycle. However, these different dosages and duration consistently relate to monotherapy, not to the triple combination according to the claims. The OD also concurs with OI that the description disclose both sequential and simultaneous administration of liposomal irinotecan, 5-FU and LV. However, the OD notes that whenever more details are provided for the triple combination (e.g. on pages 13 and 14), the description consistently specifies in the same paragraph the same dosages, the same order of administration and the same cycle duration as in claim 1 of the MR, which in turn adhere to the dosage regimen according to Arm C of Example 7.

3.3.5. With regard to the type of patient, the OD considers that the treatment of this specific group of GEM-resistant pancreatic cancer patients is rendered plausible by the common knowledge that combinations comprising (non-liposomal) irinotecan, 5-FU and LV were already successfully used at the time of application in the treatment of GEM-resistant pancreatic cancer. This previous knowledge is indicated in paragraph 4 of page 1 of the application as originally filed, with the support of the convergent

disclosures of D2, D3 , D5 and D6, all relating to the so called FOLFIRI regimens mentioned in the application as filed. As the liposomal preparation of irinotecan according to the patent in suit is only directed to improve the therapeutic index (see e.g. page 9, lines 5-10), there are no reasons to doubt that the claimed combination including liposomal irinotecan would also be suitable for the treatment of GEM-resistant PC. This view is further supported by the knowledge from the same documents D3 (page 1644, left column, last sentence of 2nd paragraph), D5 (page 1659, left column, second paragraph) and D6 (abstract) that, having gemcitabine and irinotecan very different mechanism of action, there was no cross-resistance between these active compounds. Thus, the skilled person knew that the failure of GEM-therapy did not imply the failure of a therapy involving irinotecan, as confirmed by the success of the FOLFIRI treatments.

3.3.6. OI objected that according to the CLBA, II.C.3.3.1 and T2059/13, common general knowledge does not normally include patent literature and scientific articles. Accordingly D3, D5 and D6 could not be relied on for the purpose of Article 83 EPC. However, the OD considers that although D3, D5 and D6 are not monographs or textbooks, their convergent and consistent disclosure about FOLFIRI in the period spanning from 2007 to 2012, reflects the common knowledge of the skilled person at the time of the application.

3.3.7. In the opinion of the OD, the decision T1592/12 (D16) does not apply to the facts of the patent in suit. The patent at issue in T1592/12 (EP1 210 115 B1 see D21) related to a new dosage regimen for herceptin. In T1592/12, however, the reason for denying plausibility was the fact that the patent taught weekly administration of herceptin as being most preferred, whereas the claims related to subsequent doses every three weeks. In addition to that the half-life of herceptin was known to be only about 1 week (see reasons 28). Based on this technical information, the Board had reason to doubt that herceptin was suitable for treating breast cancer by administration every three weeks. Therefore, in T1592/12 there were serious doubts as to the suitability of the claimed dosing schedule for treating the claimed disease.

This appears to be different from the situation of the patent in suit, which consistently refers to a very specific combination regimen, the same of Arm C of Example 7, which is also claimed in claim 1 of the MR, and which appears to be supported and rendered plausible by the preliminary results of Example 6 in combination with the common general knowledge as indicated above. There is no inconsistency here between the results provided in Example 6, the regimen according to Example 7 and the claims of the MR, and there are no serious doubt about the efficacy of the claimed dosage regimen for the treatment of GEM-resistant PC.

3.3.8. As the plausibility hurdle appears to have been overcome by the application as originally filed, the post-published documents D17 and D19 can be taken into account as evidence that liposomal irinotecan can effectively treat GEM-resistant pancreatic cancer when administered according to the specific combination regimen of claim 1 of the MR.

4. Priority claim (Article 87 EPC).

The OD is of the opinion that the MR does not comply with the requirements of Article 87 EPC.

4.1. In the notice of opposition OI submitted that the first priority claim of the patent as granted (13.06.2012) is not valid since claim 3 of the priority document US2012/61659211P (PD1) states that "....leucovorin is administered at a dose of 200 mg/m^2" without specifying whether leucovorin is in the l form or in the l+d (racemic) form.

According to OI the term leucovorin without any further definition refers to the racemic form, and therefore, PD1 describes a different dose from that specified in claim 1 of the patent as granted, which relates to a dose of 200 mg/m^2 of leucovorin in the l form or 400 mg/m^2 of leucovorin in the l+d form. OI expressed the view that at the time of the priority date the term "leucovorin" without any further definition would have been understood to exclusively refer to the racemic form of the drug, based on the fact that D1 reported the term "levoleucovorin" to refer to the levo isomeric form.

4.2. In the MR the dose of 400 mg/m^2 has been deleted, and claim 1 has been limited to a dose of 200 mg/m^2 of leucovorin in the l form.

According to P, although PD1 refers to "leucovorin" only, this difference is not prejudicial to the validity of the priority claim to PD1, since at the time of the priority date it was well known that, being leucovorin an optically active molecule, it could exist in the l-, d- or racemic-form.

In addition, P argued that the priority document PR1 on page 11, line 19 indicates that "leucovorin acts as a biochemical co-factor" and at the priority date it was already well known (e.g. from D1) that the l-form of leucovorin was the pharmaceutically active form. Moreover, a number of documents published before the priority date, e.g. D2 (see title; page 461, right column, par. 4; page 462, left-column, par. 4) and D3 (page 1642, right column, par. 1) relating to the FOLFIRI regimen, use the generic term "leucovorin" but then clearly use the active l-form (levoleucovorin, levofolinic acid). The priority document PR1 on page 1, line 20 refers to the FOLFIRI regimen, thus based on D2 and D3, the l-form is implicitly disclosed.

According to P, the skilled person at the time of PD1 would have considered the generic term "leucovorin" to encompass "l-leucovorin" and its racemic form. Therefore, both forms appear to be implicitly, directly and unambiguously disclosed in PD1, in accordance with the approach followed in T658/91.

Consequently, the reference in claim 1 of the MR to the l-form of leucovorin is considered to find basis in PD1 as it is a limitation from two possible alternatives, i.e. l-form and racemic-form.

4.3. The OD is of the opinion that the priority claim of PD1 is not valid.

It is well established under EPO case law that the concept of disclosure is the same for both the assessment of novelty and the determination of the right to claim priority and therefore, the same tests can be applied.

According to decisions T269/87 and T1048/92, with regard to chiral compounds, the disclosure of a racemate does not anticipate the novelty of either enantiomer (see e.g. CLBA-I.C.6.2.3). Thus, the disclosure of leucovorin in PD1, is not considered a direct and unambiguous disclosure of each of the individual enantiomer of leucovorin, let alone e.g. levoleucovorin. Indeed, if the priority document PD1 were a prior art disclosure, the dose 200 mg/m^2 leucovorin in the l-form according to claim 1 of the MR would still be novel.

The decision T658/91 invoked by P does not seem to apply in present case. In fact, the prior art document at issue in T658/91 specifically taught that the described compound had an asymmetric centre and that the disclosure concerned both the single enantiomers as well as the racemic mixture. The Board considered this teaching tantamount to an individualized disclosure of the later claimed enantiomer and therefore the Board denied the novelty. In the present case, however, there is no corresponding disclosure in the priority document that leucovorin has an asymmetric centre nor is there a teaching that both enantiomers of leucovorin, as well as the racemate, are contemplated.

This approach is further confirmed by T600/95 which indicated that it is not sufficient that the compound in question belongs conceptually to a disclosed class of possible compounds, without any pointer to the individual member. It might be the case that levo-leucovorin belongs conceptually to the class covered by the term leucovorin, but this does not equate to an unambiguous disclosure.

Moreover, although it might have been obvious for the skilled person to use the active enantiomer, the concept of obviousness does not correspond to the concept of direct and unambiguous disclosure required here. Additionally, at the time of PR1 the racemic form was also commonly marketed and used, as indicated by D1, D1b as well as D5 and D6, were no specific reference is made to the l form.

Accordingly, the claimed subject-matter which describes a dose of leucovorin in the l form cannot be derived directly and unambiguously from PD1 and therefore the priority claim of PD1 is not valid.

The valid priority date is the one of PR2, i.e. 14.03.2013. Consequently, documents D4, D8, D10 and D15b cited by OI become relevant prior art because they were made available to the public before that date.

5. Inventive step

The OD is of the opinion that the MR does not comply with the requirements of Article 56 EPC.

5.1. In the notice of opposition OI raised an objection of lack of inventive step starting from D12 or D13 as the closest prior art using routine methods and/or in combination with the information available in D15b, or in alternative starting from D15b as the closest prior art in combination with the teaching of D12 or D13.

During the OP, OI indicated that D15b, D13 and D5 could all represent good starting points, however, according to OI, D15b represented the closest prior art.

5.2. Conversely, according to P, D13 is to be regarded as the closest prior art, since it contains data on the beneficial effects of a treatment regimen using a triple combination with liposomal irinotecan, 5-FU and LV on GEM resistant PC patients. P submitted that D13 contains a more comprehensive disclosure than D15b. Based on purposive considerations, in view of the reported results, and in line with T2154/14, D13 should be preferred over D15b which does not provide any result of the disclosed treatment.

Moreover, P pointed out that D13 clearly relates to nanoliposomal CPT-11 (PEP02 also known as MM-398) whereas D15b does not indicate what MM-398 stands for.

P further argued that a phase III clinical trial may imply a certain expectation of safety, but no expectations of actual efficacy. This particularly applies to D15b which aims to treat the specific patient population of GEM-refractory PC patients whose life expectations is of 6 months or less. Thus, rather than an expectations of success, there was a hope of prolonging patients survival with the regimen of D15b, according to a try and see approach.

5.3. In the opinion of the OD, D15b represents a better starting point compared to D13.

D13 (page 189, right-hand column, paragraph 2, to page 191, left-hand column, paragraph 1) discloses the PEP02 (liposomal irinotecan) monotherapy for the treatment of patients with gemcitabine refractory pancreatic cancer, and also mentions a PEP02/5-FU/LV combination treatment used in a phase I study. However, it is silent with regard

to the respective dosage of the three components of the combination treatment, and does not provide any result specifically relative to said combination treatment either. Results are only provided for a Phase II clinical trial using PEP02 as monotherapy at a dosage of 120 mg/m^2 every three weeks (see also D12, corresponding to Reference 30 in D13).

Moreover, when referring to the combination treatment of the phase I clinical trial, D13 discloses that nanoliposomal CPT-11 (irinotecan) was used in combination with "weekly" 24-hour infusion of high-dose 5-FU/leucovorin (HDFL). Thus, D13 discloses a different schedule regimen for the claimed combination, i.e. a weekly cycle as opposed to the 2-week cycle according to claim 1 of the MR.

Therefore, not only D13 does not disclose specific results for the combination treatment, but it also fails to disclose the dosage regimen according to the patent in suit.

D15b describes the protocol for a phase III clinical study of liposomal irinotecan (MM-398), alone or in combination with 5-FU and leucovorin for use in treating metastatic pancreatic cancer in patients who have failed gemcitabine based therapy. 5-FU and leucovorin without liposomal irinotecan is the active comparator. Of the two experimental Arms (A and C), Arm A represents a 3-week (Q3W) dosing cycle of MM-398 (120 mg/m^2) and Arm C represents a 2-week (Q2W) dosing cycle of MM-398 (80 mg/m^2), 5-FU (2400 mg/m^2) and leucovorin (400 mg/m^2). Therefore, Arm C is a combination treatment using the same drugs in the same amounts of claim 1 of the MR, with the only difference that the order of administration is not specified.

It is correct what stated by P that D15b does not disclose the outcome of the clinical study, however, the Boards of Appeal have on multiple occasions selected the disclosure of a clinical trial protocol with no results as the closest prior art document, e.g. T239/16 and T2506/12.

The official title of D15b, reads: "A randomized, open label phase 3 study of MM-398, with or without 5-fluorouracil and leucovorin in patients with metastatic pancreatic cancer who have failed prior gemcitabine-based therapy". Thus, based on the title, the OD considers that D15b clearly relates to the same purpose of D13 and the same purpose of the patent in suit, i.e. the treatment of GEM-resistant PC patients.

Moreover, although D15b does not indicates the meaning of MM-398, at the time of D15b it was known from D13 that the MM-398 was nanoliposomal CPT-11, also known as PEP02.

All in all, the OD considers that D15b clearly relates to the same purpose of the patent in suit, and it is the document which requires less modifications to arrive at the dosage regimen of the MR, and therefore it represents the closest prior art.

5.4. During the OP, the parties discussed inventive step using the problem and solution approach starting from D15b as the closest prior.

5.5.1. According to OI, D15 differs from claim 1 of the MR in that the order of administration of the drugs of the combination treatment is not disclosed. However, this order would be obvious in view of the same order of administration used in the FOLFIRI or CAMPTOSAR treatment, as disclosed e.g. in D2, D4, D6 and D10.

5.5.2. A further difference indicated by OI is the lack of therapeutic results. The objective technical problem could be formulated in a more ambitious or less ambitious way, i.e. as an improved or simply as an effective treatment of GEM-resistant PC, but still claim 1 of the MR would not be inventive. In fact, OI submitted that human clinical trials in general, and phase III clinical trials in particular, are only authorised once it has been shown that the treatment under investigation has a high level of safety and efficacy in earlier studies. Thus, a skilled person reading D15b knows that the regimen described therein has already demonstrated safety and efficacy in earlier human clinical studies, earlier animal studies and in other preclinical studies. Therefore, in line with established case law, such as T239/16 and T2506/12, starting from D15b the skilled person has a reasonable expectation that the technical problem would be solved.

5.6.1. P submitted that not only D15b does not disclose the order in which the drugs of the combination treatment are administered, but also does not contain any data on the outcome of the treatment, thus failing to provide actual disclosure of both safe and effective treatment. According to P, the disclosure that a particular treatment is undergoing clinical trials is merely speculative of the treatment being safe and therapeutically effective. The selection of Arm C in D15b could not be done in the expectation of a safe and effective treatment but only in "the hope" of such an effect (emphasis added) based on a "try and see" approach.

5.6.2. According to P, the objective technical problem could be formulated as a low threshold or as a more ambitious problem, and in both case the dosage regimen of claim 1 of the MR would be inventive.

The low threshold problem could be regarded as the provision of a safe and effective treatment of GEM-resistant PC. To this regard, P indicated that the post-published documents D17, D18 and D19 all confirm that the problem has been solved, providing sound data relative to both safety and efficacy.

The more ambitious problem could be regarded as the provision of an improved safe and effective treatment of GEM-resistant PC compared to the monotherapy. To this regard, P argued that liposomal irinotecan for use as recited in claim 1 of the MR was approved following a pivotal Phase III trial, referred to as NAPOLI-1 (see e.g. D17 and D19). The protocol for this trial is explained in detail in Example 7 of the patent. The results of this trial show that the claimed invention is associated with a number of

beneficial technical effects. In the NAPOLI-1 trial, and as explained in section B of Example 7, patients were separated into three arms. Patients in Arm A were administered 120 mg/m^2 of liposomal irinotecan (referred to as "MM-398" in Example 7) over 90 minutes every three weeks. Arm B was referred to as the control arm, in which patients were treated with 5-FU/LV. Patients in Arm C were administered 80 mg/m^2 of liposomal irinotecan over 90 minutes every two weeks in combination with 5-FU/LV according to claim 1 of the MR. The results from each of Arms A (monotherapy) and C (triple therapy) were individually compared with those from Arm B (5-FU/LV). The claimed dosage regimen shows clinically and statistically relevant improved efficacy with regard to all primary and secondary endpoints studied, i.e. overall survival (OS - defined in paragraph [0164] of the patent as the time from the date of patient randomisation to the date of death or the date the patient was last known alive); progression free survival (PFS - defined in paragraph [0169] of the patent as the number of months from the data of randomization to the date of death or progression, whichever occurred earlier); median time to treatment failure (TTF - defined in paragraph [0171] of the patent as the time from randomisation to either disease progression, death or study discontinuation due to toxicity); objective response rate (ORR - discussed in paragraphs [0172] and [0173] of the patent); levels of the pancreatic cancer tumour marker CA19-9 (discussed in paragraph [0174] of the patent). The efficacy data endpoints from the NAPOLI-1 trial illustrated by P are discussed in D19, and are reproduced in the table below:

	Arm A (monotherapy)	**Arm C (combination)**
Median Overall survival / months (Arm B value)	4.9 (4.2)	6.1 (4.2)
Median progression-free survival / months (Arm B value)	2.7 (1.6)	3.1 (1.5)
Median time to treatment failure / months (Arm B value)	1.7 (1.4)	2.3 (1.4)
Overall response rate / % (Arm B value)	6.0 (1)	16 (1)
Patients showing ≥50% reduction in CA19-9 levels / % (Arm B value)	24 (11)	29 (9)

Moreover, P added that in the patient reported outcome analysis there were no substantial differences in patient quality of life between the three arms, indicating that the increased efficacy of the claimed combination regimen surprisingly does not have a detrimental effect on patients' quality of life. Therefore, P submitted that these data demonstrate that the claimed combination therapy regimen is associated with therapeutic advantages, without having a detrimental effect on patients' quality of life.

Furthermore, P submitted that the improved efficacy of the claimed dosage regimen was also surprisingly associated with a lower frequency of serious treatment emergent adverse events (TEAEs) compared to liposomal irinotecan monotherapy regimen as discussed in D19 and summarized in the table below:

	Arm A (monotherapy)	**Arm B (control)**	**Arm C (combination)**
Frequency of serious TEAEs (%)	61.2	44.8	47.9
Frequency of patients experiencing severe diarrhoea (%)	21	5	13
Frequency of patients experiencing alopecia (%)	22	5	14

5.6.3. Moreover, P pointed to the fact that the skilled person reading D15b was faced with a choice between the monotherapy (Arm A) and the combination therapy (Arm C). At the time of application the skilled person would have been aware that the field of pancreatic cancer treatment was unpredictable and prone to unsuccess and, therefore, he would have adopted a conservative approach. The conservative skilled person would have been dissuaded from the combination regimen in favour of the monotherapy, in view of the fact that liposomal irinotecan monotherapy had ben already successfully tested in a phase 2 clinical trial (see D12), whereas the combination with other drugs such as 5-FU and LV went only through phase 1, and could have caused enhanced side effects and further concerns regarding safety, especially in view of the known adverse reactions associated to irinotecan, 5-FU and LV as indicated in documents D10, D20 and D1a respectively.

Additionally, P argued that none of the prior art documents suggested the efficacy and safety of liposomal irinotecan at a dose of 80 mg/m^2 against GEM-resistant PC, neither in monotherapy nor in a combination treatment with 5-FU and LV. In particular, both D7 and D12 are rather pointing towards the use of a dose of 120 mg/m^2 of liposomal irinotecan in monotherapy; D13 does not provide the doses used in the combination therapy, which in addition is administered in a 3-week cycle; D8 in table 2 discloses a dose of 80 mg/m but does not specify whether said dose was used in the pancreatic cancer patients; D22 does not specifically mention pancreatic cancer, let alone GEM-resistant, and merely discloses that the maximum tolerated dose (MTD) of PEP02 is of 80 mg/m^2 when administered in a combination treatment with 5-FU and LV in a 3-week cycle.

Based on the considerations above, P submitted that the prior art dissuaded from the use of the triple combination, as well as from the dosage of liposomal irinotecan according to the MR. Therefore, the alleged expectation of safe and effective treatment according to T239/16 was taken away by the fact that the skilled person was dissuaded from this by the prior art.

5.6.4. P further argued that the nature of compound MM-398 is not defined in D15b.

P submitted that there are no documents on file identifying MM-398 with "irinotecan sucrose octasulfate salt liposome injection" according to claim 1 of the MR, therefore, the skilled person would not have been incited to use this specific form of liposomal irinotecan.

5.7. The OD came to the following conclusions:

5.7.1. The OD does not share the view of P that the skilled person reading D15b would be faced by the choice between monotherapy and combination therapy. The teaching forming the closest prior art in D15b is not the monotherapy but the combination therapy, i.e. the dosage regimen according to Arm C.

5.7.2. The OD acknowledges that in view of the data provided in D17-D19, the dosage regimen according to the MR results in an improved treatment compared to the monotherapy. However, since the closest prior art D15b is not the monotherapy but the combination treatment according to Arm C, for the assessment of inventive step it appears irrelevant whether the combination provides an improvement over the monotherapy. Moreover, it also appears that an ambitious problem formulated as an improvement over the monotherapy cannot be taken into account, since said effect is not plausibly derivable from the application as filed (GL G-VII, 5.2; T386/89).

Therefore, the problem to be solved cannot be regarded as an improved treatment but merely as the provision of an effective (and safe) treatment.

5.7.3. As indicated by OI, human clinical trials in general and phase III clinical trials in particular are only authorised once it has been shown that the treatment under investigation has a high level of safety and efficacy in earlier preclinical and clinical studies. In line with T239/16 reasons 6.5, the OD considers that the mere fact that the dosage regimen of Arm C was being tested in a clinical study for the treatment of GEM-resistant PC (as disclosed in document D15b) leads to an expectation of success, due to the fact that clinical studies are based on data obtained by preclinical testing both in vitro and in animals and require authority approval which takes ethical considerations into account. This means in the present case that the skilled person would expect the study arm C to treat GEM-resistant PC safely and effectively, unless he was dissuaded from this by the prior art.

The OD does not share the view of P that T239/16 does not apply in the present case because the skilled person would be dissuaded from an expectation of success by the prior art. In fact, none of the documents of the prior art appear to provide a particular disincentive to the use of the triple combination, nor to the dosage 80 mg/m^2 of liposomal irinotecan according to the MR as alleged by P. In fact, it is common practice to lower the dosage of a drug when used in a combination treatment. Therefore, lowering the recommended dose of 120 mg/m^2 liposomal irinotecan monotherapy as in D7 or D12 to 80 mg/m^2 as in the combination treatment of Arm C of D15b, would not take away the expectation of successful treatment using the regimen of Arm C, especially in the presence of a synergistic effect between irinotecan and 5-FU which is clearly suggested by D4 (see e.g. page 4534, right-hand column, paragraph 2). D13 appears to support rather then provide a disincentive to the successful expectation of a combined use of liposomal irinotecan, 5FU and LV. D8 is vague with regard to the doses used on PC patients, thus it is irrelevant with regard to the reasonable expectations of the skilled person. D22 indicates an MTD of 80 mg/m^2 for PEP02 given every 3-weeks, which however is given in combination with 5-FU and LV on day 1 and day 8, i.e. in a dosage regimen different from the one according to the MR, thus this document does not cast a doubt on exceeding toxicity when using the dosage regimen of Arm C. On the other hand, the skilled person also knows from D2-D6 that triple combinations of the same drugs, i.e. irinotecan (although not liposomal), 5-FU and LV, administered in similar dosage regimens, already proved to be promising for the treatment of GEM-resistant PC.

All in all, the prior art does not undermine, but rather support, the presumption of the official authority who authorized the clinical trial of D15c that the treatment according to Arm C would work. This presumption of success is based on careful risk/benefit evaluation by the authority. As recited in reasons 6.6. of T239/16, ethical and economical considerations require that the "benefit" will arise with reasonable certainty and will not only "be hoped for". The set-up of the clinical study of D15b thus inherently creates an expectation of success.

Similar considerations are made in T2506/12 (see reasons 3.10), wherein the Board pointed out that drugs to be used in a clinical trial with human subjects are not selected based on a general "try and see" attitude, but based on existing favourable scientific data, for both ethical and economical reasons. In line with reasons 3.15 of T2506/12, the OD considers that while the outcome of a clinical trial could be success or failure, no particular reason was known which would have discouraged the person skilled in the art from carrying out the therapeutic protocol according to Arm C of D15b, to simply confirm the usefulness of the dosage regimen. Finding out in this straightforward manner that

the disclosed dosage regimen provided indeed both efficacy and safety of treatment in GEM-resistant PC patients according to the purpose of the phase 3 clinical trial, cannot be regarded as inventive.

5.7.4. The fact that the combined treatment may give cumulative side effects, in view of the known side effects associated to each drug of the combination (e.g. see D1a, D10 and D20) does not appear to represent a disincentive either. In fact, the combination of irinotecan, 5-FU and LV is already known from the FOLFIRI regimens, which are disclosed in D2-D6. In particular, D3, D5 and D6 relate to phase 2 clinical studies, for which safety and efficacy evaluations were already made. The skilled person would not expect that the use of liposomal irinotecan (instead of non-liposomal irinotecan used in FOLFIRI) would negatively impact on the safety of very similar combination dosage regimens of the same active compounds.

5.7.5. With regard to the side effects mentioned by P, i.e. nausea, vomiting and diarrhea, the OD considers that safe treatment does not equate to the absence of side effects. The mentioned side effects appear to be common side effects of many, if not all, anticancer treatments, but in a balancing act the benefits arising from the treatment weighs in favour of treatment despite said side effects. Even taking said side effects into account, the skilled person in the art would not have been deterred from, or prejudiced against, applying the dosage regimen according to Arm C of D15b.

5.7.6. The dosage regimen disclosed for Arm C of D15b not only already discloses the dosage of 80 mg/m^2 of liposomal irinotecan according to the MR, but it already discloses also its combination with a dosage of 2400 mg/m^2 of 5-FU and 400 mg/m^2 of LV in a 2-week cycle. The OD notes that D15b does not disclose 200 mg/m^2 of LV in the l form. However, rightly enough none of the parties identified this as a difference. In fact the 400 mg/m^2 of LV in D15b are equivalent to the 200 mg/m^2 of LV in the l form according to the MR. Thus, as the dosages according to the MR are already recited in D15b, the skilled person does not need to find any further motivations or pointers to use said dosages.

5.7.7. With regard to the order of administration specified in claim 1 of the MR, i.e. "the liposomal irinotecan is administered prior to the leucovorin, and the leucovorin is administered prior to the 5-FU", it appears that no particular technical effect is associated to it. Thus, the claimed sequence of administration would be one the skilled person could chose from, among those which were available at the time of application. The OD notices that the very same order (irinotecan - LV - 5-FU) is consistently mentioned in the prior art when the triple combination of the same drugs (only where irinotecan is not in liposomal form) is used in the FOLFIRI treatment (see e.g. D2-D6). Therefore, it would be obvious for the skilled person to follow the same order.

5.7.8. With regard to the identity of compound MM-398, it is true that there are no indications on file that the compound MM-398 used in D15b was irinotecan sucrose octasulfate salt liposome injection. However, even assuming that this is a further difference, this feature does not appear to be associated with any technical effect. In page 8, lines 23-28 of the original application, it is indicated that the claimed irinotecan sucrose octasulfate salt liposome injection was known from US 8,147,867, and therefore, it would just represent an alternative liposomal irinotecan which was available to the skilled person at the time of application.

5.7.9. For these reasons the OD concludes that the subject-matter of claim 1 of the MR does not involve an inventive step and therefore, does not comply with the requirements of Article 56 EPC.

AUXILIARY REQUEST 3 (AR3)

6. AR3 is discussed first here since during OP, P did not request to discuss AR1 and AR2.

7. AR3 differs from the MR in that it requires that "*the patient achieves a response which is at least stable disease*".

8. Rule 80, Article 123 and 83 EPC

8.1. OI raised no objections under Rule 80 and Article 123 EPC with regard to AR3.

8.2. The OD concludes that AR3 complies with the requirements of Rule 80 and Articles 123(2),(3).

8.3. The OD also concludes that AR3 meets the requirements of Article 83 EPC for the same reasons provided above for the MR.

9. Inventive step

The OD is of the opinion that the AR3 does not comply with the requirements of Article 56 EPC.

9.1. D15b is the closest prior art for AR3.

9.2. P submitted that the claim 1 now required the treatment not just to be effective but to reach a particular level of effect, i.e. "at least stable disease".

P argued that, as indicated in paragraphs [0063] of the patent specification and on pages 16-17 of the application as filed, responses to therapy include four different responses, i.e. pathologic complete response (pCR), complete response (CR), partial

response (PR) and stable disease (SD), which would represent the best responses. However, also progressive disease (PD, defined in paragraph [0065]) can be considered as a somehow effective treatment, since in the absence of said treatment the disease might have been progressed at a much higher rate.

Accordingly, the new threshold introduced in claim 1 of the MR may be used to formulate a new objective technical problem, i.e. to provide a safe and effective treatment of GEM-resistant PC wherein the patient achieves a response which is at least stable disease.

P submitted that there were no expectations, neither for the skilled person nor the FDA, deriving from the prior art that the dosage regimen of D15b would have provided at least stable disease.

9.3. OI responded that "progressive disease" could not be considered part of an effective treatment, and that within the meaning of an "effective" treatment the minimum to be expected is "at least stable disease". Moreover, OI argued that the mere exclusion of the failures from claim 1 of AR3 did not allow a reformulation of the problem. OI added that even if the obtention of "at least stable disease" was to be taken into account in the formulation of the problem, this would have been obvious in view of the effects already shown for FOLFIRI, e.g. in D2 (see Table 2) and D3 (see Table 1)

9.4. The OD does not agree with P that progressive disease may be considered as an effective treatment. Clearly the patent specification refers to the Response evaluation criteria in solid tumors (RECIST) as indicated on page 27, line 23-24 of the application as originally filed. RECIST is a set of published rules that define when tumors in cancer patients improve ("respond"), stay the same ("stabilize"), or worsen ("progress") during treatment. According to the RECIST criteria, "progressive disease" is characterized by worsening and clearly cannot be considered as a "somehow" successful treatment as alleged by P. The slower progression referred to by P appears rather to pertain to the group of "partial response". As a consequence, in the framework of an effective treatment, the obtention of stable disease is considered to represent the minimum level of therapeutic effect.

The introduction of the feature "*the patient achieves a response which is at least stable disease*" in claim 1 of AR3 simply excludes the failures from the claim.

However, this new feature does not imply that all patients treated with the regimen according to claim 1 will reach stable disease. It rather excludes the patients with progressive disease from the claim. Still this does not appear to represent an actual distinction from claim 1 of the MR, since the patients treated with the claimed regimen and showing progressive disease would simply not fall under the claim.

Therefore, the new feature cannot serve to reformulate the technical problem. At most, the technical problem to be solved could be re-worded as to provide a safe and effective treatment of GEM-resistant PC, where "at least some patients" reach at least stable disease. However, the expectation to achieve stable disease, at least in some of the patients, already derived from D15b for the same reasons provided above.

Moreover, reasonable expectation to achieve at least stable disease is already provided by D2-D6 for FOLFIRI. In particular Table 2 of D2 and Table 1 of D3 clearly indicates that 28-35% of the patients reach stable disease.

The OD considers that the prior art does not contain any information which would have dissuaded the person skilled in the art from trying the protocol of D15b to confirm the expected effects.

For theses reasons and for the reasons provided in items 5.7 to 5.7.8, the person skilled in the art, starting from D15b, had reasonable expectation to achieve "safe and effective treatment" of GEM-resistant PC, this inherently implying the achievement of "stable disease" in at least some of the patients, when administering said patients the dosage regimen according to Arm C of D15b, following the order of administration generally known for the FOLFIRI combination.

Therefore, Claim 1 of AR3 does not involve an inventive step over the prior art, and does not comply with the requirements of Article 56 EPC.

AUXILIARY REQUEST 1 (AR1)

10. AR1 differs from the MR in that, like AR3, it requires that "*the patient achieves a response which is at least stable disease*". AR1 differs from both the MR and AR3 in that it does not contain the limitation that "*the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*".

11. Rule 80, Article 123, 83 and 56 EPC

11.1. The OD concludes that AR1 complies with the requirements of Rule 80, Articles 123(2),(3), Article 83 EPC for the same reasons provided above for the MR.

11.2. However, AR1 does not comply with the requirements of Article 56 EPC for the same reasons provided above for both MR and AR3.

AUXILIARY REQUEST 2 (AR2)

12. AR2 (former MR filed on 24.08.2018) differs from the MR and AR3, in that it does not requires that "*the patient achieves a response which is at least stable disease*" nor that *"the liposomal irinotecan is irinotecan sucrose octasulfate salt liposome injection*".

13. Rule 80, Article 123, 83 and 56 EPC

13.1. The OD concludes that AR1 complies with the requirements of Rule 80, Articles 123(2),(3), and Article 83 EPC for the same reasons provided above for the MR.

13.2. However, the OD considers that the conclusions on lack of inventive step provided above for both MR and AR3, apply *mutatis mutandis* to AR2.

Decision

Taking account of the amendments made by the patent proprietor during opposition proceedings (i.e. MR, AR1, AR2 and AR3), the European patent EP 2 861 210 is revoked on the ground of Article 100(a) EPC because it does not meet the requirements of Article 52(1) EPC in conjunction with Article 56 EPC (Article 101(3)(b) EPC).

Article 106
Decisions subject to appeal

(1) An appeal shall lie from decisions of the Receiving Section, Examining Divisions, Opposition Divisions and the Legal Division. It shall have suspensive effect.

(2) A decision which does not terminate proceedings as regards one of the parties can only be appealed together with the final decision, unless the decision allows a separate appeal.

(3) The right to file an appeal against decisions relating to the apportionment or fixing of costs in opposition proceedings may be restricted in the Implementing Regulations.

Rule 97
Appeal against apportionment and fixing of costs

(1) The apportionment of costs of opposition proceedings cannot be the sole subject of an appeal.

(2) A decision fixing the amount of costs of opposition proceedings cannot be appealed unless the amount exceeds that of the fee for appeal.

Rule 98
Surrender or lapse of the patent

The decision of an Opposition Division may be appealed even if the European patent has been surrendered in all the designated Contracting States or has lapsed in all those States.

Article 107
Persons entitled to appeal and to be parties to appeal proceedings

Any party to proceedings adversely affected by a decision may appeal. Any other parties to the proceedings shall be parties to the appeal proceedings as of right.

Article 108
Time limit and form

Notice of appeal shall be filed, in accordance with the Implementing Regulations, at the European Patent Office within **two months** of notification of the decision. Notice of appeal shall not be deemed to have been filed until the fee for appeal has been paid. Within **four months** of notification of the decision, a statement setting out the grounds of appeal shall be filed in accordance with the Implementing Regulations.

Further information concerning the filing of an appeal

(a) Notice of appeal can be filed in accordance with Rule 1 and Rule 2(1) EPC, by delivery by hand, by post, or by technical means of communication. The filing has to comply with the details and conditions and, where appropriate, any special formal or technical requirements laid down by the President of the European Patent Office (R. 99(3) EPC).

(b) The addresses of the filing offices of the European Patent Office are as follows:

(i) European Patent Office
D-80298 Munich
Germany

Fax: +49 89 2399-4465

(ii) European Patent Office
Postbus 5818
NL-2280 HV Rijswijk (ZH)
The Netherlands

Fax: +31 70 340-3016

(iii) European Patent Office
D-10958 Berlin
Germany

Fax: +49 30 259 01-840

(c) The notice of appeal must contain the name and address of the appellant in accordance with the provisions of Rule 41(2)(c) EPC, an indication of the decision impugned, and a request defining the subject of the appeal. In the statement of grounds of appeal the appellant shall indicate the reasons for setting aside the decision impugned, or the extent to which it is to be amended, and the facts and evidence on which the appeal is based (R. 99(1) and (2) EPC). The notice of appeal and any subsequent submissions stating the grounds for appeal must be signed (R. 50(3) EPC).

(d) The fee for appeal is laid down in the Rules relating to Fees. The schedule of fees and expenses of the EPO or a reference to the current version is regularly published in the Official Journal of the European Patent Office under the heading "Guidance for the payment of fees, expenses and prices". Fee information is also published on the EPO website under www.epo.org/ fees.

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

FDA News Release

FDA approves new treatment for advanced pancreatic cancer

For Immediate Release

October 22, 2015

Release

The U.S. Food and Drug Administration today approved Onivyde (irinotecan liposome injection), in combination with fluorouracil and leucovorin, to treat patients with advanced (metastatic) pancreatic cancer who have been previously treated with gemcitabine-based chemotherapy.

According to the National Cancer Institute, there will be 48,960 new cases of pancreatic cancer diagnosed in the U.S. in 2015, and nearly the same number of deaths caused by the disease (40,560). Pancreatic cancer can be difficult to diagnose early and treatment options are limited, especially when the disease has spread to other parts of the body (metastatic disease) and surgery to remove the tumor is not possible.

"Many FDA staff who review drug applications are clinicians as well, so it's especially rewarding when we are able to expedite access to new treatments for patients with unmet needs," said Richard Pazdur, M.D., director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research. "By using the Priority Review designation for the application for Onivyde, patients will have earlier access to a drug that helps extend survival."

The FDA granted Priority Review and orphan drug designations for Onivyde. **Priority review (http://www.fda.gov/ForPatients/Approvals/Fast/ucm405405.htm)** status is granted to applications for drugs that, if approved, would be a significant improvement in safety or effectiveness in the treatment of a serious condition. **Orphan drug designation (http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/ucm2005525.htm)** provides incentives such as tax credits, user fee waivers, and eligibility for orphan drug exclusivity to assist and encourage the development of drugs for rare diseases.

The effectiveness of Onivyde was demonstrated in a three-arm, randomized, open label study of 417 patients with metastatic pancreatic adenocarcinoma whose cancer had grown after receiving the chemotherapeutic drug gemcitabine or a gemcitabine-based therapy. The study was designed to determine whether patients receiving Onivyde plus fluorouracil/leucovorin or Onivyde alone lived longer than those receiving fluorouracil/leucovorin. Patients treated with Onivyde plus

fluorouracil/leucovorin lived an average of 6.1 months, compared to 4.2 months for those treated with only fluorouracil/leucovorin. There was no survival improvement for those who received only Onivyde compared to those who received fluorouracil/leucovorin.

In addition, patients receiving Onivyde plus fluorouracil/leucovorin had a delay in the amount of time to tumor growth compared to those who received fluorouracil/leucovorin. The average time for those receiving Onivyde plus fluorouracil/leucovorin was 3.1 months compared to 1.5 months for those receiving fluorouracil/leucovorin.

The safety of Onivyde was evaluated in 398 patients who received either Onivyde with fluorouracil/leucovorin, Onivyde alone or fluorouracil/leucovorin. The most common side effects of treatment with Onivyde included diarrhea, fatigue, vomiting, nausea, decreased appetite, inflammation in the mouth (stomatitis) and fever (pyrexia). Onivyde was also found to result in low counts of infection-fighting cells (lymphopenia and neutropenia). Death due to sepsis following neutropenia has been reported in patients treated with Onivyde.

The labeling for Onivyde includes a boxed warning to alert health care professionals about the risks of severe neutropenia and diarrhea. Onivyde is not approved for use as a single agent for the treatment of patients with metastatic pancreatic cancer.

Onivyde is marketed by Merrimack Pharmaceuticals Inc. of Cambridge, Massachusetts.

The FDA, an agency within the U.S. Department of Health and Human Services, promotes and protects the public health by, among other things, assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

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Phase III Comparison of Two Irinotecan Dosing Regimens in Second-Line Therapy of Metastatic Colorectal Cancer

By Charles S. Fuchs, Melvin R. Moore, Graydon Harker, Luis Villa, David Rinaldi, and J. Randolph Hecht

Purpose: Randomized trials in fluorouracil (FU)-refractory colorectal cancer demonstrate significant survival advantages for patients receiving irinotecan. We prospectively compared the efficacy and tolerability of two irinotecan regimens (once a week for 4 weeks followed by a 2-week rest period [weekly] v once every 3 weeks) in such patients.

Patients and Methods: This multicenter, open-label, phase III study randomly assigned patients in a 1:2 ratio to irinotecan given either weekly (125 mg/m^2) or once every 3 weeks (350 mg/m^2, or 300 mg/m^2 in patients who were ≥ 70 years of age, who had Eastern Cooperative Oncology Group performance status equal to 2, or who had prior pelvic irradiation).

Results: With median follow-up of 15.8 months, there was no significant difference in 1-year survival (46% v 41%, respectively; P = .42), median survival (9.9 v 9.9 months, respectively; P = .43), or median time to progression (4.0 v 3.0 months, respectively; P = .54) between the two regimens. Grade 3/4 diarrhea occurred in 36% of patients treated weekly and in 19% of those treated once every 3 weeks (P = .002). Grade 3/4 neutropenia occurred in 29% of patients treated weekly and 34% of those treated once every 3 weeks (P = .35). Treatment-related mortality occurred in five patients (5.3%) receiving irinotecan weekly and three patients (1.6%) given therapy once every 3 weeks (P = .12). Global quality of life was not statistically different between treatment groups.

Conclusion: Irinotecan schedules of weekly and of once every 3 weeks demonstrated similar efficacy and quality of life in patients with FU-refractory, metastatic colorectal cancer. The regimen of once every 3 weeks was associated with a significantly lower incidence of severe diarrhea.

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THE CAMPTOTHECIN derivative, irinotecan (CPT-11), is a topoisomerase I inhibitor with single-agent activity in advanced colorectal cancer as well as in other solid tumors.[1-3] SN-38, the active metabolite of irinotecan, binds to and stabilizes the topoisomerase I-DNA complex, preventing the religation of DNA during replication and transcription.[4] Subsequent collision between this stable complex and an advancing replication fork results in double-stranded DNA breaks and apoptosis.

In single-agent phase I and II trials, several irinotecan schedules were evaluated. In North America, Rothenberg et al[5] administered the drug as a 90-minute intravenous infusion weekly for 4 consecutive weeks, followed by a 2-week rest period (hereafter known as "weekly"). Diarrhea and neutropenia were the principal toxicities, and the recommended dose for subsequent study was 125 mg/m^2. At the same time, investigators in Europe administered the drug once every 3 weeks with intensive loperamide therapy to ameliorate diarrhea.[6,7] Neutropenia and diarrhea also were the dose-limiting toxicities, and the recommended phase III dose was 350 mg/m^2.

In patients with fluorouracil (FU)-refractory colorectal cancer, phase II trials of both irinotecan schedules demonstrated objective response rates of 12% to 15% and median survivals of 8 to 9 months.[8-10] Once again, diarrhea and neutropenia were the principal adverse events. Subsequently, two phase III trials demonstrated statistically significant survival advantages for patients randomly assigned to irinotecan compared with patients given either best supportive care or second-line infusional FU.[1,3] In both trials, irinotecan was delivered in the every-3-weeks schedule.

Although phase II efficacy and safety results with the weekly and every-3-weeks regimens seemed to be equivalent, no formal comparison of these regimens has been performed. We therefore conducted a randomized phase III trial to compare the efficacy, safety, and effect on patient quality of life of these two schedules of irinotecan.

PATIENTS AND METHODS

Study Design and Patient Selection

This was a multicenter, open-label, randomized study comparing two irinotecan (CPT-11, Camptosar; Pharmacia Corporation, Peapack, NJ) dosing regimens in patients with metastatic colorectal cancer whose disease had recurred or progressed after FU-based therapy. The study was conducted at 29 centers across the United States.

Patients were required to have bidimensionally measurable, histologically proven, metastatic colorectal cancer. Disease progression had to have occurred during or within 6 months after FU-based chemotherapy for metastatic disease or relapse had to have occurred during or within 12 months after adjuvant FU-based chemotherapy. Patients had to be at least 18 years old and ambulatory with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2. The study included only patients with a life expectancy of at least 12 weeks and adequate hematologic reserve and hepatic and renal function, documented by WBC ≥ 3,000/mm^3, absolute neutrophil count ≥ 1,500/mm^3, hemoglobin level ≥ 9.0 g/dL, platelets ≥ 100,000/mm^3, serum bilirubin ≤ 1.5 mg/dL, AST (SGOT) ≤ 3 × upper level of institutional normal (ULIN; ≤ 5 × ULIN if liver metastases were present), and serum creatinine ≤ 2.0 mg/dL.

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Patients who had received prior irinotecan or topotecan were excluded. We also excluded individuals who had a seizure disorder, were receiving antiepileptic prophylaxis, or had a history of Gilbert's syndrome. Also, patients with metastatic involvement of the CNS, a psychiatric disorder that could interfere with treatment compliance, significant concurrent infection, or significant cardiac disease were excluded.

This study was performed in accordance with precepts established by the Helsinki Declaration, and the protocol was approved by the institutional review board of each study center. All patients gave written informed consent to participate.

Stratification, Random Assignment, and Treatment

Patients were prospectively stratified by age (< 70 years *v* ≥ 70 years), PS (0 *v* 1 or 2), history of pelvic irradiation (yes *v* no), intent of prior FU treatment (adjuvant *v* metastatic disease *v* both), and bilirubin concentration (< 1.0 mg/dL *v* ≥ 1.0 mg/dL). Within each stratification subpopulation, patients were electronically randomly assigned to either of two treatment schedules:[1] weekly irinotecan, 125 mg/m^2/wk for 4 consecutive weeks followed by a 2-week rest period, or every-3-weeks irinotecan, 350 mg/m^2 once every 3 weeks.[2] Within the every-3-weeks arm, compromised patients, defined as being aged ≥ 70 years, having ECOG PS of 2, or having had prior pelvic irradiation, received 300 mg/m^2 irinotecan on the basis of earlier studies of the every-3-weeks regimen.[1,3] Because the every-3-weeks schedule had been studied principally in Europe, random assignment was imbalanced 2:1 in favor of that regimen to increase US investigators' experience with it. In both arms of the study, irinotecan was diluted in 500 mL of 5% dextrose and infused intravenously over 90 minutes.

Patients were treated with standard regimens of antiemetics, atropine, and intensive loperamide; however, prophylactic use of atropine was not allowed for the first study infusion. Irinotecan dosage was modified based on the intensity of adverse events that occurred in the preceding course, according to product labeling.

Treatment continued until the scheduled evaluation at 12 months, disease progression, unacceptable toxicity, patient refusal, or death. In responding patients or those with stable disease, treatment could be extended beyond 12 months at the investigator's discretion.

Efficacy and Tolerability Assessments

During the first year of the study, disease was evaluated every 6 weeks through week 18 and then every 12 weeks until progression. Disease progression was defined as an increase of at least 25% in the overall tumor area or appearance of new lesions. Because the primary end point was survival, evaluation of response and progression was not reviewed by a committee of independent experts. Safety assessments and complete blood counts were performed weekly. All adverse events were reported according to the National Cancer Institute Common Toxicity Criteria.[11]

Quality of Life

Quality of life was assessed with the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-C30 (QLQ-C30) instrument (version 2.0), which includes nine symptom scales or items, five function scales, and one Global Health Status/Quality-of-Life scale.[12] Patients completed this instrument at baseline, every 6 weeks during therapy, and at treatment discontinuation.

Study End Points and Statistical Analysis

The primary end point was 1-year survival. Secondary end points included overall survival, time to progression, safety, and quality of life. Time to progression was defined as the time from random assignment to tumor progression. Overall survival was calculated from time of random assignment to date of death from any cause. If a patient had not died, duration of survival was censored on the last date of follow-up.

Survival curves for the randomly assigned population were estimated with the Kaplan-Meier[13] method and compared by a two-tailed log-rank test. With a two-sided alpha of 0.05, a power of 0.80, and a dropout rate of ≤ 5%, 300 patients were needed (100 assigned to weekly therapy and 200 to every-3-weeks therapy) to detect a 15% difference in 1-year survival.

Analysis of all efficacy and quality-of-life end points was by intention to treat. To examine the joint influence of stratification factors and other baseline characteristics on patient outcomes, we conducted Cox regression models or stratified log-rank tests.[14] Variables that were considered included PS (0 or 1 *v* 2), age (< 70 years *v* ≥ 70 years), prior pelvic irradiation (yes *v* no), prior adjuvant FU (adjuvant and metastatic *v* adjuvant only), prior FU for metastatic disease (metastatic and adjuvant *v* metastatic only), number of organs involved (1 *v* > 1), site of metastatic disease (liver and lung *v* liver only, liver and lung *v* lung only), and baseline bilirubin (< 1 mg/dL *v* ≥ 1 mg/dL), WBC (≤ 8 × 10^3/mm^3 *v* > 8 × 10^3/mm^3), hemoglobin (< 11 g/dL *v* ≥ 11 g/dL), or carcinoembryonic antigen (≤ 10 ng/mL *v* > 10 ng/mL). Model selection for identifying variables having an effect on survival was made based on a forward stepwise procedure. *P* values were .05 to enter and .06 to remove. After the prognostic model had been determined, the effect of treatment after adjustment for other prognostic factors was estimated by including treatment in the model.

Dose-intensity was defined as the amount of irinotecan delivered per unit of time (expressed in mg/m^2/wk) over the entire period of therapy for each patient. Dose intensity was calculated as the ratio of the total dose (expressed in mg/m^2) actually received by the patient divided by the actual total treatment duration expressed in weeks. The relative dose intensity was calculated as the ratio of the actual delivered dose intensity to the dose intensity planned by the protocol.

The QLQ-C30 was analyzed with the Global Health Status/Quality of Life subscale as the primary end point and the other 14 scales as secondary end points. Quality-of-life variables were compared by univariate and multivariate analyses of variance of values at baseline and during study, of score changes from baseline, and of worst postbaseline scores. Percentages were rounded to the nearest whole number and, as a result, may not add up to 100% in some instances (Table 1).

RESULTS

Patients and Treatment

A total of 291 patients were enrolled between August 1998 and January 2001. Ninety-five of these patients were randomly assigned to the weekly schedule, and 196 patients to the every-3-weeks schedule. Although efficacy analyses were conducted on an intent-to-treat basis, a total of seven patients, one on the weekly schedule and six on the every-3-weeks regimen, received no irinotecan and were excluded from the tolerability analysis. Individuals did not receive irinotecan because of withdrawal of consent in three patients, discovery of study ineligibility in two patients, withdrawal of an investigator from the study in one patient, and election of surgery instead of chemotherapy in one patient. Among patients in the every-3-weeks group who received treatment (n = 190), 105 patients (55%) were defined by the protocol as compromised (age ≥ 70 years, ECOG PS of 2, or prior pelvic irradiation) and began irinotecan at 300 mg/m^2. For the overall study population, median follow-up was 15.8 months. Last survival data were accrued in January 2002.

No significant differences in baseline clinical characteristics existed between patients on weekly therapy and those assigned to every-3-weeks treatment (Table 1). The every-3-weeks group had a somewhat higher percentage of patients with an ECOG PS of 2, but this difference was not statistically significant (*P* = .10). Among the entire study population, 34% of patients were aged 70 years or older, and 26% had received prior pelvic irradiation. The most frequent site of metastasis was the liver, followed by the lung.

Median duration of treatment was 2.8 months (range, 1.4 to 12.5 months) for patients receiving weekly therapy compared

Table 1. Pretreatment Patient Characteristics

Characteristic	Weekly Irinotecan (n = 95) %	Weekly Irinotecan (n = 95) No.	Every-3-Weeks Irinotecan (n = 196) %	Every-3-Weeks Irinotecan (n = 196) No.
Gender				
Male	62	59	58	114
Female	38	36	42	82
Age				
< 70 yrs	66	63	66	130
≥ 70 yrs	34	32	34	66
Ethnicity				
Asian/Pacific Islander	4	4	3	5
African-American	12	11	21	42
Hispanic	11	10	10	19
White	72	68	66	129
Other	2	2	1	1
ECOG PS				
0	48	46	43	85
1	46	44	45	89
2	5	5	11	22
Primary tumor site				
Colon	78	74	76	149
Rectum	22	21	24	47
Number of involved organs				
1	56	53	45	88
2	33	31	40	79
≥ 3	11	10	14	27
Sites of metastases				
Liver +/− any other site	73	69	70	138
Liver alone	38	36	29	57
Liver + any other site	35	33	41	81
Lung +/− any other site	40	38	39	77
Previous treatment				
Surgery	92	87	91	178
Pelvic irradiation	24	23	28	54
Intent of previous FU				
Adjuvant	24	23	24	47
For metastatic disease	62	59	56	109
Both purposes	13	12	18	36
Unknown	1	1	2	4
Best response to previous FU				
Complete + partial regression	6	6	11	22
Stabilization	22	21	28	55
Progression	42	40	32	63
Unknown	30	28	29	56
Tumor progression in relation to previous FU				
During FU	16	15	19	37
< 3 mos since last FU	58	55	56	110
≥ 3 mos since last FU	22	21	21	40
Unknown	4	4	5	9
Biochemical values				
Bilirubin				
Median (range), mg/dL	0.6 (0.1 to 1.5)		0.5 (0.1 to 1.7)	
Patients ≥ 1 mg/dL	14	13	12	24
CEA				
Median (range), μg/L	61.5 (3.1 to 4,720.0)		56.5 (0.4 to 7,552.5)	
Patients > 10 μg/L	85	81	76	149
Hemoglobin				
Median (range), mg/dL	12.5 (9.6 to 16.2)		12.3 (8.9 to 17.1)	
Patients < 11 mg/dL	18	17	23	45
WBC				
Median (range), × 10^3/mm^3	6.8 (3.3 to 18.3)		7.1 (2.9 to 23.4)	
Patients > 8 × 10^3/mm^3	27	26	35	69

NOTE: Percentages are rounded and may not add up to 100%.

Abbreviations: CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; FU, fluorouracil; PS, performance status.

with 2.8 months (range, 0.7 to 14.4 months) for those given every-3-weeks treatment ($P = .75$). The most frequent reasons for treatment discontinuation were progressive disease (67% in the weekly group *v* 70% in the every-3-weeks group), adverse reactions (15% in the weekly group *v* 9% in the every-3-weeks group), and treatment refusal (10% in the weekly group *v* 6% in the every-3-weeks group); no significant differences existed between the study groups in the rate of discontinuation for any of these reasons.

The median irinotecan dose intensity administered was 63 mg/m^2/wk for the weekly group and 100 mg/m^2/wk for the every-3-weeks group ($P < .0001$). Among the every-3-weeks group, median dose intensity was 96 mg/m^2/wk for the subgroup that started on 300 mg/m^2 and 114 mg/m^2/wk for the subgroup that started on 350 mg/m^2 ($P < .0001$). Median relative irinotecan dose intensities administered were 75% of the dose planned for the weekly arm and 97% of the dose planned for the every-3-weeks arm ($P < .0001$).

Efficacy

One-year survival did not differ significantly between treatment groups: 46% (95% confidence interval [CI], 36% to 56%) for the weekly group versus 41% (95% CI, 39% to 53%) for the every-3-weeks group ($P = .42$). Median overall survival also did not differ between the treatment groups: 9.9 months (95% CI, 8.0 to 13.0 months) for weekly therapy versus 9.9 months (95% CI, 8.3 to 11.6 months) for the every-3-weeks therapy (*P*, log-rank = 0.43; Fig 1).

Time to progression was similar between treatment arms (*P*, log-rank = 0.54). Median time to progression was 4.0 months (95% CI, 2.6 to 5.0 months) for patients who received weekly irinotecan and 3.0 months (95% CI, 2.7 to 4.0 months) for patients who received every-3-weeks therapy (Fig 2).

For both overall survival and time to progression, we used stepwise Cox multivariate analysis to examine the joint influence of stratification factors and other predefined baseline clinical characteristics (Table 2). Factors adversely influencing survival were ECOG PS of 2, baseline WBC more than 8 × 10^3/mm^3, baseline hemoglobin less than 11 g/dL, and metastatic involvement of more than one organ. When the irinotecan schedule was added to these variables in the model, the effect of the irinotecan regimen remained nonsignificant ($P = .93$). Factors adversely influencing time to progression were ECOG PS of 2, baseline hemoglobin less than 11 g/dL, and metastatic involvement of the lung plus other organs. When the treatment group was added to these variables in the model of time to progression, the effect of the irinotecan regimen remained nonsignificant ($P = .70$).

Among patients randomly assigned to every-3-weeks irinotecan, individuals who were 70 years or older, had an ECOG PS of 2, or had had prior pelvic irradiation received a lower starting dose (300 mg/m^2), as defined by the protocol. We repeated our analyses after excluding patients who started at 300 mg/m^2. Nonetheless, there remained no significant difference in either overall survival ($P = .44$) or time to progression ($P = .37$) between the weekly (125 mg/m^2) and every-3-weeks (350 mg/m^2) schedules.

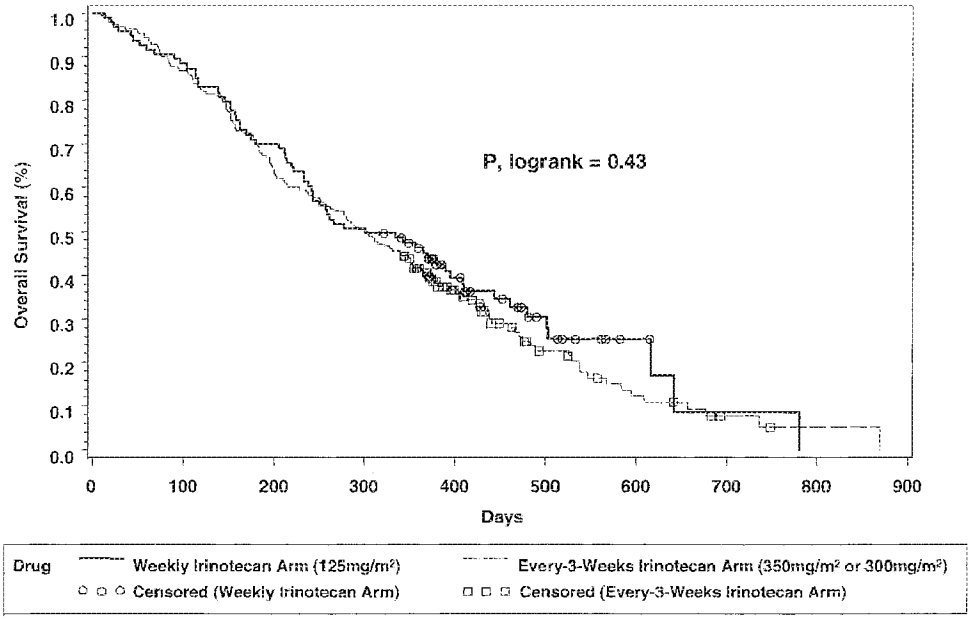

Fig 1. Probability of survival by time in patients on weekly versus every-3-weeks irinotecan. After median survival, only the censoring points are shown.

Tolerability

All patients who received at least one dose of irinotecan (n = 284 for the entire study; 94 for the weekly arm, 190 for the every-3-weeks arm) were included in the safety analysis. Over the course of therapy, the incidence of any grade 3/4 toxicity was similar between the two groups: 65% in patients given weekly treatment and 63% in those receiving every-3-weeks treatment ($P = .77$; Table 3). Severe (grade 3/4) diarrhea was significantly more common among patients given weekly therapy compared with those given irinotecan every 3 weeks (36% *v* 19%, respectively; $P = .002$). In contrast, the incidence of any cholinergic symptom during the first treatment infusion was significantly lower in the weekly group than in the every-3-weeks group (31% *v* 61%, respectively; $P < .0001$).

No other chemotherapy-related toxicity differed significantly between the treatment groups. Grade 3/4 neutropenia and grade 3/4 nausea were somewhat more common in the every-3-weeks group than in the weekly arm, although these differences did not reach statistical significance.

We conducted a stepwise logistic regression analysis to examine the joint influence of stratification factors and other predefined baseline clinical characteristics on the risks of grade 3/4 neutropenia or of grade 3/4 diarrhea (Table 4). Age 70 years or older and baseline bilirubin ≥ 1 mg/dL independently predicted occurrence of grade 3/4 neutropenia, but irinotecan schedule did not. Age 70 years or older also independently predicted occurrence of grade 3/4 diarrhea; however, treatment with the every-3-weeks schedule of iri-

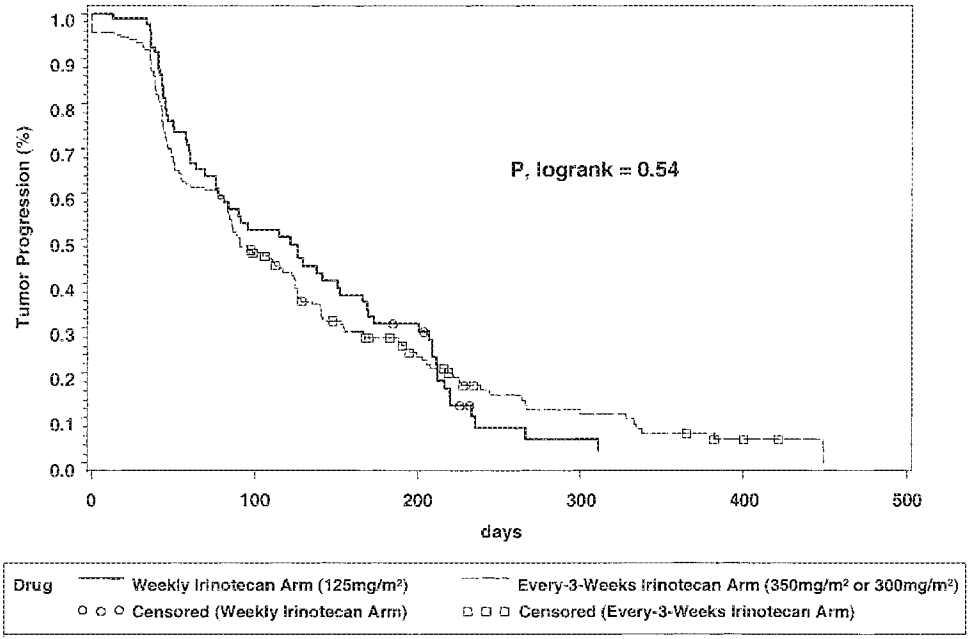

Fig 2. Time to progression in patients on weekly versus every-3-weeks irinotecan. After median time to progression, only the censoring points are shown.

Table 2. Multivariate Predictors of Overall Mortality and Tumor Progression

Characteristic	Overall Mortality		Tumor Progression	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
CEA ≤ 10 μg/L	1.0			
CEA > 10 μg/L	1.40 (0.97 to 2.00)	.07	—	NS
Hemoglobin ≥ 11 mg/dL	1.0		1.0	
Hemoglobin < 11 mg/dL	2.03 (1.48 to 2.78)	.00001	1.61 (1.14 to 2.25)	.006
ECOG PS < 2	1.0		1.0	
ECOG PS = 2	2.14 (1.35 to 3.39)	.001	1.67 (0.99 to 2.82)	.06
WBC ≤ $8 \times 10^3/mm^3$	1.0		—	NS
WBC > $8 \times 10^3/mm^3$	2.04 (1.53 to 2.73)	< .00001		
Metastases to one organ	1.0		—	NS
Metastases to > one organ	1.51 (1.14 to 1.99)	.004		
Metastases to lung only	—	NS	1.0	.03
Metastases to liver or liver plus lung			1.57 (1.04 to 2.37)	
Weekly irinotecan	1.0		1.0	
Every-3-weeks irinotecan	0.99 (0.73 to 1.33)	.93	1.06 (0.78 to 1.44)	.70

NOTE: All patient characteristics represent assessments at baseline.

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; NS, not statistically significant in the model; CEA, carcinoembryonic antigen.

notecan was associated with a significantly lower rate of grade 3/4 diarrhea ($P = .002$).

Rates of any hospitalization during therapy were similar for both treatment groups (26% for weekly *v* 30% for every 3 weeks; $P = .55$). Median duration of hospitalization also was similar between the two regimens (11 *v* 9 days, respectively; $P = .92$).

During treatment, five patients (5.3%) receiving weekly and three patients (1.6%) given every-3-weeks therapy died of causes considered by the investigator to be possibly related to the study drug ($P = .12$). Of the five deaths on the weekly arm, three were attributable to a combination of diarrhea, neutropenia, and probable sepsis; one to aspiration pneumonia; and one to unknown causes. Of the three deaths on the every-3-weeks arm, two were attributable to a combination of diarrhea, neutropenia, and probable sepsis, and one was classified as sudden death.

A significantly higher proportion of patients on the weekly schedule (61%) than on the every-3-weeks schedule (41%) required dose reduction ($P < .0001$). We examined the proportion of patients who received full doses of irinotecan during the first 4 weeks of therapy (Fig 3). During the first treatment cycle, full-dose irinotecan was received by 51% of patients in the weekly treatment arm at week 3 and 35% at week 4. In contrast, 69% of patients on the every-3-weeks schedule received a full dose at week 4. This difference in the rates of full-dose irinotecan administration between weekly and every-3-weeks therapy at week 4 was statistically significant ($P < .0001$).

Quality of Life

The global rates of compliance with the quality-of-life questionnaires were similar in the two treatment arms: 81% in the

Table 3. Frequency of Selected Adverse Events Attributable to Treatment

Adverse Event, % (n) of patients	Weekly Irinotecan (n = 94)				Every-3-Weeks Irinotecan (n = 190)				P, Grade 3/4
	Any Grade		Grade 3/4		Any Grade		Grade 3/4		
	%	No.	%	No.	%	No.	%	No.	
Any cholinergic symptom*	31	29	NA		61	115	NA		.000002†
Fatigue‡	43	40	12	11	43	81	11	21	.87
Anorexia	17	16	1	1	13	24	3	6	.43
Nausea	54	51	5	5	55	105	11	20	.15
Vomiting	40	38	6	6	42	79	13	24	.11
Diarrhea	82	77	36	34	76	144	19	36	.002
Dehydration	20	19	14	13	20	38	12	22	.59
Constipation	4	4	1	1	5	9	0	0	.33
Anemia	17	16	1	1	18	34	4	7	.28
Thrombocytopenia	3	3	0	0	4	7	1	1	.99
Neutropenia	43	40	29	27	42	79	34	65	.35
Fever§ or infection with grade 3 or 4 neutropenia	4	4	3	3	7	14	2	4	.69
Any toxicity	97	91	65	61	97	184	63	120	.77

Abbreviation: NA, not available or applicable.

* Cholinergic symptoms are defined as one or more of the following symptoms occurring during or within 24 hours after the first dose of irinotecan: lacrimation, diaphoresis, flushing, rhinorrhea, bradycardia, and abdominal cramping.

† *P* value is for the difference between frequency of any cholinergic symptom.

‡ Fatigue includes malaise, asthenia, and lethargy.

§ Fever grade corresponds to column heading, even though neutropenia grade is 3 or 4.

Table 4. Multivariate Predictors of Treatment-Related Adverse Events

Characteristic	Grade 3/4 Diarrhea		Grade 3/4 Neutropenia	
	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
Age < 70 years	1.0		1.0	
Age ≥ 70 years	1.81 (1.02 to 3.21)	.04	2.06 (1.21 to 3.51)	.008
Bilirubin < 1 mg/dL			1.0	
Bilirubin ≥ 1 mg/dL	—	NS	2.46 (1.20 to 5.04)	.01
Weekly irinotecan	1.0		1.0	
Every-3-weeks irinotecan	0.40 (0.23 to 0.71)	.002	1.36 (0.79 to 2.37)	.27

NOTE: All patient characteristics represent assessments at baseline.
Abbreviations: CI, confidence interval; NS, not statistically significant in the model.

weekly group and 86% in the every-3-weeks group ($P = .11$). Analyses of mean changes from baseline in the scores on the Global Health Status/Quality of Life subscale of the quality-of-life questionnaire demonstrated no significant differences between patients given irinotecan in the weekly or every-3-weeks schedules (Fig 4). Univariate and multivariate analyses of variance revealed no significant difference between the dosage arms in quality-of-life scores at baseline or during the study, or in worst postbaseline scores, in any of the EORTC QLQ-C30 symptoms, or in item or function scales.

DISCUSSION

This prospective, randomized, multicenter trial provides comparative data on the efficacy, tolerability, and effect on patient quality of life between the weekly versus the every-3-weeks schedules of irinotecan in 291 patients with FU-refractory colorectal cancer. The study found no significant differences in efficacy between the schedules in this population. Both schedules offered similar survival and time to tumor progression. The study was statistically powered to detect a 15% difference in 1-year survival; a smaller difference is unlikely to be clinically meaningful in the second-line treatment of colorectal cancer.

Toxicity patterns of the two schedules were somewhat distinctive. Severe (grade 3/4) diarrhea was significantly more common with the weekly schedule than with the every-3-weeks regimen. In contrast, grade 3/4 neutropenia was slightly more frequent in the every-3-weeks group, although this difference was not statistically significant. However, the rates of fever with neutropenia were virtually identical between the two treatment schedules, and the rates of hospitalization did not differ between the groups.

Acute cholinergic symptoms during the first dose of irinotecan were significantly more common with the every-3-weeks schedule, presumably as a result of the higher dose of drug administered. Nonetheless, this toxicity can be abrogated by the administration of prophylactic atropine, which was not permitted during the first dose in this study. Severe (grade 3/4) nausea was relatively uncommon in our patients, although the rate of severe nausea was marginally higher with the every-3-weeks regimen. Despite the apparent disparities in toxicity, quality-of-life scores did not differ significantly between the two irinotecan dosage groups.

A higher treatment-related mortality rate was noted among patients receiving weekly irinotecan (5.3%) than among those given the every-3-weeks schedule (1.6%), although this difference was not statistically significant. In the weekly group, at least three of five treatment-related deaths were caused by diarrhea and dehydration. Although the event rate in the weekly irinotecan group appears high, early experience with the weekly (Roswell Park) schedule of FU and high-dose leucovorin indicated a 6.4% mortality rate attributable to severe diarrhea and dehydration.[15] Clearly, patients treated with weekly irinotecan

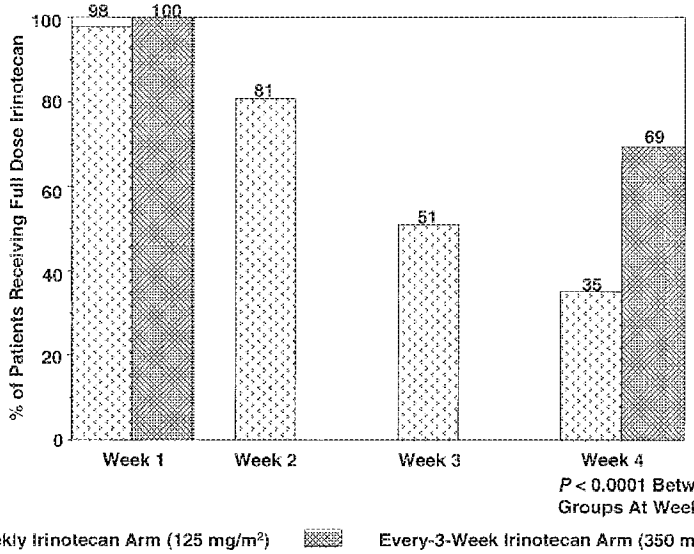

Fig 3. Percentage of patients on full dose of irinotecan at selected time points.

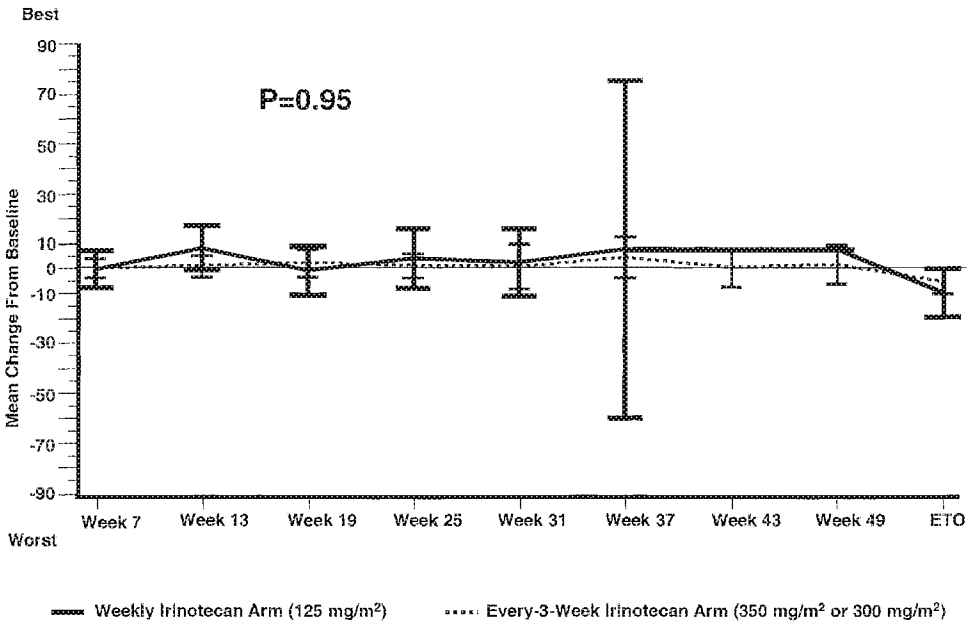

Fig 4. Global Health Status/Quality of Life scores: mean changes from baseline in patients on weekly versus every-3-weeks irinotecan.

require close monitoring, rapid institution of antidiarrheal therapy, and administration of parenteral fluids should significant diarrhea persist.

Patients enrolled onto this study appear to be representative of the general population of patients with colorectal cancer who receive second-line irinotecan.[1,3,5,9,10] The median survival (~10 months) in this study appears comparable to the findings in other phase II and III trials of irinotecan in similar populations.[1,3,5,9,10] In our trial, baseline clinical characteristics of the patients assigned to the two treatment groups were similar except for a slightly greater proportion of patients with an ECOG PS of 2 in the every-3-weeks group. However, this difference did not appear to influence the results; when PS was included in multivariate analysis, there continued to be no significant difference in survival or time to progression between the two treatment schedules.

During the conduct of this study, two randomized trials reported that the combination of irinotecan with FU and leucovorin prolonged survival in patients with previously untreated metastatic colorectal cancer.[16,17] The schedules used in those trials differ considerably; whereas Saltz et al[17] delivered bolus FU, leucovorin, and irinotecan weekly for 4 weeks every 6 weeks, Douillard et al[16] principally delivered higher doses of irinotecan with infusional FU and leucovorin every 2 weeks. Of note, the rate of grade 3/4 diarrhea was 23% for the weekly three-drug combination compared with 13% for the every-2-weeks schedule of Douillard et al. Although no formal comparison of the weekly and every 2-weeks schedules of irinotecan, FU, and leucovorin has been reported, these findings are consistent with the higher rate of grade 3/4 diarrhea observed with weekly irinotecan alone in our trial. Such observations could suggest that regimens using less frequent irinotecan dosing may be associated with less severe treatment-related diarrhea. As an additional consideration, future studies may examine whether weekly schedules that deliver irinotecan for 2 consecutive weeks followed by a 1-week rest, rather than for 4 consecutive weeks followed by a 2-week rest, could offer a means for improving patient tolerability.

ACKNOWLEDGMENT

We acknowledge the contributions of the patients, our fellow investigators, the nurses and other caregivers in this study, and James P. McGovren, PhD, Ambrose Kwok, MSc, and Robert Marlowe, BA.

APPENDIX

The appendix is available online at www.jco.org.

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use GEMZAR safely and effectively. See full prescribing information for GEMZAR.

GEMZAR (gemcitabine for injection) Powder, Lyophilized, For Solution For Intravenous Use

Initial U.S. Approval: 1996

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

Gemzar® is a nucleoside metabolic inhibitor indicated for:

- Ovarian cancer in combination with carboplatin (1.1)
- Breast cancer in combination with paclitaxel (1.2)
- Non-small cell lung cancer in combination with cisplatin (1.3)
- Pancreatic cancer as a single-agent (1.4)

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

Gemzar is for intravenous use only.

- Ovarian cancer: 1000 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.1)
- Breast cancer: 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.2)
- Non-small cell lung cancer: 4-week schedule, 1000 mg/m^2 over 30 minutes on Days 1, 8, and 15 of each 28-day cycle: 3-week schedule; 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.3)
- Pancreatic cancer: 1000 mg/m^2 over 30 minutes once weekly for up to 7 weeks (or until toxicity necessitates reducing or holding a dose), followed by a week of rest from treatment. Subsequent cycles should consist of infusions once weekly for 3 consecutive weeks out of every 4 weeks (2.4)
- Dose Reductions or discontinuation may be needed based on toxicities (2.1-2.4)

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

- 200 mg vial for injection (3)
- 1 g vial for injection (3)

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

Patients with a known hypersensitivity to gemcitabine (4)

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

- Infusion time and dose frequency: Increased toxicity with infusion time >60 minutes or dosing more frequently than once weekly. (5.1)
- Hematology: Monitor for myelosuppression, which can be dose-limiting. (5.2, 5.7)
- Pulmonary toxicity: Discontinue Gemzar immediately for severe pulmonary toxicity. (5.3)
- Renal: Monitor renal function prior to initiation of therapy and periodically thereafter. Use with caution in patients with renal impairment. Cases of hemolytic uremic syndrome (HUS) and/or renal failure, some fatal, have occurred. Discontinue Gemzar for HUS or severe renal toxicity. (5.4)
- Hepatic: Monitor hepatic function prior to initiation of therapy and periodically thereafter. Use with caution in patients with hepatic impairment. Serious hepatotoxicity, including liver failure and death, have occurred. Discontinue Gemzar for severe hepatic toxicity. (5.5)
- Pregnancy: Can cause fetal harm. Advise women of potential risk to the fetus. (5.6, 8.1)
- Radiation toxicity. May cause severe and life-threatening toxicity. (5.8)

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

The most common adverse reactions for the single-agent (≥20%) are nausea and vomiting, anemia, ALT, AST, neutropenia, leukopenia, alkaline phosphatase, proteinuria, fever, hematuria, rash, thrombocytopenia, dyspnea (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Eli Lilly and Company at 1-800-LillyRx (1-800-545 5979) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 00/0000

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Ovarian Cancer

Gemzar in combination with carboplatin is indicated for the treatment of patients with advanced ovarian cancer that has relapsed at least 6 months after completion of platinum-based therapy.

1.2 Breast Cancer

Gemzar in combination with paclitaxel is indicated for the first-line treatment of patients with metastatic breast cancer after failure of prior anthracycline-containing adjuvant chemotherapy, unless anthracyclines were clinically contraindicated.

1.3 Non-Small Cell Lung Cancer

Gemzar is indicated in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (Stage IIIA or IIIB), or metastatic (Stage IV) non-small cell lung cancer.

1.4 Pancreatic Cancer

Gemzar is indicated as first-line treatment for patients with locally advanced (nonresectable Stage II or Stage III) or metastatic (Stage IV) adenocarcinoma of the pancreas. Gemzar is indicated for patients previously treated with 5-FU.

2 DOSAGE AND ADMINISTRATION

Gemzar is for intravenous use only. Gemzar may be administered on an outpatient basis.

2.1 Ovarian Cancer

Gemzar should be administered intravenously at a dose of 1000 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle. Carboplatin AUC 4 should be administered intravenously on Day 1 after Gemzar administration. Patients should be monitored prior to each dose with a complete blood count, including differential counts. Patients should have an absolute granulocyte count $\geq$1500 x 10^6/L and a platelet count $\geq$100,000 x 10^6/L prior to each cycle.

Dose Modifications

Gemzar dosage adjustment for hematological toxicity within a cycle of treatment is based on the granulocyte and platelet counts taken on Day 8 of therapy. If marrow suppression is detected, Gemzar dosage should be modified according to guidelines in Table 1.

Table 1: Day 8 Dosage Reduction Guidelines for Gemzar in Combination with Carboplatin

Absolute granulocyte count (x 10^6/L)		Platelet count (x 10^6/L)	% of full dose
$\geq$1500	And	$\geq$100,000	100
1000-1499	and/or	75,000-99,999	50
<1000	and/or	<75,000	Hold

In general, for severe (Grade 3 or 4) non-hematological toxicity, except nausea/vomiting, therapy with Gemzar should be held or decreased by 50% depending on the judgment of the treating physician. For carboplatin dosage adjustment, see manufacturer's prescribing information.

Dose adjustment for Gemzar in combination with carboplatin for subsequent cycles is based upon observed toxicity. The dose of Gemzar in subsequent cycles should be reduced to 800 mg/m^2 on Days 1 and 8 in case of any of the following hematologic toxicities:

- Absolute granulocyte count <500 x 10^6/L for more than 5 days
- Absolute granulocyte count <100 x 10^6/L for more than 3 days
- Febrile neutropenia
- Platelets <25,000 x 10^6/L
- Cycle delay of more than one week due to toxicity

If any of the above toxicities recur after the initial dose reduction, for the subsequent cycle, Gemzar should be given on Day 1 only at 800 mg/m^2.

2.2 Breast Cancer

Gemzar should be administered intravenously at a dose of 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle. Paclitaxel should be administered at 175 mg/m^2 on Day 1 as a 3-hour intravenous infusion before Gemzar administration. Patients should be monitored prior to each dose with a complete blood count, including differential counts. Patients should have an absolute granulocyte count $\geq$1500 x 10^6/L and a platelet count $\geq$100,000 x 10^6/L prior to each cycle.

Dose Modifications

Gemzar dosage adjustment for hematological toxicity is based on the granulocyte and platelet counts taken on Day 8 of therapy. If marrow suppression is detected, Gemzar dosage should be modified according to the guidelines in Table 2.

Table 2: Day 8 Dosage Reduction Guidelines for Gemzar in Combination with Paclitaxel

Absolute granulocyte count ($\times 10^6$/L)		Platelet count ($\times 10^6$/L)	% of full dose
≥1200	And	>75,000	100
1000-1199	Or	50,000-75,000	75
700-999	And	≥50,000	50
<700	Or	<50,000	Hold

In general, for severe (Grade 3 or 4) non-hematological toxicity, except alopecia and nausea/vomiting, therapy with Gemzar should be held or decreased by 50% depending on the judgment of the treating physician. For paclitaxel dosage adjustment, see manufacturer's prescribing information.

2.3 Non-Small Cell Lung Cancer

Two schedules have been investigated and the optimum schedule has not been determined *[see Clinical Studies (14.3)]*. With the 4-week schedule, Gemzar should be administered intravenously at 1000 mg/m^2 over 30 minutes on Days 1, 8, and 15 of each 28-day cycle. Cisplatin should be administered intravenously at 100 mg/m^2 on Day 1 after the infusion of Gemzar. With the 3-week schedule, Gemzar should be administered intravenously at 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle. Cisplatin at a dose of 100 mg/m^2 should be administered intravenously after the infusion of Gemzar on Day 1. See prescribing information for cisplatin administration and hydration guidelines.

Dose Modifications

Dosage adjustments for hematologic toxicity may be required for Gemzar and for cisplatin. Gemzar dosage adjustment for hematological toxicity is based on the granulocyte and platelet counts taken on the day of therapy. Patients receiving Gemzar should be monitored prior to each dose with a complete blood count (CBC), including differential and platelet counts. If marrow suppression is detected, therapy should be modified or suspended according to the guidelines in Table 3. For cisplatin dosage adjustment, see manufacturer's prescribing information.

In general, for severe (Grade 3 or 4) non-hematological toxicity, except alopecia and nausea/vomiting, therapy with Gemzar plus cisplatin should be held or decreased by 50% depending on the judgment of the treating physician. During combination therapy with cisplatin, serum creatinine, serum potassium, serum calcium, and serum magnesium should be carefully monitored (Grade 3/4 serum creatinine toxicity for Gemzar plus cisplatin was 5% versus 2% for cisplatin alone).

2.4 Pancreatic Cancer

Gemzar should be administered by intravenous infusion at a dose of 1000 mg/m^2 over 30 minutes once weekly for up to 7 weeks (or until toxicity necessitates reducing or holding a dose), followed by a week of rest from treatment. Subsequent cycles should consist of infusions once weekly for 3 consecutive weeks out of every 4 weeks.

Dose Modifications

Dosage adjustment is based upon the degree of hematologic toxicity experienced by the patient *[see Warnings and Precautions (5.2)]*. Clearance in women and the elderly is reduced and women were somewhat less able to progress to subsequent cycles *[see Warnings and Precautions (5.2) and Clinical Pharmacology (12.3)]*.

Patients receiving Gemzar should be monitored prior to each dose with a complete blood count (CBC), including differential and platelet count. If marrow suppression is detected, therapy should be modified or suspended according to the guidelines in Table 3.

Table 3: Dosage Reduction Guidelines

Absolute granulocyte count ($\times 10^6$/L)		Platelet count ($\times 10^6$/L)	% of full dose
≥1000	And	≥100,000	100
500-999	Or	50,000-99,999	75
<500	Or	<50,000	Hold

Laboratory evaluation of renal and hepatic function, including transaminases and serum creatinine, should be performed prior to initiation of therapy and periodically thereafter. Gemzar should be administered with caution in patients with evidence of significant renal or hepatic impairment as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations.

Patients treated with Gemzar who complete an entire cycle of therapy may have the dose for subsequent cycles increased by 25%, provided that the absolute granulocyte count (AGC) and platelet nadirs exceed 1500 x 10^6/L and 100,000 x 10^6/L, respectively, and if non-hematologic toxicity has not been greater than WHO Grade 1. If patients tolerate the subsequent course of Gemzar at the increased dose, the dose for the next cycle can be further increased by 20%, provided again that the AGC and platelet nadirs exceed 1500 x 10^6/L and 100,000 x 10^6/L, respectively, and that non-hematologic toxicity has not been greater than WHO Grade 1.

2.5 Preparation and Administration Precautions

Caution should be exercised in handling and preparing Gemzar solutions. The use of gloves is recommended. If Gemzar solution contacts the skin or mucosa, immediately wash the skin thoroughly with soap and water or rinse the mucosa with copious amounts of water. Although acute dermal irritation has not been observed in animal studies, 2 of 3 rabbits exhibited drug-related systemic toxicities (death, hypoactivity, nasal discharge, shallow breathing) due to dermal absorption.

Procedures for proper handling and disposal of anti-cancer drugs should be considered. Several guidelines on this subject have been published *[see References (15)]*.

2.6 Preparation for Intravenous Infusion Administration

The recommended diluent for reconstitution of Gemzar is 0.9% Sodium Chloride Injection without preservatives. Due to solubility considerations, the maximum concentration for Gemzar upon reconstitution is 40 mg/mL. Reconstitution at concentrations greater than 40 mg/mL may result in incomplete dissolution, and should be avoided.

To reconstitute, add 5 mL of 0.9% Sodium Chloride Injection to the 200-mg vial or 25 mL of 0.9% Sodium Chloride Injection to the 1-g vial. Shake to dissolve. These dilutions each yield a gemcitabine concentration of 38 mg/mL which includes accounting for the displacement volume of the lyophilized powder (0.26 mL for the 200-mg vial or 1.3 mL for the 1-g vial). The total volume upon reconstitution will be 5.26 mL or 26.3 mL, respectively. Complete withdrawal of the vial contents will provide 200 mg or 1 g of gemcitabine, respectively. Prior to administration the appropriate amount of drug must be diluted with 0.9% Sodium Chloride Injection. Final concentrations may be as low as 0.1 mg/mL.

Reconstituted Gemzar is a clear, colorless to light straw-colored solution. After reconstitution with 0.9% Sodium Chloride Injection, the pH of the resulting solution lies in the range of 2.7 to 3.3. The solution should be inspected visually for particulate matter and discoloration prior to administration, whenever solution or container permit. If particulate matter or discoloration is found, do not administer.

When prepared as directed, Gemzar solutions are stable for 24 hours at controlled room temperature 20° to 25°C (68° to 77°F) [see USP Controlled Room Temperature]. Discard unused portion. Solutions of reconstituted Gemzar should not be refrigerated, as crystallization may occur.

The compatibility of Gemzar with other drugs has not been studied. No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets.

3 DOSAGE FORMS AND STRENGTHS

Gemzar (gemcitabine for injection, USP) is a white to off-white lyophilized powder available in sterile single-use vials containing 200 mg or 1 g gemcitabine.

4 CONTRAINDICATIONS

Gemzar is contraindicated in those patients with a known hypersensitivity to the drug.

5 WARNINGS AND PRECAUTIONS

Patients receiving therapy with Gemzar should be monitored closely by a physician experienced in the use of cancer chemotherapeutic agents.

5.1 Infusion Time

Caution — Prolongation of the infusion time beyond 60 minutes and more frequent than weekly dosing have been shown to increase toxicity *[see Clinical Studies (14.5)]*.

5.2 Hematology

Gemzar can suppress bone marrow function as manifested by leukopenia, thrombocytopenia, and anemia *[see Adverse Reactions (6.1)]*, and myelosuppression is usually the dose-limiting toxicity. Patients should be monitored for myelosuppression during therapy *[see Dosage and Administration (2.1, 2.2, 2.3, and 2.4)]*.

5.3 Pulmonary

Pulmonary toxicity has been reported with the use of Gemzar. In cases of severe lung toxicity, Gemzar therapy should be discontinued immediately and appropriate supportive care measures instituted *[see Adverse Reactions (6.1 and 6.2)]*.

5.4 Renal

Hemolytic Uremic Syndrome (HUS) and/or renal failure have been reported following one or more doses of Gemzar. Renal failure leading to death or requiring dialysis, despite discontinuation of therapy, has been reported. The majority of the cases of renal failure leading to death were due to HUS *[see Adverse Reactions (6.1 and 6.2)]*.

Gemzar should be used with caution in patients with preexisting renal impairment as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations *[see Use in Specific Populations (8.6)]*.

5.5 Hepatic

Serious hepatotoxicity, including liver failure and death, has been reported in patients receiving Gemzar alone or in combination with other potentially hepatotoxic drugs *[see Adverse Reactions (6.1 and 6.2)]*.

Gemzar should be used with caution in patients with preexisting hepatic insufficiency as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations. Administration of Gemzar in patients with concurrent liver metastases or a preexisting medical history of hepatitis, alcoholism, or liver cirrhosis may lead to exacerbation of the underlying hepatic insufficiency *[see Use in Specific Populations (8.7)]*.

5.6 Pregnancy

Gemzar can cause fetal harm when administered to a pregnant woman. In pre-clinical studies in mice and rabbits, gemcitabine was teratogenic, embryotoxic, and fetotoxic. There are no adequate and well-controlled studies of Gemzar in pregnant women. If this

drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus *[see Use in Specific Populations (8.1)]*.

5.7 Laboratory Tests

Patients receiving Gemzar should be monitored prior to each dose with a complete blood count (CBC), including differential and platelet count. Suspension or modification of therapy should be considered when marrow suppression is detected *[see Dosage and Administration (2.1, 2.2, 2.3, and 2.4)]*.

Laboratory evaluation of renal and hepatic function should be performed prior to initiation of therapy and periodically thereafter *[see Dosage and Administration (2.4)]*.

5.8 Radiation Therapy

A pattern of tissue injury typically associated with radiation toxicity has been reported in association with concurrent and non-concurrent use of Gemzar.

Non-concurrent (given >7 days apart) — Analysis of the data does not indicate enhanced toxicity when Gemzar is administered more than 7 days before or after radiation, other than radiation recall. Data suggest that Gemzar can be started after the acute effects of radiation have resolved or at least one week after radiation.

Concurrent (given together or ≤7 days apart) — Preclinical and clinical studies have shown that Gemzar has radiosensitizing activity. Toxicity associated with this multimodality therapy is dependent on many different factors, including dose of Gemzar, frequency of Gemzar administration, dose of radiation, radiotherapy planning technique, the target tissue, and target volume. In a single trial, where Gemzar at a dose of 1000 mg/m^2 was administered concurrently for up to 6 consecutive weeks with therapeutic thoracic radiation to patients with non-small cell lung cancer, significant toxicity in the form of severe, and potentially life-threatening mucositis, especially esophagitis and pneumonitis was observed, particularly in patients receiving large volumes of radiotherapy [median treatment volumes 4795 cm^3]. Subsequent studies have been reported and suggest that Gemzar administered at lower doses with concurrent radiotherapy has predictable and less severe toxicity. However, the optimum regimen for safe administration of Gemzar with therapeutic doses of radiation has not yet been determined in all tumor types.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

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.

Most adverse reactions are reversible and do not need to result in discontinuation, although doses may need to be withheld or reduced.

Gemzar has been used in a wide variety of malignancies, both as a single-agent and in combination with other cytotoxic drugs.

Single-Agent Use:

Myelosuppression is the principal dose-limiting toxicity with Gemzar therapy. Dosage adjustments for hematologic toxicity are frequently needed *[see Dosage and Administration (2.1, 2.2, 2.3, and 2.4)]*.

The data in Table 4 are based on 979 patients receiving Gemzar as a single-agent administered weekly as a 30-minute infusion for treatment of a wide variety of malignancies. The Gemzar starting doses ranged from 800 to 1250 mg/m^2. Data are also shown for the subset of patients with pancreatic cancer treated in 5 clinical studies. The frequency of all grades and severe (WHO Grade 3 or 4) adverse reactions were generally similar in the single-agent safety database of 979 patients and the subset of patients with pancreatic cancer. Adverse reactions reported in the single-agent safety database resulted in discontinuation of Gemzar therapy in about 10% of patients. In the comparative trial in pancreatic cancer, the discontinuation rate for adverse reactions was 14.3% for the Gemzar arm and 4.8% for the 5-FU arm. All WHO-graded laboratory adverse reactions are listed in Table 4, regardless of causality.
Non-laboratory adverse reactions listed in Table 4 or discussed below were those reported, regardless of causality, for at least 10% of all patients, except the categories of Extravasation, Allergic, and Cardiovascular and certain specific adverse reactions under the Renal, Pulmonary, and Infection categories.

Table 4: Selected WHO-Graded Adverse Reactions in Patients Receiving Single-Agent Gemzar

	WHO Grades (% incidence)[a]						
	All Patients[b]			Pancreatic Cancer Patients[c]			Discontinuations (%)[d]
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4	All Patients
Laboratory[e]							
Hematologic							
Anemia	68	7	1	73	8	2	<1
Leukopenia	62	9	<1	64	8	1	<1
Neutropenia	63	19	6	61	17	7	-
Thrombocytopenia	24	4	1	36	7	<1	<1
Hepatic							<1
ALT	68	8	2	72	10	1	
AST	67	6	2	78	12	5	
Alkaline Phosphatase	55	7	2	77	16	4	

Bilirubin	13	2	<1	26	6	2	
Renal							<1
Proteinuria	45	<1	0	32	<1	0	
Hematuria	35	<1	0	23	0	0	
BUN	16	0	0	15	0	0	
Creatinine	8	<1	0	6	0	0	
Non-laboratory[f]							
Nausea and Vomiting	69	13	1	71	10	2	<1
Fever	41	2	0	38	2	0	<1
Rash	30	<1	0	28	<1	0	<1
Dyspnea	23	3	<1	10	0	<1	<1
Diarrhea	19	1	0	30	3	0	0
Hemorrhage	17	<1	<1	4	2	<1	<1
Infection	16	1	<1	10	2	<1	<1
Alopecia	15	<1	0	16	0	0	0
Stomatitis	11	<1	0	10	<1	0	<1
Somnolence	11	<1	<1	11	2	<1	<1
Paresthesias	10	<1	0	10	<1	0	0

[a] Grade based on criteria from the World Health Organization (WHO).

[b] N=699-974; all patients with laboratory or non-laboratory data.

[c] N=161-241; all pancreatic cancer patients with laboratory or non-laboratory data.

[d] N=979.

[e] Regardless of causality.

[f] Table includes non-laboratory data with incidence for all patients ≥10%. For approximately 60% of the patients, non-laboratory adverse reactions were graded only if assessed to be possibly drug-related.

Hematologic — In studies in pancreatic cancer myelosuppression is the dose-limiting toxicity with Gemzar, but <1% of patients discontinued therapy for either anemia, leukopenia, or thrombocytopenia. Red blood cell transfusions were required by 19% of patients. The incidence of sepsis was less than 1%. Petechiae or mild blood loss (hemorrhage), from any cause, was reported in 16% of patients; less than 1% of patients required platelet transfusions. Patients should be monitored for myelosuppression during Gemzar therapy and dosage modified or suspended according to the degree of hematologic toxicity *[see Dosage and Administration (2.1, 2.2, 2.3, and 2.4)]*.

Gastrointestinal — Nausea and vomiting were commonly reported (69%) but were usually of mild to moderate severity. Severe nausea and vomiting (WHO Grade 3/4) occurred in <15% of patients. Diarrhea was reported by 19% of patients, and stomatitis by 11% of patients.

Hepatic — In clinical trials, Gemzar was associated with transient elevations of one or both serum transaminases in approximately 70% of patients, but there was no evidence of increasing hepatic toxicity with either longer duration of exposure to Gemzar or with greater total cumulative dose. Serious hepatotoxicity, including liver failure and death, has been reported very rarely in patients receiving Gemzar alone or in combination with other potentially hepatotoxic drugs *[see Adverse Reactions (6.2)]*.

Renal — In clinical trials, mild proteinuria and hematuria were commonly reported. Clinical findings consistent with the Hemolytic Uremic Syndrome (HUS) were reported in 6 of 2429 patients (0.25%) receiving Gemzar in clinical trials. Four patients developed HUS on Gemzar therapy, 2 immediately posttherapy. The diagnosis of HUS should be considered if the patient develops anemia with evidence of microangiopathic hemolysis, elevation of bilirubin or LDH, reticulocytosis, severe thrombocytopenia, and/or evidence of renal failure (elevation of serum creatinine or BUN). Gemzar therapy should be discontinued immediately. Renal failure may not be reversible even with discontinuation of therapy and dialysis may be required *[see Adverse Reactions (6.2)]*.

Fever — The overall incidence of fever was 41%. This is in contrast to the incidence of infection (16%) and indicates that Gemzar may cause fever in the absence of clinical infection. Fever was frequently associated with other flu-like symptoms and was usually mild and clinically manageable.

Rash — Rash was reported in 30% of patients. The rash was typically a macular or finely granular maculopapular pruritic eruption of mild to moderate severity involving the trunk and extremities. Pruritus was reported for 13% of patients.

Pulmonary — In clinical trials, dyspnea, unrelated to underlying disease, has been reported in association with Gemzar therapy. Dyspnea was occasionally accompanied by bronchospasm. Pulmonary toxicity has been reported with the use of Gemzar *[see Adverse Reactions (6.2)]*. The etiology of these effects is unknown. If such effects develop, Gemzar should be discontinued. Early use of supportive care measures may help ameliorate these conditions.

Edema — Edema (13%), peripheral edema (20%), and generalized edema (<1%) were reported. Less than 1% of patients discontinued due to edema.

Flu-like Symptoms — "Flu syndrome" was reported for 19% of patients. Individual symptoms of fever, asthenia, anorexia, headache, cough, chills, and myalgia were commonly reported. Fever and asthenia were also reported frequently as isolated symptoms. Insomnia, rhinitis, sweating, and malaise were reported infrequently. Less than 1% of patients discontinued due to flu-like symptoms.

Infection — Infections were reported for 16% of patients. Sepsis was rarely reported (<1%).

Alopecia — Hair loss, usually minimal, was reported by 15% of patients.

Neurotoxicity — There was a 10% incidence of mild paresthesias and a <1% rate of severe paresthesias.

Extravasation — Injection-site related events were reported for 4% of patients. There were no reports of injection site necrosis. Gemzar is not a vesicant.

Allergic — Bronchospasm was reported for less than 2% of patients. Anaphylactoid reaction has been reported rarely. Gemzar should not be administered to patients with a known hypersensitivity to this drug *[see Contraindications (4)]*.

Cardiovascular — During clinical trials, 2% of patients discontinued therapy with Gemzar due to cardiovascular events such as myocardial infarction, cerebrovascular accident, arrhythmia, and hypertension. Many of these patients had a prior history of cardiovascular disease *[see Adverse Reactions (6.2)]*.

Combination Use in Non-Small Cell Lung Cancer:

In the Gemzar plus cisplatin versus cisplatin study, dose adjustments occurred with 35% of Gemzar injections and 17% of cisplatin injections on the combination arm, versus 6% on the cisplatin-only arm. Dose adjustments were required in greater than 90% of patients on the combination, versus 16% on cisplatin. Study discontinuations for possibly drug-related adverse reactions occurred in 15% of patients on the combination arm and 8% of patients on the cisplatin arm. With a median of 4 cycles of Gemzar plus cisplatin treatment, 94 of 262 patients (36%) experienced a total of 149 hospitalizations due to possibly treatment-related adverse reactions. With a median of 2 cycles of cisplatin treatment, 61 of 260 patients (23%) experienced 78 hospitalizations due to possibly treatment-related adverse reactions.

In the Gemzar plus cisplatin versus etoposide plus cisplatin study, dose adjustments occurred with 20% of Gemzar injections and 16% of cisplatin injections in the Gemzar plus cisplatin arm compared with 20% of etoposide injections and 15% of cisplatin injections in the etoposide plus cisplatin arm. With a median of 5 cycles of Gemzar plus cisplatin treatment, 15 of 69 patients (22%) experienced 15 hospitalizations due to possibly treatment-related adverse reactions. With a median of 4 cycles of etoposide plus cisplatin treatment, 18 of 66 patients (27%) experienced 22 hospitalizations due to possibly treatment-related adverse reactions. In patients who completed more than one cycle, dose adjustments were reported in 81% of the Gemzar plus cisplatin patients, compared with 68% on the etoposide plus cisplatin arm. Study discontinuations for possibly drug-related adverse reactions occurred in 14% of patients on the Gemzar plus cisplatin arm and in 8% of patients on the etoposide plus cisplatin arm. The incidence of myelosuppression was increased in frequency with Gemzar plus cisplatin treatment (~90%) compared to that with the Gemzar monotherapy (~60%). With combination therapy Gemzar dosage adjustments for hematologic toxicity were required more often while cisplatin dose adjustments were less frequently required.

Table 5 presents the safety data from the Gemzar plus cisplatin versus cisplatin study in non-small cell lung cancer. The NCI Common Toxicity Criteria (CTC) were used. The two-drug combination was more myelosuppressive with 4 (1.5%) possibly treatment-related deaths, including 3 resulting from myelosuppression with infection and one case of renal failure associated with pancytopenia and infection. No deaths due to treatment were reported on the cisplatin arm. Nine cases of febrile neutropenia were reported on the combination therapy arm compared to 2 on the cisplatin arm. More patients required RBC and platelet transfusions on the Gemzar plus cisplatin arm.

Myelosuppression occurred more frequently on the combination arm, and in 4 possibly treatment-related deaths myelosuppression was observed. Sepsis was reported in 4% of patients on the Gemzar plus cisplatin arm compared to 1% on the cisplatin arm. Platelet transfusions were required in 21% of patients on the combination arm and <1% of patients on the cisplatin arm. Hemorrhagic events occurred in 14% of patients on the combination arm and 4% on the cisplatin arm. However, severe hemorrhagic events were rare. Red blood cell transfusions were required in 39% of the patients on the Gemzar plus cisplatin arm, versus 13% on the cisplatin arm. The data suggest cumulative anemia with continued Gemzar plus cisplatin use.

Nausea and vomiting despite the use of antiemetics occurred more often with Gemzar plus cisplatin therapy (78%) than with cisplatin alone (71%). In studies with single-agent Gemzar, a lower incidence of nausea and vomiting (58% to 69%) was reported. Renal function abnormalities, hypomagnesemia, neuromotor, neurocortical, and neurocerebellar toxicity occurred more often with Gemzar plus cisplatin than with cisplatin monotherapy. Neurohearing toxicity was similar on both arms.

Cardiac dysrrhythmias of Grade 3 or greater were reported in 7 (3%) patients treated with Gemzar plus cisplatin compared to one (<1%) Grade 3 dysrrhythmia reported with cisplatin therapy. Hypomagnesemia and hypokalemia were associated with one Grade 4 arrhythmia on the Gemzar plus cisplatin combination arm.

Table 6 presents data from the randomized study of Gemzar plus cisplatin versus etoposide plus cisplatin in 135 patients with NSCLC. One death (1.5%) was reported on the Gemzar plus cisplatin arm due to febrile neutropenia associated with renal failure which was possibly treatment-related. No deaths related to treatment occurred on the etoposide plus cisplatin arm. The overall incidence of Grade 4 neutropenia on the Gemzar plus cisplatin arm was less than on the etoposide plus cisplatin arm (28% versus 56%). Sepsis was experienced by 2% of patients on both treatment arms. Grade 3 anemia and Grade 3/4 thrombocytopenia were more common on the Gemzar plus cisplatin arm. RBC transfusions were given to 29% of the patients who received Gemzar plus cisplatin versus 21% of patients who received etoposide plus cisplatin. Platelet transfusions were given to 3% of the patients who received Gemzar plus cisplatin versus 8% of patients who received etoposide plus cisplatin. Grade 3/4 nausea and vomiting were also more common on the Gemzar plus cisplatin arm. On the Gemzar plus cisplatin arm, 7% of participants were hospitalized due to febrile neutropenia compared to 12% on the etoposide plus cisplatin arm. More than twice as many patients had dose reductions or omissions of a scheduled dose of Gemzar as compared to etoposide, which may explain the differences in the incidence of neutropenia and febrile neutropenia between treatment arms. Flu syndrome was reported by 3% of patients on the

Gemzar plus cisplatin arm with none reported on the comparator arm. Eight patients (12%) on the Gemzar plus cisplatin arm reported edema compared to one patient (2%) on the etoposide plus cisplatin arm.

Table 5: Selected CTC-Graded Adverse Reactions From Comparative Trial of Gemzar Plus Cisplatin Versus Single-Agent Cisplatin in NSCLC

CTC Grades (% incidence)[a]

	Gemzar plus Cisplatin[b]			Cisplatin[c]		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[d]						
Hematologic						
Anemia	89	22	3	67	6	1
RBC Transfusion[e]	39			13		
Leukopenia	82	35	11	25	2	1
Neutropenia	79	22	35	20	3	1
Thrombocytopenia	85	25	25	13	3	1
Platelet Transfusions[e]	21			<1		
Lymphocytes	75	25	18	51	12	5
Hepatic						
Transaminase	22	2	1	10	1	0
Alkaline Phosphatase	19	1	0	13	0	0
Renal						
Proteinuria	23	0	0	18	0	0
Hematuria	15	0	0	13	0	0
Creatinine	38	4	<1	31	2	<1
Other Laboratory						
Hyperglycemia	30	4	0	23	3	0
Hypomagnesemia	30	4	3	17	2	0
Hypocalcemia	18	2	0	7	0	<1
Non-laboratory[f]						
Nausea	93	25	2	87	20	<1
Vomiting	78	11	12	71	10	9
Alopecia	53	1	0	33	0	0
Neuro Motor	35	12	0	15	3	0
Neuro Hearing	25	6	0	21	6	0
Diarrhea	24	2	2	13	0	0
Neuro Sensory	23	1	0	18	1	0
Infection	18	3	2	12	1	0
Fever	16	0	0	5	0	0
Neuro Cortical	16	3	1	9	1	0
Neuro Mood	16	1	0	10	1	0
Local	15	0	0	6	0	0
Neuro Headache	14	0	0	7	0	0
Stomatitis	14	1	0	5	0	0
Hemorrhage	14	1	0	4	0	0
Dyspnea	12	4	3	11	3	2
Hypotension	12	1	0	7	1	0
Rash	11	0	0	3	0	0

[a] Grade based on Common Toxicity Criteria (CTC). Table includes data for adverse reactions with incidence ≥10% in either arm.

[b] N=217-253; all Gemzar plus cisplatin patients with laboratory or non-laboratory data. Gemzar at 1000 mg/m^2 on Days 1, 8, and 15 and cisplatin at 100 mg/m^2 on Day 1 every 28 days.

[c] N=213-248; all cisplatin patients with laboratory or non-laboratory data. Cisplatin at 100 mg/m^2 on Day 1 every 28 days.

[d] Regardless of causality.

[e] Percent of patients receiving transfusions. Percent transfusions are not CTC-graded events.

[f] Non-laboratory events were graded only if assessed to be possibly drug-related.

Table 6: Selected WHO-Graded Adverse Reactions From Comparative Trial of Gemzar Plus Cisplatin Versus Etoposide Plus Cisplatin in NSCLC

WHO Grades (% incidence)[a]

	Gemzar plus Cisplatin[b]	Etoposide plus Cisplatin[c]

	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[d]						
Hematologic						
Anemia	88	22	0	77	13	2
RBC Transfusions[e]	29			21		
Leukopenia	86	26	3	87	36	7
Neutropenia	88	36	28	87	20	56
Thrombocytopenia	81	39	16	45	8	5
Platelet Transfusions[e]	3			8		
Hepatic						
ALT	6	0	0	12	0	0
AST	3	0	0	11	0	0
Alkaline Phosphatase	16	0	0	11	0	0
Bilirubin	0	0	0	0	0	0
Renal						
Proteinuria	12	0	0	5	0	0
Hematuria	22	0	0	10	0	0
BUN	6	0	0	4	0	0
Creatinine	2	0	0	2	0	0
Non-laboratory[f,g]						
Nausea and Vomiting	96	35	4	86	19	7
Fever	6	0	0	3	0	0
Rash	10	0	0	3	0	0
Dyspnea	1	0	1	3	0	0
Diarrhea	14	1	1	13	0	2
Hemorrhage	9	0	3	3	0	3
Infection	28	3	1	21	8	0
Alopecia	77	13	0	92	51	0
Stomatitis	20	4	0	18	2	0
Somnolence	3	0	0	3	2	0
Paresthesias	38	0	0	16	2	0

[a] Grade based on criteria from the World Health Organization (WHO).

[b] N=67-69; all Gemzar plus cisplatin patients with laboratory or non-laboratory data. Gemzar at 1250 mg/m^2 on Days 1 and 8 and cisplatin at 100 mg/m^2 on Day 1 every 21 days.

[c] N=57-63; all cisplatin plus etoposide patients with laboratory or non-laboratory data. Cisplatin at 100 mg/m^2 on Day 1 and intravenous etoposide at 100 mg/m^2 on Days 1, 2, and 3 every 21 days.

[d] Regardless of causality.

[e] Percent of patients receiving transfusions. Percent transfusions are not WHO-graded events.

[f] Non-laboratory events were graded only if assessed to be possibly drug-related.

[g] Pain data were not collected.

Combination Use in Breast Cancer:

In the Gemzar plus paclitaxel versus paclitaxel study, dose reductions occurred with 8% of Gemzar injections and 5% of paclitaxel injections on the combination arm, versus 2% on the paclitaxel arm. On the combination arm, 7% of Gemzar doses were omitted and <1% of paclitaxel doses were omitted, compared to <1% of paclitaxel doses on the paclitaxel arm. A total of 18 patients (7%) on the Gemzar plus paclitaxel arm and 12 (5%) on the paclitaxel arm discontinued the study because of adverse reactions. There were two deaths on study or within 30 days after study drug discontinuation that were possibly drug-related, one on each arm.

Table 7 presents the safety data occurrences of ≥10% (all grades) from the Gemzar plus paclitaxel versus paclitaxel study in breast cancer.

Table 7: Adverse Reactions From Comparative Trial of Gemzar Plus Paclitaxel Versus Single-Agent Paclitaxel in Breast Cancer[a]

CTC Grades (% incidence)

	Gemzar plus Paclitaxel (N=262)			**Paclitaxel (N=259)**		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[b]						
Hematologic						
Anemia	69	6	1	51	3	<1

Neutropenia	69	31	17	31	4	7
Thrombocytopenia	26	5	<1	7	<1	<1
Leukopenia	21	10	1	12	2	0
Hepatobiliary						
ALT	18	5	<1	6	<1	0
AST	16	2	0	5	<1	0
Non-laboratory[c]						
Alopecia	90	14	4	92	19	3
Neuropathy-sensory	64	5	<1	58	3	0
Nausea	50	1	0	31	2	0
Fatigue	40	6	<1	28	1	<1
Myalgia	33	4	0	33	3	<1
Vomiting	29	2	0	15	2	0
Arthralgia	24	3	0	22	2	<1
Diarrhea	20	3	0	13	2	0
Anorexia	17	0	0	12	<1	0
Neuropathy-motor	15	2	<1	10	<1	0
Stomatitis/pharyngitis	13	1	<1	8	<1	0
Fever	13	<1	0	3	0	0
Rash/desquamation	11	<1	<1	5	0	0

[a] Grade based on Common Toxicity Criteria (CTC) Version 2.0 (all grades ≥10%).

[b] Regardless of causality.

[c] Non-laboratory events were graded only if assessed to be possibly drug-related.

The following are the clinically relevant adverse reactions that occurred in >1% and <10% (all grades) of patients on either arm. In parentheses are the incidences of Grade 3 and 4 adverse reactions (Gemzar plus paclitaxel versus paclitaxel): febrile neutropenia (5.0% versus 1.2%), infection (0.8% versus 0.8%), dyspnea (1.9% versus 0), and allergic reaction/hypersensitivity (0 versus 0.8%).

No differences in the incidence of laboratory and non-laboratory events were observed in patients 65 years or older, as compared to patients younger than 65.

Combination Use in Ovarian Cancer:

In the Gemzar plus carboplatin versus carboplatin study, dose reductions occurred with 10.4% of Gemzar injections and 1.8% of carboplatin injections on the combination arm, versus 3.8% on the carboplatin alone arm. On the combination arm, 13.7% of Gemzar doses were omitted and 0.2% of carboplatin doses were omitted, compared to 0% of carboplatin doses on the carboplatin alone arm. There were no differences in discontinuations due to adverse reactions between arms (10.9% versus 9.8%, respectively).

Table 8 presents the adverse reactions (all grades) occurring in ≥10% of patients in the ovarian cancer study.

Table 8: Adverse Reactions From Comparative Trial of Gemzar Plus Carboplatin Versus Single-Agent Carboplatin in Ovarian Cancer[a]

CTC Grades (% incidence)

	Gemzar plus Carboplatin (N=175)			**Carboplatin (N=174)**		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[b]						
Hematologic						
Neutropenia	90	42	29	58	11	1
Anemia	86	22	6	75	9	2
Leukopenia	86	48	5	70	6	<1
Thrombocytopenia	78	30	5	57	10	1
RBC Transfusions[c]	38			15		
Platelet Transfusions[c]	9			3		
Non-laboratory[b]						
Nausea	69	6	0	61	3	0
Alopecia	49	0	0	17	0	0
Vomiting	46	6	0	36	2	<1
Constipation	42	6	1	37	3	0
Fatigue	40	3	<1	32	5	0
Neuropathy-sensory	29	1	0	27	2	0
Diarrhea	25	3	0	14	<1	0
Stomatitis/pharyngitis	22	<1	0	13	0	0

Anorexia	16	1	0	13	0	0

[a] Grade based on Common Toxicity Criteria (CTC) Version 2.0 (all grades ≥10%).

[b] Regardless of causality.

[c] Percent of patients receiving transfusions. Transfusions are not CTC-graded events. Blood transfusions included both packed red blood cells and whole blood.

In addition to blood product transfusions as listed in Table 8, myelosuppression was also managed with hematopoietic agents. These agents were administered more frequently with combination therapy than with monotherapy (granulocyte growth factors: 23.6% and 10.1%, respectively; erythropoietic agents: 7.3% and 3.9%, respectively).

The following are the clinically relevant adverse reactions, regardless of causality, that occurred in >1% and <10% (all grades) of patients on either arm. In parentheses are the incidences of Grade 3 and 4 adverse reactions (Gemzar plus carboplatin versus carboplatin): AST or ALT elevation (0 versus 1.2%), dyspnea (3.4% versus 2.9%), febrile neutropenia (1.1% versus 0), hemorrhagic event (2.3% versus 1.1%), hypersensitivity reaction (2.3% versus 2.9%), motor neuropathy (1.1% versus 0.6%), and rash/desquamation (0.6% versus 0).

No differences in the incidence of laboratory and non-laboratory events were observed in patients 65 years or older, as compared to patients younger than 65.

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of Gemzar. 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.

These adverse reactions have occurred after Gemzar single-agent use and Gemzar in combination with other cytotoxic agents. Decisions to include these events are based on the seriousness of the event, frequency of reporting, or potential causal connection to Gemzar.

Cardiovascular — Congestive heart failure and myocardial infarction have been reported very rarely with the use of Gemzar. Arrhythmias, predominantly supraventricular in nature, have been reported very rarely.

Vascular Disorders — Clinical signs of peripheral vasculitis and gangrene have been reported very rarely.

Skin — Cellulitis and non-serious injection site reactions in the absence of extravasation have been rarely reported. Severe skin reactions, including desquamation and bullous skin eruptions, have been reported very rarely.

Hepatic — Increased liver function tests including elevations in aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase, and bilirubin levels have been reported rarely. Serious hepatotoxicity including liver failure and death has been reported very rarely in patients receiving Gemzar alone or in combination with other potentially hepatotoxic drugs. Hepatic veno-occlusive disease has been reported.

Pulmonary — Parenchymal toxicity, including interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, and adult respiratory distress syndrome (ARDS), has been reported rarely following one or more doses of Gemzar administered to patients with various malignancies. Some patients experienced the onset of pulmonary symptoms up to 2 weeks after the last Gemzar dose. Respiratory failure and death occurred very rarely in some patients despite discontinuation of therapy.

Renal — Hemolytic Uremic Syndrome (HUS) and/or renal failure have been reported following one or more doses of Gemzar. Renal failure leading to death or requiring dialysis, despite discontinuation of therapy, has been rarely reported. The majority of the cases of renal failure leading to death were due to HUS.

Injury, Poisoning, and Procedural Complications — Radiation recall reactions have been reported *[see Warnings and Precautions (5.8)]*.

7 DRUG INTERACTIONS

No specific drug interaction studies have been conducted. Information is available on the pharmacodynamics and pharmacokinetics of Gemzar in combination with cisplatin, paclitaxel, or carboplatin *[see Clinical Pharmacology (12.2 and 12.3)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D. See 'Warnings and Precautions' section.

Gemzar can cause fetal harm when administered to a pregnant woman. Based on its mechanism of action, Gemzar is expected to result in adverse reproductive effects. There are no adequate and well-controlled studies of Gemzar in pregnant women. Gemcitabine is embryotoxic causing fetal malformations (cleft palate, incomplete ossification) at doses of 1.5 mg/kg/day in mice (about 1/200 the recommended human dose on a mg/m^2 basis). Gemcitabine is fetotoxic causing fetal malformations (fused pulmonary artery, absence of gall bladder) at doses of 0.1 mg/kg/day in rabbits (about 1/600 the recommended human dose on a mg/m^2 basis). Embryotoxicity was characterized by decreased fetal viability, reduced live litter sizes, and developmental delays. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus *[see Warnings and Precautions (5.6)]*.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Gemzar, a decision should be made whether 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 Gemzar in pediatric patients has not been established. Gemzar was evaluated in a Phase 1 trial in pediatric patients with refractory leukemia and determined that the maximum tolerated dose was 10 mg/m^2/min for 360 minutes three times weekly followed by a one-week rest period. Gemzar was also evaluated in a Phase 2 trial in patients with relapsed acute lymphoblastic leukemia (22 patients) and acute myelogenous leukemia (10 patients) using 10 mg/m^2/min for 360 minutes three times weekly followed by a one-week rest period. Toxicities observed included bone marrow suppression, febrile neutropenia, elevation of serum transaminases, nausea, and rash/desquamation, which were similar to those reported in adults. No meaningful clinical activity was observed in this Phase 2 trial.

8.5 Geriatric Use

Gemzar clearance is affected by age *[see Clinical Pharmacology (12.3)]*. There is no evidence, however, that unusual dose adjustments *[see Dosage and Administration (2.1, 2.2, 2.3, and 2.4)]* are necessary in patients over 65, and in general, adverse reaction rates in the single-agent safety database of 979 patients were similar in patients above and below 65. Grade 3/4 thrombocytopenia was more common in the elderly. In the randomized clinical trial of Gemzar in combination with carboplatin for recurrent ovarian cancer *[see Clinical Studies (14.1)]*, 125 women treated with Gemzar plus carboplatin were <65 years and 50 were ≥65 years. Similar effectiveness was observed between older and younger women. There was significantly higher Grade 3/4 neutropenia in women 65 years of age or older. Overall, there were no other substantial differences in toxicity profile of Gemzar plus carboplatin based on age.

8.6 Renal

Hemolytic Uremic Syndrome (HUS) and/or renal failure have been reported following one or more doses of Gemzar. Renal failure leading to death or requiring dialysis, despite discontinuation of therapy, has been reported. The majority of the cases of renal failure leading to death were due to HUS *[see Adverse Reactions (6.1 and 6.2)]*.

Gemzar should be used with caution in patients with preexisting renal impairment as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations *[see Warnings and Precautions (5.4)]*.

8.7 Hepatic

Serious hepatotoxicity, including liver failure and death, has been reported in patients receiving Gemzar alone or in combination with other potentially hepatotoxic drugs *[see Adverse Reactions (6.1 and 6.2)]*.

Gemzar should be used with caution in patients with preexisting hepatic insufficiency as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations. Administration of Gemzar in patients with concurrent liver metastases or a preexisting medical history of hepatitis, alcoholism, or liver cirrhosis may lead to exacerbation of the underlying hepatic insufficiency *[see Warnings and Precautions (5.5)]*.

8.8 Gender

Gemzar clearance is affected by gender *[see Clinical Pharmacology (12.3)]*. In the single-agent safety database (N=979 patients), however, there is no evidence that unusual dose adjustments *[see Dosage and Administration (2)]* are necessary in women. In general, in single-agent studies of Gemzar, adverse reaction rates were similar in men and women, but women, especially older women, were more likely not to proceed to a subsequent cycle and to experience Grade 3/4 neutropenia and thrombocytopenia. There was a greater tendency in women, especially older women, not to proceed to the next cycle.

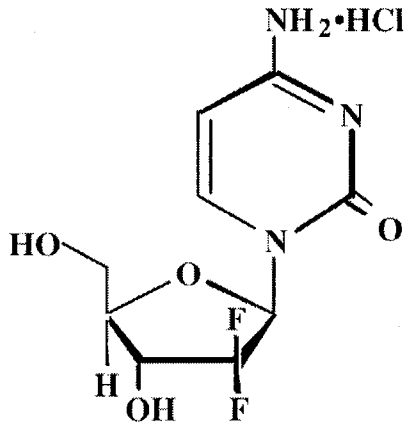
10 OVERDOSAGE

There is no known antidote for overdoses of Gemzar. Myelosuppression, paresthesias, and severe rash were the principal toxicities seen when a single dose as high as 5700 mg/m^2 was administered by intravenous infusion over 30 minutes every 2 weeks to several patients in a Phase 1 study. In the event of suspected overdose, the patient should be monitored with appropriate blood counts and should receive supportive therapy, as necessary.

11 DESCRIPTION

Gemzar (gemcitabine for injection, USP) is a nucleoside metabolic inhibitor that exhibits antitumor activity. Gemcitabine HCl is 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer).

The structural formula is as follows:



The empirical formula for gemcitabine HCl is $C_9H_{11}F_2N_3O_4$ • HCl. It has a molecular weight of 299.66.

Gemcitabine HCl is a white to off-white solid. It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

The clinical formulation is supplied in a sterile form for intravenous use only. Vials of Gemzar contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

12.2 Pharmacodynamics

Gemcitabine demonstrated dose-dependent synergistic activity with cisplatin *in vitro*. No effect of cisplatin on gemcitabine triphosphate accumulation or DNA double-strand breaks was observed. *In vivo*, gemcitabine showed activity in combination with cisplatin against the LX-1 and CALU-6 human lung xenografts, but minimal activity was seen with the NCI-H460 or NCI-H520 xenografts. Gemcitabine was synergistic with cisplatin in the Lewis lung murine xenograft. Sequential exposure to gemcitabine 4 hours before cisplatin produced the greatest interaction.

12.3 Pharmacokinetics

Absorption and Distribution

The pharmacokinetics of gemcitabine were examined in 353 patients, with various solid tumors. Pharmacokinetic parameters were derived using data from patients treated for varying durations of therapy given weekly with periodic rest weeks and using both short infusions (<70 minutes) and long infusions (70 to 285 minutes). The total Gemzar dose varied from 500 to 3600 mg/m^2.

The volume of distribution was increased with infusion length. Volume of distribution of gemcitabine was 50 L/m^2 following infusions lasting <70 minutes. For long infusions, the volume of distribution rose to 370 L/m^2.

Gemcitabine pharmacokinetics are linear and are described by a 2-compartment model. Population pharmacokinetic analyses of combined single and multiple dose studies showed that the volume of distribution of gemcitabine was significantly influenced by duration of infusion and gender. Gemcitabine plasma protein binding is negligible.

Metabolism

Gemcitabine disposition was studied in 5 patients who received a single 1000 mg/m^2/30 minute infusion of radiolabeled drug. Within one (1) week, 92% to 98% of the dose was recovered, almost entirely in the urine. Gemcitabine (<10%) and the inactive uracil metabolite, 2′-deoxy-2′,2′-difluorouridine (dFdU), accounted for 99% of the excreted dose. The metabolite dFdU is also found in plasma.

The active metabolite, gemcitabine triphosphate, can be extracted from peripheral blood mononuclear cells. The half-life of the terminal phase for gemcitabine triphosphate from mononuclear cells ranges from 1.7 to 19.4 hours.

Excretion

Clearance of gemcitabine was affected by age and gender. The lower clearance in women and the elderly results in higher concentrations of gemcitabine for any given dose. Differences in either clearance or volume of distribution based on patient characteristics or the duration of infusion result in changes in half-life and plasma concentrations. Table 9 shows plasma clearance and half-life of gemcitabine following short infusions for typical patients by age and gender.

Table 9: Gemcitabine Clearance and Half-Life for the "Typical" Patient

Age	Clearance Men ($L/hr/m^2$)	Clearance Women ($L/hr/m^2$)	Half-Life[a] Men (min)	Half-Life[a] Women (min)
29	92.2	69.4	42	49
45	75.7	57.0	48	57
65	55.1	41.5	61	73
79	40.7	30.7	79	94

[a] Half-life for patients receiving a short infusion (<70 min).

Gemcitabine half-life for short infusions ranged from 42 to 94 minutes, and the value for long infusions varied from 245 to 638 minutes, depending on age and gender, reflecting a greatly increased volume of distribution with longer infusions.

Drug Interactions

When Gemzar (1250 mg/m^2 on Days 1 and 8) and cisplatin (75 mg/m^2 on Day 1) were administered in NSCLC patients, the clearance of gemcitabine on Day 1 was 128 $L/hr/m^2$ and on Day 8 was 107 $L/hr/m^2$. The clearance of cisplatin in the same study was reported to be 3.94 $mL/min/m^2$ with a corresponding half-life of 134 hours *[see Drug Interactions (7)]*. Analysis of data from metastatic breast cancer patients shows that, on average, Gemzar has little or no effect on the pharmacokinetics (clearance and half-life) of paclitaxel and paclitaxel has little or no effect on the pharmacokinetics of Gemzar. Data from NSCLC patients demonstrate that Gemzar and carboplatin given in combination does not alter the pharmacokinetics of Gemzar or carboplatin compared to administration of either single-agent. However, due to wide confidence intervals and small sample size, interpatient variability may be observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies to evaluate the carcinogenic potential of Gemzar have not been conducted. Gemcitabine induced forward mutations *in vitro* in a mouse lymphoma (L5178Y) assay and was clastogenic in an *in vivo* mouse micronucleus assay. Gemcitabine was negative when tested using the Ames, *in vivo* sister chromatid exchange, and *in vitro* chromosomal aberration assays, and did not cause unscheduled DNA synthesis *in vitro*. Gemcitabine IP doses of 0.5 mg/kg/day (about 1/700 the human dose on a mg/m^2 basis) in male mice had an effect on fertility with moderate to severe hypospermatogenesis, decreased fertility, and decreased implantations. In female mice, fertility was not affected but maternal toxicities were observed at 1.5 mg/kg/day administered intravenously (about 1/200 the human dose on a mg/m^2 basis) and fetotoxicity or embryolethality was observed at 0.25 mg/kg/day administered intravenously (about 1/1300 the human dose on a mg/m^2 basis).

14 CLINICAL STUDIES

14.1 Ovarian Cancer

Gemzar was studied in a randomized Phase 3 study of 356 patients with advanced ovarian cancer that had relapsed at least 6 months after first-line platinum-based therapy. Patients were randomized to receive either Gemzar 1000 mg/m^2 on Days 1 and 8 of a 21-day cycle and carboplatin AUC 4 administered after Gemzar on Day 1 of each cycle or single-agent carboplatin AUC 5 administered on Day 1 of each 21-day cycle as the control arm. The primary endpoint of this study was progression free survival (PFS).

Patient characteristics are shown in Table 10. The addition of Gemzar to carboplatin resulted in statistically significant improvement in PFS and overall response rate as shown in Table 11 and Figure 1. Approximately 75% of patients in each arm received poststudy chemotherapy. Only 13 of 120 patients with documented poststudy chemotherapy regimen in the carboplatin arm received Gemzar after progression. There was not a significant difference in overall survival between arms.

Table 10: Gemzar Plus Carboplatin Versus Carboplatin in Ovarian Cancer - Baseline Demographics and Clinical Characteristics

	Gemzar/Carboplatin	Carboplatin
Number of randomized patients	178	178
Median age, years Range	59 36 to 78	58 21 to 81
Baseline ECOG performance status 0-1[a]	94%	95%
Disease Status Evaluable	7.9%	2.8%

Bidimensionally measurable	91.6%	95.5%
Platinum-free interval[b]		
6-12 months	39.9%	39.9%
>12 months	59.0%	59.6%
First-line therapy		
Platinum-taxane combination	70.2%	71.3%
Platinum-non-taxane combination	28.7%	27.5%
Platinum monotherapy	1.1%	1.1%

[a] Nine patients (5 on the Gemzar plus carboplatin arm and 4 on the carboplatin arm) did not have baseline Eastern Cooperative Oncology Group (ECOG) performance status recorded.

[b] Three patients (2 on the Gemzar plus carboplatin arm and 1 on the carboplatin arm) had a platinum-free interval of less than 6 months.

Table 11: Gemzar Plus Carboplatin Versus Carboplatin in Ovarian Cancer - Results of Efficacy Analysis

	Gemzar/Carboplatin (N=178)	Carboplatin (N=178)	
PFS			
Median (95%, C.I.) months	8.6 (8.0, 9.7)	5.8 (5.2, 7.1)	p=0.0038[d]
Hazard Ratio (95%, C.I.)	0.72 (0.57, 0.90)		
Overall Survival			
Median (95%, C.I.) months	18.0 (16.2, 20.3)	17.3 (15.2, 19.3)	p=0.8977[d]
Hazard Ratio (95%, C.I.)	0.98 (0.78, 1.24)		
Adjusted[a] Hazard Ratio (95%, C.I.)	0.86 (0.67, 1.10)		
Investigator Reviewed			
Overall Response Rate	47.2%	30.9%	p=0.0016[e]
CR	14.6%	6.2%	
PR+PRNM[b]	32.6%	24.7%	
Independently Reviewed			
Overall Response Rate[c,f]	46.3%	35.6%	p=0.11[e]
CR	9.1%	4.0%	
PR+PRNM	37.2%	31.7%	

[a] Treatment adjusted for performance status, tumor area, and platinum-free interval.

[b] Partial response non-measurable disease

[c] Independent reviewers could not evaluate disease demonstrated by sonography or physical exam.

[d] Log Rank, unadjusted

[e] Chi Square

[f] Independently reviewed cohort - Gemzar/Carboplatin N=121, Carboplatin N=101

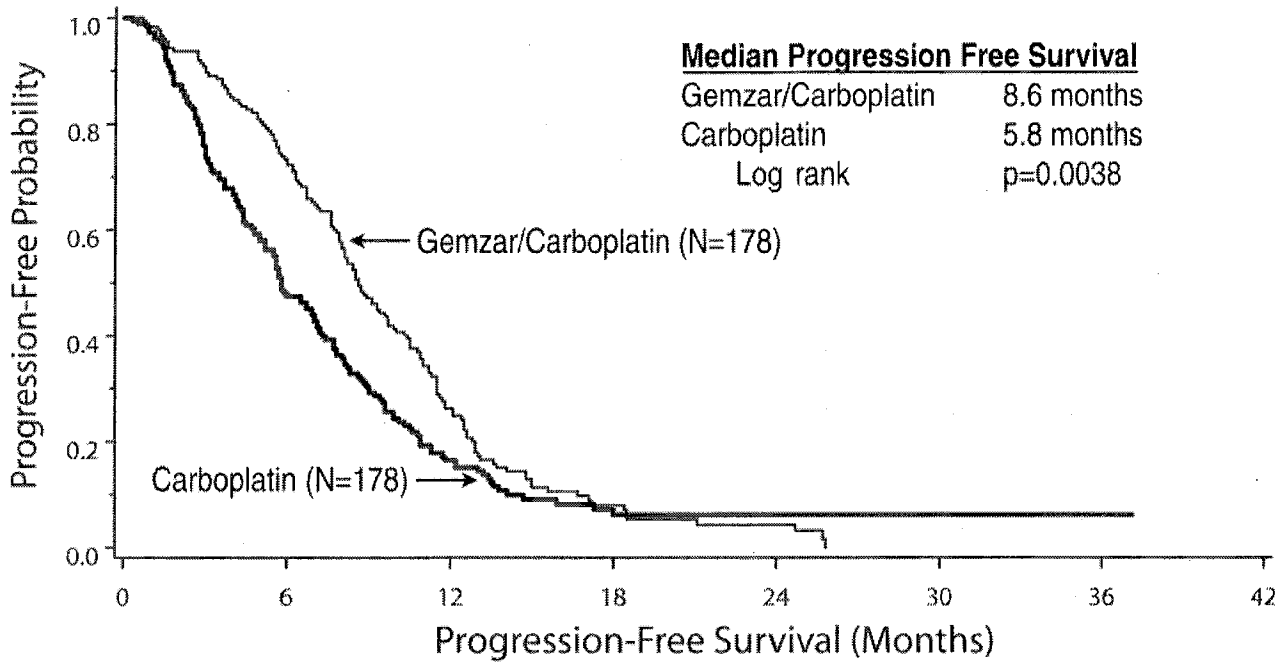


Figure 1: Kaplan-Meier Curve of Progression Free Survival in Gemzar Plus Carboplatin Versus Carboplatin in Ovarian Cancer (N=356)

14.2 Breast Cancer

Data from a multi-national, randomized Phase 3 study (529 patients) support the use of Gemzar in combination with paclitaxel for treatment of breast cancer patients who have received prior adjuvant/neoadjuvant anthracycline chemotherapy unless clinically contraindicated. Gemzar 1250 mg/m^2 was administered on Days 1 and 8 of a 21-day cycle with paclitaxel 175 mg/m^2 administered prior to Gemzar on Day 1 of each cycle. Single-agent paclitaxel 175 mg/m^2 was administered on Day 1 of each 21-day cycle as the control arm.

The addition of Gemzar to paclitaxel resulted in statistically significant improvement in time to documented disease progression and overall response rate compared to monotherapy with paclitaxel as shown in Table 12 and Figure 2. Final survival analysis results at 440 events were Hazard Ratio of 0.86 (95%, CI: 0.71 – 1.04) for the ITT population, as shown in Table 12.

Table 12: Gemzar Plus Paclitaxel Versus Paclitaxel in Breast Cancer

	Gemzar/Paclitaxel	Paclitaxel	
Number of patients	267	262	
Median age, years Range	53 26 to 83	52 26 to 75	
Metastatic disease	97.0%	96.9%	
Baseline KPS[a] ≥90	70.4%	74.4%	
Number of tumor sites 1-2 ≥3	 56.6% 43.4%	 58.8% 41.2%	
Visceral disease	73.4%	72.9%	
Prior anthracycline	96.6%	95.8%	
Overall Survival[b]			
Median (95%, CI)	18.6 (16.5, 20.7)	15.8 (14.1, 17.3)	
Hazard Ratio (95%, CI)	0.86 (0.71,1.04)		
Time to Documented Disease Progression[c] Median (95%, C.I.), months	5.2 (4.2, 5.6)	2.9 (2.6, 3.7)	p<0.0001
Hazard Ratio (95%, C.I.)	0.650 (0.524, 0.805)		p<0.0001
Overall Response Rate[c] (95%, C.I.)	40.8% (34.9, 46.7)	22.1% (17.1, 27.2)	p<0.0001

[a] Karnofsky Performance Status.

[b] Based on the ITT population

[c] These represent reconciliation of investigator and Independent Review Committee assessments according to a predefined algorithm.

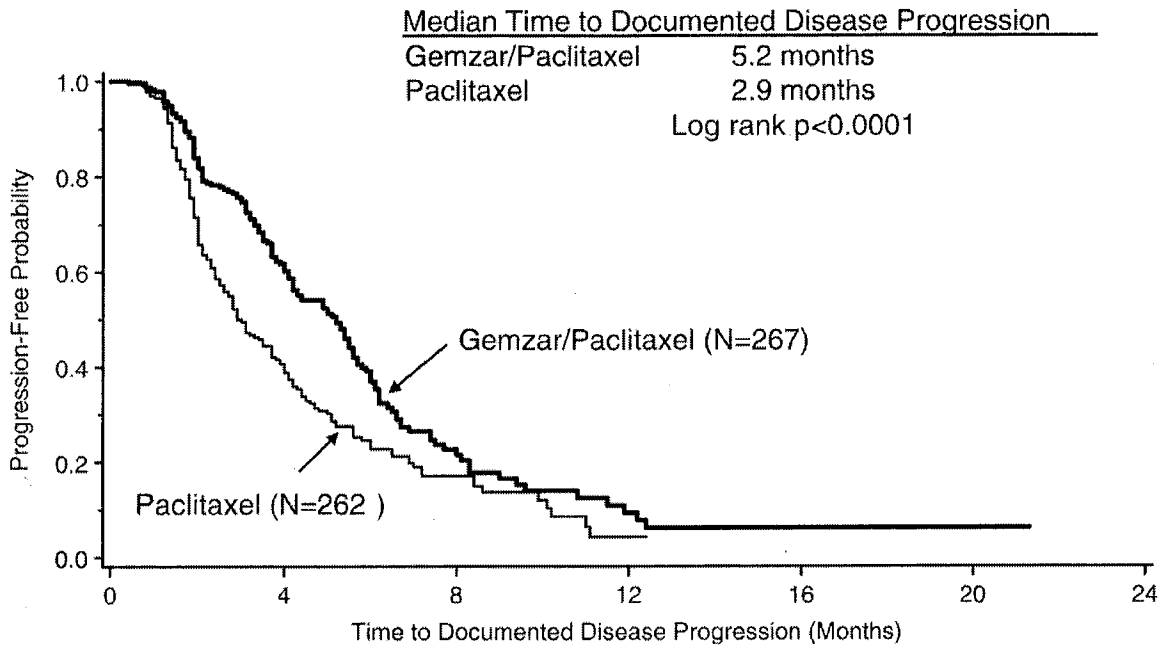


Figure 2: Kaplan-Meier Curve of Time to Documented Disease Progression in Gemzar Plus Paclitaxel Versus Paclitaxel Breast Cancer Study (N=529)

14.3 Non-Small Cell Lung Cancer (NSCLC)

Data from 2 randomized clinical studies (657 patients) support the use of Gemzar in combination with cisplatin for the first-line treatment of patients with locally advanced or metastatic NSCLC.

Gemzar plus cisplatin versus cisplatin: This study was conducted in Europe, the US, and Canada in 522 patients with inoperable Stage IIIA, IIIB, or IV NSCLC who had not received prior chemotherapy. Gemzar 1000 mg/m^2 was administered on Days 1, 8, and 15 of a 28-day cycle with cisplatin 100 mg/m^2 administered on Day 1 of each cycle. Single-agent cisplatin 100 mg/m^2 was administered on Day 1 of each 28-day cycle. The primary endpoint was survival. Patient demographics are shown in Table 13. An imbalance with regard to histology was observed with 48% of patients on the cisplatin arm and 37% of patients on the Gemzar plus cisplatin arm having adenocarcinoma.

The Kaplan-Meier survival curve is shown in Figure 3. Median survival time on the Gemzar plus cisplatin arm was 9.0 months compared to 7.6 months on the single-agent cisplatin arm (Log rank p=0.008, two-sided). Median time to disease progression was 5.2 months on the Gemzar plus cisplatin arm compared to 3.7 months on the cisplatin arm (Log rank p=0.009, two-sided). The objective response rate on the Gemzar plus cisplatin arm was 26% compared to 10% with cisplatin (Fisher's Exact p<0.0001, two-sided). No difference between treatment arms with regard to duration of response was observed.

Gemzar plus cisplatin versus etoposide plus cisplatin: A second, multicenter, study in Stage IIIB or IV NSCLC randomized 135 patients to Gemzar 1250 mg/m^2 on Days 1 and 8, and cisplatin 100 mg/m^2 on Day 1 of a 21-day cycle or to intravenous etoposide 100 mg/m^2 on Days 1, 2, and 3 and cisplatin 100 mg/m^2 on Day 1 of a 21-day cycle (Table 13).

There was no significant difference in survival between the two treatment arms (Log rank p=0.18, two-sided). The median survival was 8.7 months for the Gemzar plus cisplatin arm versus 7.0 months for the etoposide plus cisplatin arm. Median time to disease progression for the Gemzar plus cisplatin arm was 5.0 months compared to 4.1 months on the etoposide plus cisplatin arm (Log rank p=0.015, two-sided). The objective response rate for the Gemzar plus cisplatin arm was 33% compared to 14% on the etoposide plus cisplatin arm (Fisher's Exact p=0.01, two-sided).

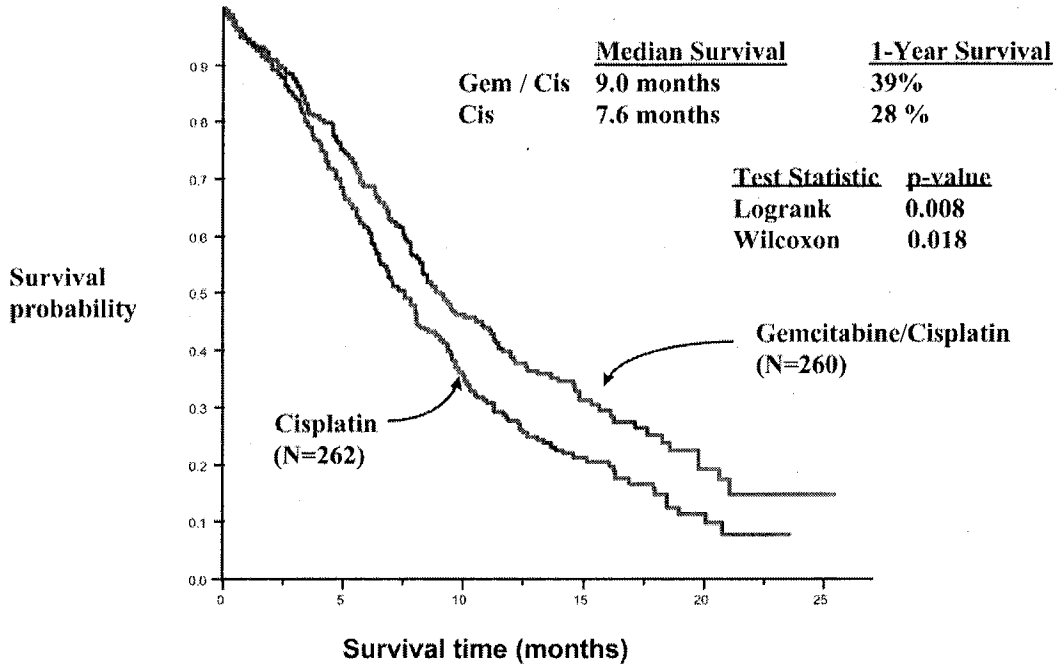


Figure 3: Kaplan-Meier Survival Curve in Gemzar Plus Cisplatin Versus Cisplatin NSCLC Study (N=522)

Table 13: Randomized Trials of Combination Therapy With Gemzar Plus Cisplatin in NSCLC

Trial	28-day Schedule[a]			21-day Schedule[b]		
Treatment Arm	Gemzar/Cisplatin	Cisplatin		Gemzar/Cisplatin	Cisplatin/Etoposide	
Number of patients	260	262		69	66	
Male	182	186		64	61	
Female	78	76		5	5	
Median age, years	62	63		58	60	
Range	36 to 88	35 to 79		33 to 76	35 to 75	
Stage IIIA	7%	7%		N/A[c]	N/A[c]	
Stage IIIB	26%	23%		48%	52%	
Stage IV	67%	70%		52%	49%	
Baseline KPS[d] 70 to 80	41%	44%		45%	52%	
Baseline KPS[d] 90 to 100	57%	55%		55%	49%	
Survival			p=0.008			p=0.18
Median, months	9.0	7.6		8.7	7.0	
(95%, C.I.) months	8.2, 11.0	6.6, 8.8		7.8, 10.1	6.0, 9.7	
Time to Disease Progression			p=0.009			p=0.015
Median, months	5.2	3.7		5.0	4.1	
(95%, C.I.) months	4.2, 5.7	3.0, 4.3		4.2, 6.4	2.4, 4.5	
Tumor Response	26%	10%	p<0.0001[e]	33%	14%	p=0.01[e]

[a] 28-day schedule — Gemzar plus cisplatin: Gemzar 1000 mg/m^2 on Days 1, 8, and 15 and cisplatin 100 mg/m^2 on Day 1 every 28 days; Single-agent cisplatin: cisplatin 100 mg/m^2 on Day 1 every 28 days.

[b] 21-day schedule — Gemzar plus cisplatin: Gemzar 1250 mg/m^2 on Days 1 and 8 and cisplatin 100 mg/m^2 on Day 1 every 21 days; Etoposide plus Cisplatin: cisplatin 100 mg/m^2 on Day 1 and intravenous etoposide 100 mg/m^2 on Days 1, 2, and 3 every 21 days.

[c] N/A Not applicable.

[d] Karnofsky Performance Status.

[e] p-value for tumor response was calculated using the two-sided Fisher's Exact test for difference in binomial proportions. All other p-values were calculated using the Log rank test for difference in overall time to an event.

14.4 Pancreatic Cancer

Data from 2 clinical trials evaluated the use of Gemzar in patients with locally advanced or metastatic pancreatic cancer. The first trial compared Gemzar to 5-Fluorouracil (5-FU) in patients who had received no prior chemotherapy. A second trial studied the use of Gemzar in pancreatic cancer patients previously treated with 5-FU or a 5-FU-containing regimen. In both studies, the first cycle

of Gemzar was administered intravenously at a dose of 1000 mg/m^2 over 30 minutes once weekly for up to 7 weeks (or until toxicity necessitated holding a dose) followed by a week of rest from treatment with Gemzar. Subsequent cycles consisted of injections once weekly for 3 consecutive weeks out of every 4 weeks.

The primary efficacy parameter in these studies was "clinical benefit response," which is a measure of clinical improvement based on analgesic consumption, pain intensity, performance status, and weight change. Definitions for improvement in these variables were formulated prospectively during the design of the 2 trials. A patient was considered a clinical benefit responder if either:

i) the patient showed a ≥50% reduction in pain intensity (Memorial Pain Assessment Card) or analgesic consumption, or a 20-point or greater improvement in performance status (Karnofsky Performance Status) for a period of at least 4 consecutive weeks, without showing any sustained worsening in any of the other parameters. Sustained worsening was defined as 4 consecutive weeks with either any increase in pain intensity or analgesic consumption or a 20-point decrease in performance status occurring during the first 12 weeks of therapy.

OR:

ii) the patient was stable on all of the aforementioned parameters, and showed a marked, sustained weight gain (≥7% increase maintained for ≥4 weeks) not due to fluid accumulation.

The first study was a multicenter (17 sites in US and Canada), prospective, single-blinded, two-arm, randomized, comparison of Gemzar and 5-FU in patients with locally advanced or metastatic pancreatic cancer who had received no prior treatment with chemotherapy. 5-FU was administered intravenously at a weekly dose of 600 mg/m^2 for 30 minutes. The results from this randomized trial are shown in Table 14. Patients treated with Gemzar had statistically significant increases in clinical benefit response, survival, and time to disease progression compared to 5-FU. The Kaplan-Meier curve for survival is shown in Figure 4. No confirmed objective tumor responses were observed with either treatment.

Table 14: Gemzar Versus 5-FU in Pancreatic Cancer

	Gemzar	5-FU	
Number of patients	63	63	
Male	34	34	
Female	29	29	
Median age	62 years	61 years	
Range	37 to 79	36 to 77	
Stage IV disease	71.4%	76.2%	
Baseline KPS[a] ≤70	69.8%	68.3%	
Clinical benefit response	22.2% (N[c]=14)	4.8% (N[c]=3)	p=0.004[e]
Survival			p=0.0009
Median	5.7 months	4.2 months	
6-month probability[b]	(N=30) 46%	(N=19) 29%	
9-month probability[b]	(N=14) 24%	(N=4) 5%	
1-year probability[b]	(N=9) 18%	(N=2) 2%	
Range	0.2 to 18.6 months	0.4 to 15.1+[d] months	
95% C.I. of the median	4.7 to 6.9 months	3.1 to 5.1 months	
Time to Disease Progression			p=0.0013
Median	2.1 months	0.9 months	
Range	0.1+[d] to 9.4 months	0.1 to 12.0+[d] months	
95% C.I. of the median	1.9 to 3.4 months	0.9 to 1.1 months	

[a] Karnofsky Performance Status.

[b] Kaplan-Meier estimates.

[c] N=number of patients.

[d] No progression at last visit; remains alive.

[e] The p-value for clinical benefit response was calculated using the two-sided test for difference in binomial proportions. All other p-values were calculated using the Log rank test for difference in overall time to an event.

Clinical benefit response was achieved by 14 patients treated with Gemzar and 3 patients treated with 5-FU. One patient on the Gemzar arm showed improvement in all 3 primary parameters (pain intensity, analgesic consumption, and performance status). Eleven patients on the Gemzar arm and 2 patients on the 5-FU arm showed improvement in analgesic consumption and/or pain intensity with stable performance status. Two patients on the Gemzar arm showed improvement in analgesic consumption or pain intensity with improvement in performance status. One patient on the 5-FU arm was stable with regard to pain intensity and analgesic consumption with improvement in performance status. No patient on either arm achieved a clinical benefit response based on weight gain.

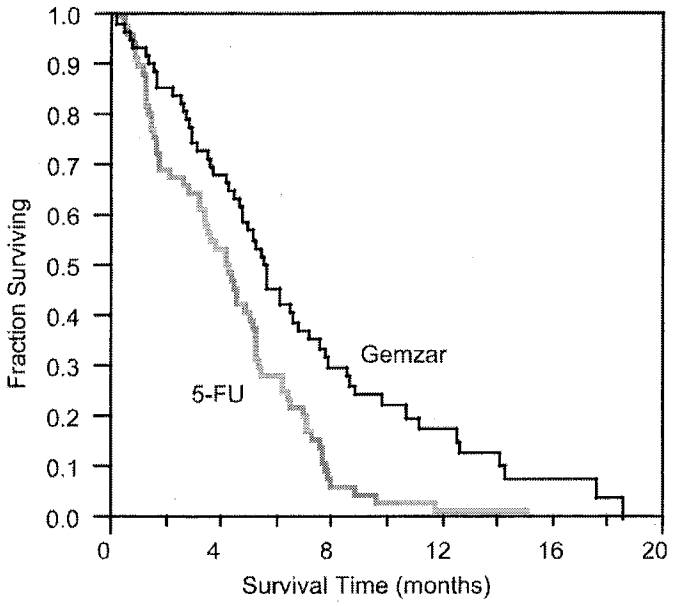


Figure 4: Kaplan-Meier Survival Curve

The second trial was a multicenter (17 US and Canadian centers), open-label study of Gemzar in 63 patients with advanced pancreatic cancer previously treated with 5-FU or a 5-FU-containing regimen. The study showed a clinical benefit response rate of 27% and median survival of 3.9 months.

14.5 Other Clinical Studies

When Gemzar was administered more frequently than once weekly or with infusions longer than 60 minutes, increased toxicity was observed. Results of a Phase 1 study of Gemzar to assess the maximum tolerated dose (MTD) on a daily x 5 schedule showed that patients developed significant hypotension and severe flu-like symptoms that were intolerable at doses above 10 mg/m^2. The incidence and severity of these events were dose-related. Other Phase 1 studies using a twice-weekly schedule reached MTDs of only 65 mg/m^2 (30-minute infusion) and 150 mg/m^2 (5-minute bolus). The dose-limiting toxicities were thrombocytopenia and flu-like symptoms, particularly asthenia. In a Phase 1 study to assess the maximum tolerated infusion time, clinically significant toxicity, defined as myelosuppression, was seen with weekly doses of 300 mg/m^2 at or above a 270-minute infusion time. The half-life of gemcitabine is influenced by the length of the infusion *[see Clinical Pharmacology (12.3)]* and the toxicity appears to be increased if Gemzar is administered more frequently than once weekly or with infusions longer than 60 minutes *[see Warnings and Precautions (5.1)]*.

15 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.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Gemzar (gemcitabine for injection, USP), is available in sterile single-use vials individually packaged in a carton containing:
200 mg white to off-white, lyophilized powder in a 10-mL size sterile single-use vial - NDC 0002-7501-01 (No. 7501)
1 g white to off-white, lyophilized powder in a 50-mL size sterile single-use vial - NDC 0002-7502-01 (No. 7502)

16.2 Storage and Handling

Unopened vials of Gemzar are stable until the expiration date indicated on the package when stored at controlled room temperature 20° to 25°C (68° to 77°F) and that allows for excursions between 15° and 30°C (59° and 86°F) [See USP Controlled Room Temperature] *[see Dosage and Administration (2.5 and 2.6)]*.

17 PATIENT COUNSELING INFORMATION

17.1 Low Blood Cell Counts

Patients should be adequately informed of the risk of low blood cell counts and instructed to immediately contact their physician should any sign of infection develop including fever. Patients should also contact their physician if bleeding or symptoms of anemia occur *[see Warnings and Precautions (5.2)]*.

17.2 Pregnancy

There are no adequate and well-controlled studies of Gemzar in pregnant women. Based on animal studies Gemzar can cause fetal harm when administered to a pregnant woman. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the risks to the fetus need to be discussed with their physician *[see Warnings and Precautions (5.6) and Use in Specific Populations (8.1)]*.

17.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Gemzar, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother *[see Use in Specific Populations (8.3)]*.

Literature revised Month dd, yyyy

Eli Lilly and Company, Indianapolis, IN 46285, USA

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Gemzar safely and effectively. See full prescribing information for Gemzar.

GEMZAR (gemcitabine for injection) Powder, Lyophilized, For Solution For Intravenous Use

Initial U.S. Approval: 1996

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

Dosage and Administration:
Dose Modifications for Non-Hematologic Adverse Reactions (2.5) 05/2014

Warnings and Precautions:
Capillary Leak Syndrome (5.8) 05/2013
Posterior Reversible Encephalopathy Syndrome (5.9) 05/2014

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

Gemzar® is a nucleoside metabolic inhibitor indicated:
- in combination with carboplatin, for the treatment of advanced ovarian cancer that has relapsed at least 6 months after completion of platinum-based therapy (1.1)
- in combination with paclitaxel, for first-line treatment of metastatic breast cancer after failure of prior anthracycline-containing adjuvant chemotherapy, unless anthracyclines were clinically contraindicated (1.2)
- in combination with cisplatin for the treatment of non-small cell lung cancer (1.3)
- as a single agent for the treatment of pancreatic cancer (1.4)

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

Gemzar is for intravenous use only.
- Ovarian Cancer: 1000 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.1)
- Breast Cancer: 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.2)
- Non-Small Cell Lung Cancer: 1000 mg/m^2 over 30 minutes on Days 1, 8, and 15 of each 28-day cycle or 1250 mg/m^2 over 30 minutes on Days 1 and 8 of each 21-day cycle (2.3)
- Pancreatic Cancer: 1000 mg/m^2 over 30 minutes once weekly for the first 7 weeks, then one week rest, then once weekly for 3 weeks of each 28-day cycle (2.4)

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

- 200 mg/single-use vial (3)
- 1 g/single-use vial (3)

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

Patients with a known hypersensitivity to gemcitabine (4)

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

- Schedule-dependent toxicity: Increased toxicity with infusion time greater than 60 minutes or dosing more frequently than once weekly. (5.1)
- Myelosuppression: Monitor for myelosuppression prior to each cycle and reduce or withhold dose for severe myelosuppression. (5.2, 5.7)
- Pulmonary Toxicity and Respiratory Failure: Discontinue Gemzar immediately for unexplained new or worsening dyspnea or evidence of severe pulmonary toxicity. (5.3)
- Hemolytic-Uremic Syndrome (HUS): Monitor renal function prior to initiation and during therapy. Discontinue Gemzar for HUS or severe renal impairment. (5.4)
- Hepatic Toxicity: Monitor hepatic function prior to initiation and during therapy. Discontinue Gemzar for severe hepatic toxicity. (5.5)
- Embryofetal Toxicity: Can cause fetal harm. Advise women of potential risk to the fetus. (5.6, 8.1)
- Exacerbation of Radiation Therapy Toxicity: May cause severe and life-threatening toxicity when administered during or within 7 days of radiation therapy. (5.7)
- Capillary Leak Syndrome: Discontinue Gemzar. (5.8)
- Posterior reversible encephalopathy syndrome (PRES): Discontinue Gemzar. (5.9)

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

The most common adverse reactions for the single agent (≥20%) are nausea/vomiting, anemia, hepatic transaminitis, neutropenia, increased alkaline phosphatase, proteinuria, fever, hematuria, rash, thrombocytopenia, dyspnea, and peripheral edema (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Eli Lilly and Company at 1-800-LillyRx (1-800-545-5979) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 5/2014

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Ovarian Cancer

Gemzar in combination with carboplatin is indicated for the treatment of patients with advanced ovarian cancer that has relapsed at least 6 months after completion of platinum-based therapy.

1.2 Breast Cancer

Gemzar in combination with paclitaxel is indicated for the first-line treatment of patients with metastatic breast cancer after failure of prior anthracycline-containing adjuvant chemotherapy, unless anthracyclines were clinically contraindicated.

1.3 Non-Small Cell Lung Cancer

Gemzar is indicated in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (Stage IIIA or IIIB), or metastatic (Stage IV) non-small cell lung cancer.

1.4 Pancreatic Cancer

Gemzar is indicated as first-line treatment for patients with locally advanced (nonresectable Stage II or Stage III) or metastatic (Stage IV) adenocarcinoma of the pancreas. Gemzar is indicated for patients previously treated with 5-FU.

2 DOSAGE AND ADMINISTRATION

2.1 Ovarian Cancer

Recommended Dose and Schedule

The recommended dose of Gemzar is 1000 mg/m^2 as an intravenous infusion over 30 minutes on Days 1 and 8 of each 21-day cycle, in combination with carboplatin AUC 4 intravenously after Gemzar administration on Day 1 of each 21-day cycle. Refer to carboplatin prescribing information for additional information.

Dose Modifications

Recommended Gemzar dose modifications for myelosuppression are described Table 1 and Table 2 *[see Warnings and Precautions (5.2)]*. Refer to Dosage and Administration (2.5) for recommendations for non-hematologic adverse reactions.

Table 1: Dosage Reduction Guidelines for Gemzar for Myelosuppression on Day of Treatment in Ovarian Cancer

Treatment Day	Absolute granulocyte count (x 10^6/L)		Platelet count (x 10^6/L)	% of full dose
Day 1	≥1500	and	≥100,000	100%
	<1500	or	<100,000	Delay Treatment Cycle
Day 8	≥1500	and	≥100,000	100
	1000-1499	or	75,000-99,999	50
	<1000	or	<75,000	Hold

Table 2: Gemzar Dose Modification for Myelosuppression in Previous Cycle In Ovarian Cancer

Occurrence	Myelosuppression During Treatment Cycle	Dose Modification
Initial Occurrence	Absolute granulocyte count less than 500 x 10^6/L for more than 5 days Absolute granulocyte count less than 100 x 10^6/L for more than 3 days Febrile neutropenia Platelets less than 25,000x10^6/L Cycle delay of more than one week due to toxicity	Permanently reduce Gemzar to 800 mg/m^2 on Days 1 and 8
Subsequent Occurrence	If any of the above toxicities occur after the initial dose reduction	Permanently reduce Gemzar dose to 800 mg/m^2 on Day 1 only

2.2 Breast Cancer

Recommended Dose and Schedule

The recommended dose of Gemzar is 1250 mg/m^2 intravenously over 30 minutes on Days 1 and 8 of each 21-day cycle that includes paclitaxel. Paclitaxel should be administered at 175 mg/m^2 on Day 1 as a 3 hour intravenous infusion before Gemzar administration.

Dose Modifications

Recommended dose modifications for Gemzar for myelosuppression are described in Table 3 *[see Warnings and Precautions (5.2)]*. Refer to Dosage and Administration (2.5) for recommendations for non-hematologic adverse reactions.

Table 3: Recommended Dose Reductions for Gemzar for Myelosuppression on Day of Treatment in Breast Cancer

Treatment Day	Absolute granulocyte count ($\times 10^6$/L)		Platelet count ($\times 10^6$/L)	% of full dose
Day 1	≥1500	and	≥100,000	100%
	less than 1500	or	less than 100,000	Hold
Day 8	≥1200	and	>75,000	100%
	1000-1199	or	50,000-75,000	75%
	700-999	and	≥50,000	50%
	<700	or	<50,000	Hold

2.3 Non-Small Cell Lung Cancer

Recommended Dose and Schedule

Every 4-week schedule

The recommended dose of Gemzar is 1000 mg/m^2 intravenously over 30 minutes on Days 1, 8, and 15 in combination with cisplatin therapy. Administer cisplatin intravenously at 100 mg/m^2 on Day 1 after the infusion of Gemzar.

Every 3-week schedule

The recommended dose of Gemzar is 1250 mg/m^2 intravenously over 30 minutes on Days 1 and 8 in combination with cisplatin therapy. Administer cisplatin intravenously at 100 mg/m^2 on Day 1 after the infusion of Gemzar.

Dose Modifications

Recommended dose modifications for Gemzar myelosuppression are described in Table 4 *[see Warnings and Precautions (5.2)]*. Refer to Dosage and Administration (2.5) for Gemzar recommendations for non-hematologic adverse reactions.

2.4 Pancreatic Cancer

Recommended Dose and Schedule

The recommended dose of Gemzar is 1000 mg/m^2 over 30 minutes intravenously. The recommended treatment schedule

- Weeks 1-8: weekly dosing for the first 7 weeks followed by one week rest.
- After week 8: weekly dosing on Days 1, 8, and 15 of 28-day cycles.

Dose Modifications

Recommended dose modifications for Gemzar for myelosuppression are described in Table 4 *[see Warnings and Precautions (5.2)]*. Refer to Dosage and Administration (2.5) for recommendations for non-hematologic adverse reactions.

Patients receiving Gemzar should be monitored prior to each dose with a complete blood count (CBC), including differential and platelet count. If marrow suppression is detected, therapy should be modified or suspended according to the guidelines in Table 4.

Table 4: Recommended Dose Reductions for Gemzar for Myelosuppression in Pancreatic Cancer and Non-Small Cell Lung Cancer

Absolute granulocyte count ($\times 10^6$/L)		Platelet count ($\times 10^6$/L)	% of full dose
≥1000	And	≥100,000	100
500-999	Or	50,000-99,999	75
<500	Or	<50,000	Hold

2.5 Dose Modifications for Non-Hematologic Adverse Reactions

Permanently discontinue Gemzar for any of the following

- Unexplained dyspnea or other evidence of severe pulmonary toxicity
- Severe hepatic toxicity
- Hemolytic-Uremic Syndrome
- Capillary Leak Syndrome
- Posterior reversible encephalopathy syndrome

Withhold Gemzar or reduce dose by 50% for other severe (Grade 3 or 4) non-hematological toxicity until resolved. No dose modifications are recommended for alopecia, nausea, or vomiting.

2.6 Preparation and Administration Precautions

Exercise caution and wear gloves when preparing Gemzar solutions. Immediately wash the skin thoroughly or rinse the mucosa with copious amounts of water if Gemzar contacts the skin or mucus membranes. Death has occurred in animal studies due to dermal absorption. For further guidance on handling Gemzar go to "OSHA Hazardous Drugs" (refer to antineoplastic weblinks including OSHA Technical Manual) at OSHA. http://www.osha.gov/SLTC/hazardousdrugs/index.html

2.7 Preparation for Intravenous Infusion Administration

Reconstitute the vials with 0.9% Sodium Chloride Injection without preservatives.

Add 5 mL to the 200-mg vial or 25 mL to the 1-g vial. These dilutions each yield a Gemzar concentration of 38 mg/mL. Complete withdrawal of the vial contents will provide 200 mg or 1 g of Gemzar. Prior to administration the appropriate amount of drug must be diluted with 0.9% Sodium Chloride Injection. Final concentrations may be as low as 0.1 mg/mL.

Reconstituted Gemzar is a clear, colorless to light straw-colored solution. Inspect visually prior to administration and discard for particulate matter or discoloration. Gemzar solutions are stable for 24 hours at controlled room temperature of 20° to 25°C (68° to 77°F). Do not refrigerate as crystallization can occur.

No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets.

3 DOSAGE FORMS AND STRENGTHS

Gemzar (gemcitabine for injection USP) is a white to off-white lyophilized powder available in sterile single-use vials containing 200 mg or 1 g gemcitabine.

4 CONTRAINDICATIONS

Gemzar is contraindicated in patients with a known hypersensitivity to gemcitabine.

5 WARNINGS AND PRECAUTIONS

5.1 Schedule-dependent Toxicity

In clinical trials evaluating the maximum tolerated dose of Gemzar, prolongation of the infusion time beyond 60 minutes or more frequent than weekly dosing resulted in an increased incidence of clinically significant hypotension, severe flu-like symptoms, myelosuppression, and asthenia. The half-life of Gemzar is influenced by the length of the infusion *[see Clinical Pharmacology (12.3)]*.

5.2 Myelosuppression

Myelosuppression manifested by neutropenia, thrombocytopenia, and anemia occurs with Gemzar as a single agent and the risks are increased when Gemzar is combined with other cytotoxic drugs. In clinical trials, Grade 3-4 neutropenia, anemia, and thrombocytopenia occurred in 25%, 8%, and 5%, respectively of patients receiving single-agent. The frequencies of Grade 3-4 neutropenia, anemia, and thrombocytopenia varied from 48% to 71%, 8 to 28%, and 5 to 55%, respectively, in patients receiving Gemzar in combination with another drug.

5.3 Pulmonary Toxicity and Respiratory Failure

Pulmonary toxicity, including interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, and adult respiratory distress syndrome (ARDS), has been reported. In some cases, these pulmonary events can lead to fatal respiratory failure despite discontinuation of therapy. The onset of pulmonary symptoms may occur up to 2 weeks after the last dose of Gemzar. Discontinue Gemzar in patients who develop unexplained dyspnea, with or without bronchospasm, or have any evidence of pulmonary toxicity *[see Adverse Reactions (6.1 and 6.2)]*.

5.4 Hemolytic Uremic Syndrome

Hemolytic Uremic Syndrome to include fatalities from renal failure or the requirement for dialysis can occur in patients treated with Gemzar. In clinical trials, HUS was reported in 6 of 2429 patients (0.25%). Most fatal cases of renal failure were due to HUS *[see Adverse Reactions (6.1 and 6.2)]*. Assess renal function prior to initiation of Gemzar and periodically during treatment. Consider the diagnosis of HUS in patients who develops anemia with evidence of microangiopathic hemolysis, elevation of bilirubin or LDH, or reticulocytosis; severe thrombocytopenia; or evidence of renal failure (elevation of serum creatinine or BUN) *[see Dosage and Administration (2.5) and Use In Specific Populations (8.6)]*. Permanently discontinue Gemzar in patients with HUS or severe renal impairment. Renal failure may not be reversible even with discontinuation of therapy. Renal failure may not be reversible even with discontinuation of therapy.

5.5 Hepatic Toxicity

Drug-induced liver injury, including liver failure and death, has been reported in patients receiving Gemzar alone or in combination with other potentially hepatotoxic drugs *[see Adverse Reactions (6.1 and 6.2)]*. Administration of Gemzar in patients with concurrent liver metastases or a pre-existing medical history or hepatitis, alcoholism, or liver cirrhosis can lead to exacerbation of the underlying hepatic insufficiency *[see Use in Specific Populations (8.7)]*. Assess hepatic function prior to initiation of Gemzar and periodically during treatment. Discontinue Gemzar in patients that develop severe liver injury.

5.6 Embryofetal Toxicity

Gemzar can cause fetal harm when administered to a pregnant woman, based on its mechanism of action. Gemcitabine was teratogenic, embryotoxic, and fetotoxic in mice and rabbits. If this drug is used during pregnancy, or if a woman becomes pregnant while taking Gemzar, the patient should be apprised of the potential hazard to a fetus. *[see Use In Specific Populations (8.1)]*

5.7 Exacerbation of Radiation Therapy Toxicity

Gemzar is not indicated for use in combination with radiation therapy.

Concurrent (given together or ≤7 days apart) — Life-threatening mucositis, especially esophagitis and pneumonitis occurred in a trial in which Gemzar was administered at a dose of 1000 mg/m^2 to patients with non-small cell lung cancer for up to 6 consecutive weeks concurrently with thoracic radiation.

Non-concurrent (given >7 days apart) — Excessive toxicity has not been observed when Gemzar is administered more than 7 days before or after radiation. Radiation recall has been reported in patients who receive Gemzar after prior radiation.

5.8 Capillary Leak Syndrome

Capillary leak syndrome (CLS) with severe consequences has been reported in patients receiving Gemzar as a single agent or in combination with other chemotherapeutic agents. Discontinue Gemzar if CLS develops during therapy.

5.9 Posterior Reversible Encephalopathy Syndrome

Posterior reversible encephalopathy syndrome (PRES) has been reported in patients receiving Gemzar as a single agent or in combination with other chemotherapeutic agents. PRES can present with headache, seizure, lethargy, hypertension, confusion, blindness, and other visual and neurologic disturbances. Confirm the diagnosis of PRES with magnetic resonance imaging (MRI) and discontinue Gemzar if PRES develops during therapy.

6 ADVERSE REACTIONS

The following serious adverse reactions are discussed in greater detail in another section of the label

- Schedule-Dependent Toxicity *[see Warnings and Precautions (5.1)]*
- Myelosuppression *[see Warnings and Precautions (5.2)]*
- Pulmonary Toxicity and Respiratory Failure *[see Warnings and Precautions (5.3)]*
- Hemolytic Uremic Syndrome *[see Warnings and Precautions (5.4)]*
- Hepatic Toxicity *[see Warnings and Precautions (5.5)]*
- Embryo-fetal Toxicity *[see Warnings and Precautions (5.6), Use in Specific Populations (8.1), and Nonclinical Toxicology (13.1)]*
- Exacerbation of Radiation Toxicity *[see Warnings and Precautions (5.7)]*
- Capillary Leak Syndrome *[see Warnings and Precautions (5.8)]*
- Posterior Reversible Encephalopathy Syndrome *[see Warnings and Precautions (5.9)]*

6.1 Clinical Trials Experience

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 clinical practice.

Single-Agent Use:

The data described below reflect exposure to Gemzar as a single agent administered at doses between 800 mg/m^2 to 1250 mg/m^2 over 30 minutes intravenously, once weekly, in 979 patients with a variety of malignancies. The most common (≥20%) adverse reactions of single-agent Gemzar are nausea/vomiting, anemia, increased ALT, increased AST, neutropenia, increased alkaline phosphatase, proteinuria, fever, hematuria, rash, thrombocytopenia, dyspnea, and edema. The most common (≥5%) Grade 3 or 4 adverse reactions were neutropenia, nausea/vomiting; increased ALT, increase alkaline phosphatase, anemia, increased AST, and thrombocytopenia. Approximately 10% of the 979 patients discontinued Gemzar due to adverse reactions. Adverse reactions resulting in discontinuation of Gemzar in 2% of 979 patients were cardiovascular adverse events (myocardial infarction, cerebrovascular accident, arrhythmia, and hypertension) and adverse reactions resulting in discontinuation of Gemzar in less than 1% of the 979 patients were anemia, thrombocytopenia, hepatic dysfunction, renal dysfunction, nausea/vomiting, fever, rash, dyspnea, hemorrhage, infection, stomatitis, somnolence, flu-like syndrome, and edema.

Table 5 presents the incidence of adverse reactions reported in 979 patients with various malignancies receiving single-agent Gemzar across 5 clinical trials. Table 5 includes all clinical adverse reactions, reported in at least 10% of patients. A listing of clinically significant adverse reactions is provided following the table.

Table 5: Selected Per-Patient Incidence of Adverse Events in Patients Receiving Single-Agent Gemzar[a]

	All Patients[b]		
	All Grades	Grade 3	Grade 4
Laboratory[c]			
Hematologic			
Anemia	68	7	1
Neutropenia	63	19	6
Thrombocytopenia	24	4	1
Hepatic			
Increased ALT	68	8	2
Increased AST	67	6	2
Increased Alkaline Phosphatase	55	7	2
Hyperbilirubinemia	13	2	<1
Renal			
Proteinuria	45	<1	0
Hematuria	35	<1	0
Increased BUN	16	0	0
Increased Creatinine	8	<1	0
Non-laboratory[d]			
Nausea and Vomiting	69	13	1
Fever	41	2	0

Rash	30	<1	0
Dyspnea	23	3	<1
Diarrhea	19	1	0
Hemorrhage	17	<1	<1
Infection	16	1	<1
Alopecia	15	<1	0
Stomatitis	11	<1	0
Somnolence	11	<1	<1
Paresthesias	10	<1	0

[a] Grade based on criteria from the World Health Organization (WHO).
[b] N=699-974; all patients with laboratory or non-laboratory data.
[c] Regardless of causality.
[d] For approximately 60% of patients, non-laboratory adverse events were graded only if assessed to be possibly drug-related.

- Transfusion requirements — Red blood cell transfusions (19%); platelet transfusions (<1%)
- Fever — Fever occurred in the absence of clinical infection and frequently in combination with other flu-like symptoms.
- Pulmonary — Dyspnea unrelated to underlying disease and sometimes accompanied by bronchospasm.
- Edema — Edema (13%), peripheral edema (20%), and generalized edema (<1%); <1% of patients. discontinued Gemzar due to edema.
- Flu-like Symptoms — Characterized by fever, asthenia, anorexia, headache, cough, chills, myalgia, asthenia insomnia, rhinitis, sweating, and/or malaise (19%); <1% of patients discontinued Gemzar due to flu-like symptoms
- Infection — Sepsis (<1%)
- Extravasation — Injection-site reactions (4%)
- Allergic — Bronchospasm (<2%); anaphylactoid reactions *[see Contraindications (4)]*.

Non-Small Cell Lung Cancer:

Table 6 presents the incidence of selected adverse reactions, occurring in ≥10% of Gemzar-treated patients and at a higher incidence in the Gemzar plus cisplatin arm, reported in a randomized trial of Gemzar plus cisplatin (n=262) administered in 28-day cycles as compared to cisplatin alone (n=260) in patients receiving first-line treatment for locally advanced or metastatic non-small cell lung cancer (NSCLC) *[see Clinical Studies (14.3)]*.

Patients randomized to Gemzar plus cisplatin received a median of 4 cycles of treatment and those randomized to cisplatin received a median of 2 cycles of treatment. In this trial, the requirement for dose adjustments (>90% versus 16%), discontinuation of treatment for adverse reactions (15% versus 8%), and the proportion of patients hospitalized (36% versus 23%) were all higher for patients receiving Gemzar plus cisplatin arm compared to those receiving cisplatin alone. The incidence of febrile neutropenia (9/262 versus 2/260), sepsis (4% versus 1%), Grade 3 cardiac dysrhythmias (3% versus <1%) were all higher in the Gemzar plus cisplatin arm compared to the cisplatin alone arm. The two-drug combination was more myelosuppressive with 4 (1.5%) possibly treatment-related deaths, including 3 resulting from myelosuppression with infection and one case of renal failure associated with pancytopenia and infection. No deaths due to treatment were reported on the cisplatin arm.

Table 6: Per-Patient Incidence of Selected Adverse Reactions from Randomized Trial of Gemzar plus Cisplatin versus Single-Agent Cisplatin in Patients with NSCLC Occurring at Higher Incidence in Gemzar-Treated Patients [Between Arm Difference of ≥5% (All Grades) or ≥2% (Grades 3-4)][a]

	Gemzar plus Cisplatin[b]			Cisplatin[c]		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[d]						
Hematologic						
Anemia	89	22	3	67	6	1
RBC Transfusion[e]	39			13		
Neutropenia	79	22	35	20	3	1
Thrombocytopenia	85	25	25	13	3	1
Platelet Transfusions[e]	21			<1		
Lymphopenia	75	25	18	51	12	5
Hepatic						
Increased Transaminases	22	2	1	10	1	0
Increased Alkaline Phosphatase	19	1	0	13	0	0
Renal						
Proteinuria	23	0	0	18	0	0
Hematuria	15	0	0	13	0	0
Elevated creatinine	38	4	<1	31	2	<1
Other Laboratory						

Hyperglycemia	30	4	0	23	3	0
Hypomagnesemia	30	4	3	17	2	0
Hypocalcemia	18	2	0	7	0	<1
Non-laboratory[f]						
Nausea	93	25	2	87	20	<1
Vomiting	78	11	12	71	10	9
Alopecia	53	1	0	33	0	0
Neuro Motor	35	12	0	15	3	0
Diarrhea	24	2	2	13	0	0
Neuro Sensory	23	1	0	18	1	0
Infection	18	3	2	12	1	0
Fever	16	0	0	5	0	0
Neuro Cortical	16	3	1	9	1	0
Neuro Mood	16	1	0	10	1	0
Local	15	0	0	6	0	0
Neuro Headache	14	0	0	7	0	0
Stomatitis	14	1	0	5	0	0
Hemorrhage	14	1	0	4	0	0
Hypotension	12	1	0	7	1	0
Rash	11	0	0	3	0	0

[a] National Cancer Institute Common Toxicity Criteria (CTC) for severity grading.

[b] N=217-253; all Gemzar plus cisplatin patients with laboratory or non-laboratory data Gemzar at 1000 mg/m^2 on Days 1, 8, and 15 and cisplatin at 100 mg/m^2 on Day 1 every 28 days.

[c] N=213-248; all cisplatin patients with laboratory or non-laboratory data. Cisplatin at 100 mg/m^2 on Day 1 every 28 days.

[d] Regardless of causality.

[e] Percent of patients receiving transfusions. Percent transfusions are not CTC-graded events.

[f] Non-laboratory events were graded only if assessed to be possibly drug-related.

Table 7 presents the incidence of selected adverse reactions, occurring in ≥10% of Gemzar-treated patients and at a higher incidence in the Gemzar plus cisplatin arm, reported in a randomized trial of Gemzar plus cisplatin (n=69) administered in 21-day cycles as compared to etoposide plus cisplatin alone (n=66) in patients receiving first-line treatment for locally advanced or metastatic non-small cell lung cancer (NSCLC) *[see Clinical Studies (14.3)]*. A listing of clinically significant adverse reactions is provided following the table.

Patients in the Gemzar cisplatin (GC) arm received a median of 5 cycles and those in the etoposide/cisplatin (EC) arm received a median of 4 cycles. The majority of patients receiving more than one cycle of treatment required dose adjustments; 81% in the (GC) arm and 68% in the (EC) arm. The incidence of hospitalizations for treatment-related adverse events was 22% (GC) and 27% in the (EC) arm. The proportion of discontinuation of treatment for treatment-related adverse reactions was higher for patients in the (GC) arm (14% versus 8%). The proportion of patients hospitalized for febrile neutropenia was lower in the (GC) arm (7% versus 12%). There was one death attributed to treatment, a patient with febrile neutropenia and renal failure, which occurred in the Gemzar/cisplatin arm.

Table 7: Per-Patient Incidence of Selected Adverse Reactions in Randomized Trial of Gemzar plus Cisplatin versus Etoposide plus Cisplatin in Patients with NSCLC[a]

	Gemzar plus Cisplatin[b]			**Etoposide plus Cisplatin[c]**		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[d]						
Hematologic						
Anemia	88	22	0	77	13	2
RBC Transfusions[e]	29	-	-	21	-	-
Neutropenia	88	36	28	87	20	56
Thrombocytopenia	81	39	16	45	8	5
Platelet Transfusions[e]	3	-	-	8	-	-
Hepatic						
Increased ALT	6	0	0	12	0	0
Increased AST	3	0	0	11	0	0
Increased Alkaline	16	0	0	11	0	0
Phosphatase						
Bilirubin	0	0	0	0	0	0
Renal						
Proteinuria	12	0	0	5	0	0

Hematuria	22	0	0	10	0	0
BUN	6	0	0	4	0	0
Creatinine	2	0	0	2	0	0
Non-laboratory[f,g]						
Nausea and Vomiting	96	35	4	86	19	7
Fever	6	0	0	3	0	0
Rash	10	0	0	3	0	0
Dyspnea	1	0	1	3	0	0
Diarrhea	14	1	1	13	0	2
Hemorrhage	9	0	3	3	0	3
Infection	28	3	1	21	8	0
Alopecia	77	13	0	92	51	0
Stomatitis	20	4	0	18	2	0
Somnolence	3	0	0	3	2	0
Paresthesias	38	0	0	16	2	0

[a] Grade based on criteria from the World Health Organization (WHO).

[b] N=67-69; all Gemzar plus cisplatin patients with laboratory or non-laboratory data. Gemzar at 1250 mg/m^2 on Days 1 and 8 and cisplatin at 100 mg/m^2 on Day 1 every 21 days.

[c] N=57-63; all cisplatin plus etoposide patients with laboratory or non-laboratory data. Cisplatin at 100 mg/m^2 on Day 1 and intravenous etoposide at 100 mg/m^2 on Days 1, 2, and 3 every 21 days.

[d] Regardless of causality.

[e] WHO grading scale not applicable to proportion of patients with transfusions

[f] Non-laboratory events were graded only if assessed to be possibly drug-related.

[g] Pain data were not collected.

- Flu-like syndrome: 3% in the Gemzar/cisplatin arm versus none in the etoposide/cisplatin arm.
- Edema: 12% in the Gemzar/cisplatin arm versus 2% in the etoposide/cisplatin arm.

Breast Cancer

Table 8 presents the incidence of selected adverse reactions, occurring in ≥10% of Gemzar-treated patients and at a higher incidence in the Gemzar plus paclitaxel arm, reported in a randomized trial of Gemzar plus paclitaxel (n=262) compared to paclitaxel alone (n=259) for the first-line treatment of metastatic breast cancer (MBC) in women who received anthracycline-containing chemotherapy in the adjuvant/neo-adjuvant setting or for whom anthracyclines were contraindicated. *[see Clinical Studies (14.2)]*.

The requirement for dose reduction of paclitaxel were higher for patients in the Gemzar/paclitaxel arm (5% versus 2%). The number of paclitaxel doses omitted (<1%), the proportion of patients discontinuing treatment for treatment-related adverse reactions (7% versus 5%), and the number of treatment-related deaths (1 patient in each arm) were similar between the two arms.

Table 8: Per-Patient Incidence of Selected Adverse Reactions from Comparative Trial of Gemzar plus Paclitaxel versus Single-Agent Paclitaxel in Breast Cancer[a] Occurring at Higher Incidence in Gemzar-Treated Patients

[Between Arm Difference of ≥5% (All Grades) or ≥2% (Grades 3-4)]

	Gemzar plus Paclitaxel (N=262)			**Paclitaxel (N=259)**		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[b]						
Hematologic						
Anemia	69	6	1	51	3	<1
Neutropenia	69	31	17	31	4	7
Thrombocytopenia	26	5	<1	7	<1	<1
Hepatobiliary						
Increased ALT	18	5	<1	6	<1	0
Increased AST	16	2	0	5	<1	0
Non-laboratory[c]						
Alopecia	90	14	4	92	19	3
Neuropathy-sensory	64	5	<1	58	3	0
Nausea	50	1	0	31	2	0
Fatigue	40	6	<1	28	1	<1
Vomiting	29	2	0	15	2	0
Diarrhea	20	3	0	13	2	0
Anorexia	17	0	0	12	<1	0
Neuropathy-motor	15	2	<1	10	<1	0

Stomatitis/pharyngitis	13	1	<1	8	<1	0
Fever	13	<1	0	3	0	0
Rash/desquamation	11	<1	<1	5	0	0

[a] Severity grade based on National Cancer Institute Common Toxicity Criteria (CTC) Version 2.0

[b] Regardless of causality.

[c] Non-laboratory events were graded only if assessed to be possibly drug-related.

The following clinically relevant, Grade 3 or 4 adverse reactions occurred with a higher incidence in the Gemzar plus paclitaxel arm compared with the paclitaxel arm: febrile neutropenia (5.0% versus 1.2%) and dyspnea (1.9% versus 0).

Ovarian Cancer

Table 9 presents the incidence of selected adverse reactions, occurring in ≥10% of gemcitabine-treated patients and at a higher incidence in the Gemzar plus carboplatin arm, reported in a randomized trial of Gemzar plus carboplatin (n=175) compared to carboplatin alone (n=174) for the second-line treatment of ovarian cancer in women with disease that had relapsed more than 6 months following first-line platinum-based chemotherapy. *[see Clinical Studies (14.1)]*. Additional clinically significant adverse reactions, occurring in less than 10% of patients, are provided following Table 9.

The proportion of patients with dose adjustments for carboplatin (1.8% versus 3.8%), doses of carboplatin omitted (0.2% versus 0), and discontinuing treatment for treatment-related adverse reactions (10.9% versus 9.8%), were similar between arms. Dose adjustment for Gemzar occurred in 10.4% of patients and Gemzar dose was omitted in 13.7% of patients in the Gemzar /carboplatin arm.

Table 9: Per-Patient Incidence of Adverse Reactions in Randomized Trial of Gemzar plus Carboplatin versus Carboplatin in Ovarian Cancer Occurring at Higher Incidence in Gemzar-Treated Patients [Between Arm Difference of ≥5% (All Grades) or ≥2% (Grades 3-4)]

	Gemzar plus Carboplatin (N=175)			**Carboplatin (N=174)**		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Laboratory[b]						
Hematologic						
Neutropenia	90	42	29	58	11	1
Anemia	86	22	6	75	9	2
Thrombocytopenia	78	30	5	57	10	1
RBC Transfusions[c]	38			15		
Platelet Transfusions[c]	9			3		
Non-laboratory[b]						
Nausea	69	6	0	61	3	0
Alopecia	49	0	0	17	0	0
Vomiting	46	6	0	36	2	<1
Constipation	42	6	1	37	3	0
Fatigue	40	3	<1	32	5	0
Diarrhea	25	3	0	14	<1	0
Stomatitis/pharyngitis	22	<1	0	13	0	0

[a] Grade based on Common Toxicity Criteria (CTC) Version 2.0.

[b] Regardless of causality.

[c] Percent of patients receiving transfusions. Transfusions are not CTC-graded events. Blood transfusions included both packed red blood cells and whole blood.

Hematopoietic growth factors were administered more frequently in the Gemzar-containing arm: granulocyte growth factors (23.6% and 10.1%) and erythropoietic agents (7.3% and 3.9%).

The following clinically relevant, Grade 3 and 4 adverse reactions occurred more frequently in the Gemzar plus carboplatin arm: dyspnea (3.4% versus 2.9%), febrile neutropenia (1.1% versus 0), hemorrhagic event (2.3% versus 1.1 %), motor neuropathy (1.1% versus 0.6%), and rash/desquamation (0.6% versus 0).

6.2 Post-Marketing Experience

The following adverse reactions have been identified during post-approval use of Gemzar. 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.

Cardiovascular — Congestive heart failure, myocardial infarction, Arrhythmias, supraventricular arrhythmias.

Vascular Disorders — Peripheral vasculitis, gangrene, and capillary leak syndrome *[see Warnings and Precautions (5.9)]*

Skin — Cellulitis, severe skin reactions, including desquamation and bullous skin eruptions

Hepatic — Hepatic failure, hepatic veno-occlusive disease

Pulmonary — Interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, and adult respiratory distress syndrome (ARDS)

Nervous System — Posterior reversible encephalopathy syndrome (PRES) *[see Warnings and Precautions (5.9)]*

7 DRUG INTERACTIONS

No drug interaction studies have been conducted.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D. *[see Warnings and Precautions (5.6)]*

Risk Summary

Gemzar can cause fetal harm when administered to a pregnant woman. Based on its mechanism of action, Gemzar is expected to result in adverse reproductive effects. Gemcitabine was teratogenic, embryotoxic, and fetotoxic in mice and rabbits. If Gemzar is used during pregnancy, or if the patient becomes pregnant while taking Gemzar, the patient should be apprised of the potential hazard to a fetus.

Animal Data

Gemcitabine is embryotoxic causing fetal malformations (cleft palate, incomplete ossification) at doses of 1.5 mg/kg/day in mice (approximately 0.005 times the recommended human dose on a mg/m^2 basis). Gemcitabine is fetotoxic causing fetal malformations (fused pulmonary artery, absence of gall bladder) at doses of 0.1 mg/kg/day in rabbits (about 0.002 times the recommended human dose on a mg/m^2 basis). Embryotoxicity was characterized by decreased fetal viability, reduced live litter sizes, and developmental delays. *[see Warnings and Precautions (5.6)]*

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Gemzar, a decision should be made whether 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 Gemzar have not been established in pediatric patients. The safety and pharmacokinetics of gemcitabine were evaluated in a trial in pediatric patients with refractory leukemia. The maximum tolerated dose was 10 mg/m^2/min for 360 minutes three times weekly followed by a one-week rest period. The safety and activity of Gemzar were evaluated in a trial of pediatric patients with relapsed acute lymphoblastic leukemia (22 patients) and acute myelogenous leukemia (10 patients) at a dose of 10 mg/m^2/min administered over 360 minutes three times weekly followed by a one-week rest period. Toxicities observed included bone marrow suppression, febrile neutropenia, elevation of serum transaminases, nausea, and rash/desquamation. No meaningful clinical activity was observed in this trial.

8.5 Geriatric Use

In clinical studies of GEMZAR, enrolling 979 patients with various cancers who received GEMZAR as a single agent, no overall differences in safety were observed between patients aged 65 and older and younger patients, with the exception of a higher rate of Grade 3-4 thrombocytopenia in older patients as compared to younger patients. In a randomized trial in women with ovarian cancer, 175 women received GEMZAR plus carboplatin, of which 29% were age 65 years or older. Similar effectiveness was observed between older and younger women. There was significantly higher Grade 3/4 neutropenia in women 65 years of age or older.

GEMZAR clearance is affected by age, however there are no recommended dose adjustments based on patients' age *[see Clinical Pharmacology (12.3)]*.

8.6 Renal Impairment

No clinical studies have been conducted with gemcitabine in patients with decreased renal function.

8.7 Hepatic Impairment

No clinical studies have been conducted with gemcitabine in patients with decreased hepatic function.

8.8 Gender

Gemzar clearance is affected by gender *[see Clinical Pharmacology (12.3)]*. In single-agent studies of Gemzar, women, especially older women, were more likely not to proceed to a subsequent cycle and to experience Grade 3/4 neutropenia and thrombocytopenia.

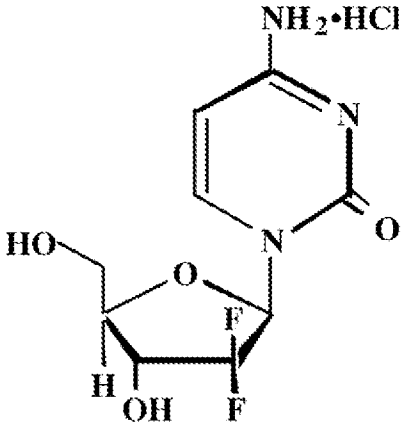
10 OVERDOSAGE

Myelosuppression, paresthesias, and severe rash were the principal toxicities seen when a single dose as high as 5700 mg/m^2 was administered by intravenous infusion over 30 minutes every 2 weeks to several patients in a dose-escalation study.

11 DESCRIPTION

Gemzar (gemcitabine for injection, USP) is a nucleoside metabolic inhibitor that exhibits antitumor activity. Gemcitabine HCl is 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer).

The structural formula is as follows:



The empirical formula for gemcitabine HCl is $C_9H_{11}F_2N_3O_4$ • HCl. It has a molecular weight of 299.66.

Gemcitabine HCl is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

Gemzar is supplied in a sterile form for intravenous use only. Vials of Gemzar contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Gemcitabine kills cells undergoing DNA synthesis and blocks the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolized by nucleoside kinases to diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. Gemcitabine diphosphate inhibits ribonucleotide reductase, an enzyme responsible for catalyzing the reactions that generate deoxynucleoside triphosphates for DNA synthesis, resulting in reductions in deoxynucleotide concentrations, including dCTP. Gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP by the action of the diphosphate enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands, which eventually results in the initiation of apoptotic cell death.

12.3 Pharmacokinetics

Absorption and Distribution

The pharmacokinetics of gemcitabine were examined in 353 patients, with various solid tumors. Pharmacokinetic parameters were derived using data from patients treated for varying durations of therapy given weekly with periodic rest weeks and using both short infusions (<70 minutes) and long infusions (70 to 285 minutes). The total Gemzar dose varied from 500 to 3600 mg/m^2.

The volume of distribution was increased with infusion length. Volume of distribution of gemcitabine was 50 L/m^2 following infusions lasting <70 minutes. For long infusions, the volume of distribution rose to 370 L/m^2.

Gemcitabine pharmacokinetics are linear and are described by a 2-compartment model. Population pharmacokinetic analyses of combined single and multiple dose studies showed that the volume of distribution of gemcitabine was significantly influenced by duration of infusion and gender. Gemcitabine plasma protein binding is negligible.

Metabolism

Gemcitabine disposition was studied in 5 patients who received a single 1000 mg/m^2/30 minute infusion of radiolabeled drug. Within one (1) week, 92% to 98% of the dose was recovered, almost entirely in the urine. Gemcitabine (<10%) and the inactive uracil metabolite, 2′-deoxy-2′,2′-difluorouridine (dFdU), accounted for 99% of the excreted dose. The metabolite dFdU is also found in plasma.

The active metabolite, gemcitabine triphosphate, can be extracted from peripheral blood mononuclear cells. The half-life of the terminal phase for gemcitabine triphosphate from mononuclear cells ranges from 1.7 to 19.4 hours.

Elimination

Clearance of gemcitabine was affected by age and gender. The lower clearance in women and the elderly results in higher concentrations of gemcitabine for any given dose. Differences in either clearance or volume of distribution based on patient characteristics or the duration of infusion result in changes in half-life and plasma concentrations. Table 10 shows plasma clearance and half-life of gemcitabine following short infusions for typical patients by age and gender.

Table 10: Gemcitabine Clearance and Half-Life for the "Typical" Patient

Age	Clearance Men (L/hr/m^2)	Clearance Women (L/hr/m^2)	Half-Life[a] Men (min)	Half-Life[a] Women (min)
29	92.2	69.4	42	49
45	75.7	57.0	48	57

65	55.1	41.5	61	73
79	40.7	30.7	79	94

[a] Half-life for patients receiving <70 minute infusion.

Gemcitabine half-life for short infusions ranged from 42 to 94 minutes, and the value for long infusions varied from 245 to 638 minutes, depending on age and gender, reflecting a greatly increased volume of distribution with longer infusions.

Drug Interactions

When Gemzar (1250 mg/m^2 on Days 1 and 8) and cisplatin (75 mg/m^2 on Day 1) were administered in NSCLC patients, the clearance of gemcitabine on Day 1 was 128 L/hr/m^2 and on Day 8 was 107 L/hr/m^2. Analysis of data from metastatic breast cancer patients shows that, on average, Gemzar has little or no effect on the pharmacokinetics (clearance and half-life) of paclitaxel and paclitaxel has little or no effect on the pharmacokinetics of gemcitabine. Data from NSCLC patients demonstrate that Gemzar and carboplatin given in combination does not alter the pharmacokinetics of gemcitabine or carboplatin compared to administration of either single agent. However, due to wide confidence intervals and small sample size, interpatient variability may be observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies to evaluate the carcinogenic potential of Gemzar have not been conducted. Gemcitabine was mutagenic in an *in vitro* mouse lymphoma (L5178Y) assay and was clastogenic in an *in vivo* mouse micronucleus assay. Gemcitabine IP doses of 0.5 mg/kg/day (about 1/700 the human dose on a mg/m^2 basis) in male mice had an effect on fertility with moderate to severe hypospermatogenesis, decreased fertility, and decreased implantations. In female mice, fertility was not affected but maternal toxicities were observed at 1.5 mg/kg/day administered intravenously (about 1/200 the human dose on a mg/m^2 basis) and fetotoxicity or embryolethality was observed at 0.25 mg/kg/day administered intravenously (about 1/1300 the human dose on a mg/m^2 basis).

14 CLINICAL STUDIES

14.1 Ovarian Cancer

The safety and efficacy of Gemzar was studied in a randomized trial of 356 women with advanced ovarian cancer that had relapsed at least 6 months after first-line platinum-based therapy. Patients were randomized to receive either Gemzar 1000 mg/m^2 on Days 1 and 8 of a 21-day cycle and carboplatin AUC 4 administered after Gemzar infusion on Day 1 of each cycle (n=178) or to carboplatin AUC 5 administered on Day 1 of each 21-day cycle (n=178). The primary efficacy outcome measure was progression free survival (PFS).

Patient characteristics are shown in Table 11. The addition of Gemzar to carboplatin resulted in statistically significant improvements in PFS and overall response rate as shown in Table 12 and Figure 1. Approximately 75% of patients in each arm received additional chemotherapy for disease progression; 13 of 120 patients in the carboplatin alone arm received Gemzar for treatment of disease progression. There was no significant difference in overall survival between the treatment arms.

Table 11: Randomized Trial of Gemzar plus Carboplatin versus Carboplatin in Ovarian Cancer - Baseline Demographics and Clinical Characteristics

	Gemzar/Carboplatin	Carboplatin
Number of randomized patients	178	178
Median age, years Range	59 36 to 78	58 21 to 81
Baseline ECOG performance status 0-1[a]	94%	95%
Disease Status Evaluable Bidimensionally measurable	 8% 92%	 3% 96%
Platinum-free interval[b] 6-12 months >12 months	 40% 59%	 40% 60%
First-line therapy Platinum-taxane combination Platinum-non-taxane combination Platinum monotherapy	 70% 29% 1%	 71% 28% 1%

[a] 5 patients on Gemzar plus carboplatin arm and 4 patients on carboplatin arm with no baseline Eastern Cooperative Oncology Group (ECOG) performance status.

[b] 2 on Gemzar plus carboplatin arm and 1 on carboplatin arm had platinum-free interval <6 months.

Table 12: Randomized Trial of Gemzar plus Carboplatin versus Carboplatin in Ovarian Cancer - Efficacy Outcomes

	Gemzar/Carboplatin (N=178)	Carboplatin (N=178)
Progression-free Survival		

Median (95% CI[a]) months	8.6 (8.0, 9.7)	5.8 (5.2, 7.1)
Hazard Ratio (95% CI)	0.72 (0.57, 0.90)	
p-value[b]	p=0.0038	
Overall Survival		
Median (95% CI) months	18.0 (16.2, 20.3)	17.3 (15.2, 19.3)
Hazard Ratio (95% CI)	0.98 (0.78, 1.24)	
p-value[b]	p=0.8977	
Investigator Reviewed Overall Response Rate	47.2%	30.9%
p-value[e]	p=0.0016	
CR[d]	14.6%	6.2%
PR plus PRNM[e]	32.6%	24.7%
Independently Reviewed Overall Response Rate[f]	46.3%	35.6%
p-value[e]	p=0.11	
CR[d]	9.1%	4.0%
PR plus PRNM[e]	37.2%	31.7%

[a] CI=confidence interval
[b] Complete response
[c] Partial response plus partial response, non-measurable disease
[d] log Rank, unadjusted
[e] chi Square
[f] Independently reviewed cohort - Gemzar/carboplatin (n=121), carboplatin (n=101); independent reviewers unable to measure disease detected by sonography or physical exam

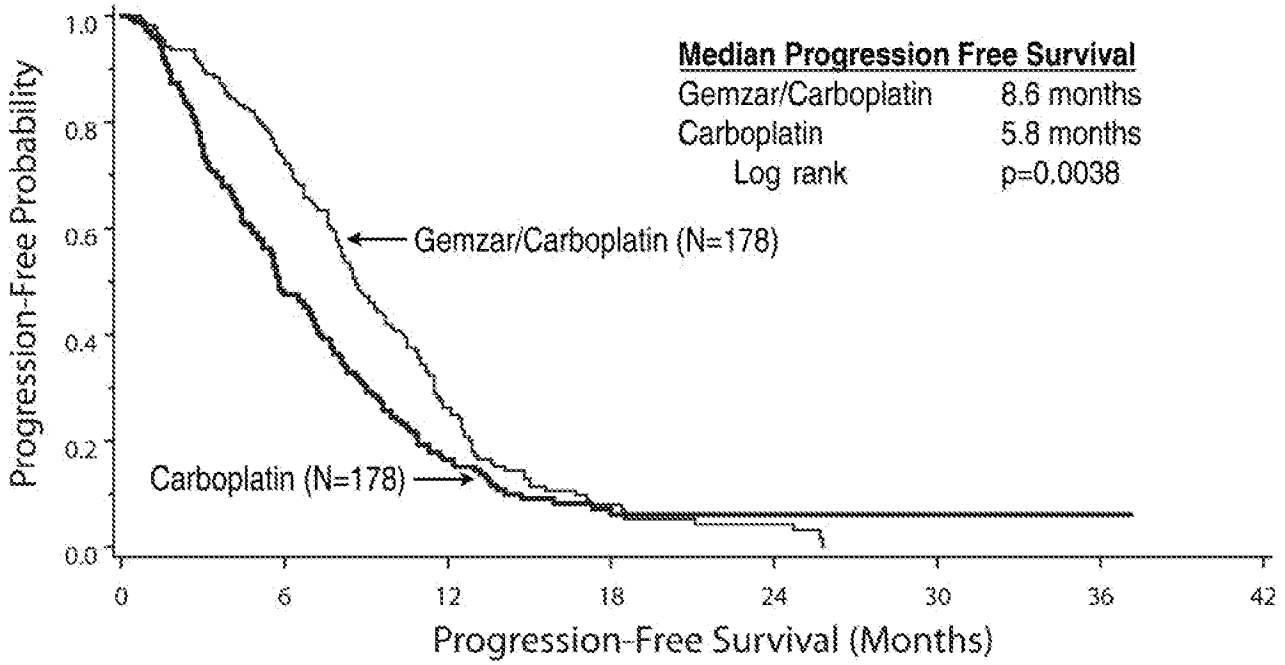


Figure 1: Kaplan-Meier Curve of Progression Free Survival in Gemzar plus Carboplatin versus Carboplatin in Ovarian Cancer (N=356)

14.2 Breast Cancer

The safety and efficacy of Gemzar were evaluated in a multi-national, randomized, open-label trial conducted in women receiving initial treatment for metastatic breast cancer in women who have received prior adjuvant/neoadjuvant anthracycline chemotherapy unless clinically contraindicated. Patients were randomized to receive Gemzar 1250 mg/m^2 on Days 1 and 8 of a 21-day cycle and paclitaxel 175 mg/m^2 administered prior to Gemzar on Day 1 of each cycle (n=267) or to receive paclitaxel 175 mg/m^2 was administered on Day 1 of each 21-day cycle (n=262). The primary efficacy outcome measure was time to documented disease progression.

A total of 529 patients were enrolled; 267 were randomized to Gemzar and paclitaxel and 262 to paclitaxel alone. Demographic and baseline characteristics were similar between treatment arms (see Table 13). Efficacy results are presented in Table

13 and Figure 2. The addition of Gemzar to paclitaxel resulted in statistically significant improvement in time to documented disease progression and overall response rate compared to paclitaxel alone. There was no significant difference in overall survival.

Table 13: Randomized Trial of Gemzar plus Paclitaxel versus Paclitaxel in Breast Cancer

	Gemzar/Paclitaxel	Paclitaxel
Number of patients	267	262
Demographic/Entry Characteristics		
Median age (years) Range	53 26 to 83	52 26 to 75
Metastatic disease	97%	97%
Baseline KPS[a] ≥90	70%	74%
Number of tumor sites 1-2 ≥3	 57% 43%	 59% 41%
Visceral disease	73%	73%
Prior anthracycline	97%	96%
Efficacy Outcomes		
Time to Documented Disease Progression[b]		
Median in months (95% CI)	5.2 (4.2, 5.6)	2.9 (2.6, 3.7)
Hazard Ratio (95% CI)	0.650 (0.524, 0.805)	
p-value	p<0.0001	
Overall Survival[c]		
Median Survival in months (95% CI)	18.6 (16.5, 20.7)	15.8 (14.1, 17.3)
Hazard Ratio (95% CI)	0.86 (0.71, 1.04)	
p-value	Not Significant	
Overall Response Rate (95% CI)	40.8% (34.9, 46.7)	22.1% (17.1, 27.2)
p-value	p<0.0001	

[a] Karnofsky Performance Status.

[b] These represent reconciliation of investigator and Independent Review Committee assessments according to a predefined algorithm.

[c] Based on the ITT population

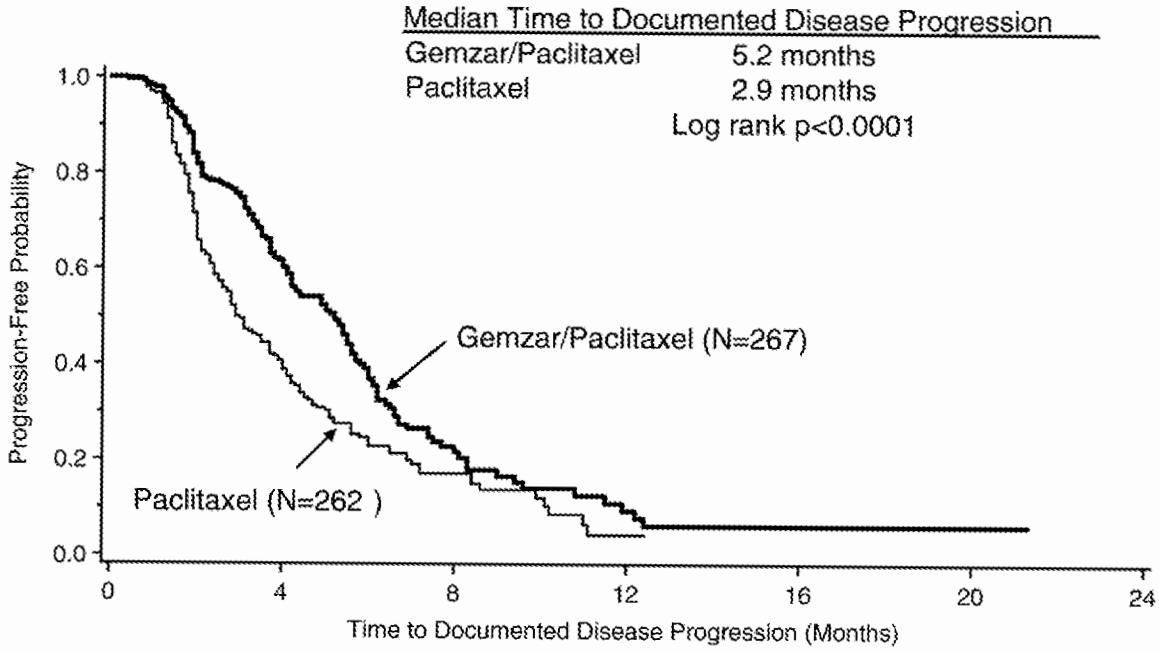


Figure 2: Kaplan-Meier Curve of Time to Documented Disease Progression in Gemzar plus Paclitaxel versus Paclitaxel Breast Cancer Study (N=529)

14.3 Non-Small Cell Lung Cancer (NSCLC)

The safety and efficacy of Gemzar was evaluated in two randomized, multicenter trials.

28-Day Schedule

A multinational, randomized trial compared Gemzar plus cisplatin to cisplatin alone in the treatment of patients with inoperable Stage IIIA, IIIB, or IV NSCLC who had not received prior chemotherapy. Patients were randomized to receive Gemzar 1000 mg/m^2 on Days 1, 8, and 15 of a 28-day cycle with cisplatin 100 mg/m^2 administered on Day 1 of each cycle or to receive cisplatin 100 mg/m^2 on Day 1 of each 28-day cycle. The primary efficacy outcome measure was overall survival. A total of 522 patients were enrolled at clinical centers in Europe, the US, and Canada. Patient demographics and baseline characteristics (shown in Table 13) were similar between arms with the exception of histologic subtype of NSCLC, with 48% of patients on the cisplatin arm and 37% of patients on the Gemzar plus cisplatin arm having adenocarcinoma. Efficacy results are presented in Table 13 and Figure 3 for overall survival.

21-Day Schedule

A randomized (1:1), multicenter trial was conducted in 135 patients with Stage IIIB or IV NSCLC. Patients were randomized to receive Gemzar 1250 mg/m^2 on Days 1 and 8, and cisplatin 100 mg/m^2 on Day 1 of a 21-day cycle or to receive etoposide 100 mg/m^2 intravenously on Days 1, 2, and 3 and cisplatin 100 mg/m^2 on Day 1 of a 21 -day cycle.

There was no significant difference in survival between the two treatment arms (Log rank p=0.18, two-sided). The median survival was 8.7 months for the Gemzar plus cisplatin arm versus 7.0 months for the etoposide plus cisplatin arm. Median time to disease progression for the Gemzar plus cisplatin arm was 5.0 months compared to 4.1 months on the etoposide plus cisplatin arm (Log rank p=0.015, two-sided). The objective response rate for the Gemzar plus cisplatin arm was 33% compared to 14% on the etoposide plus cisplatin arm (Fisher's Exact p=0.01, two-sided).

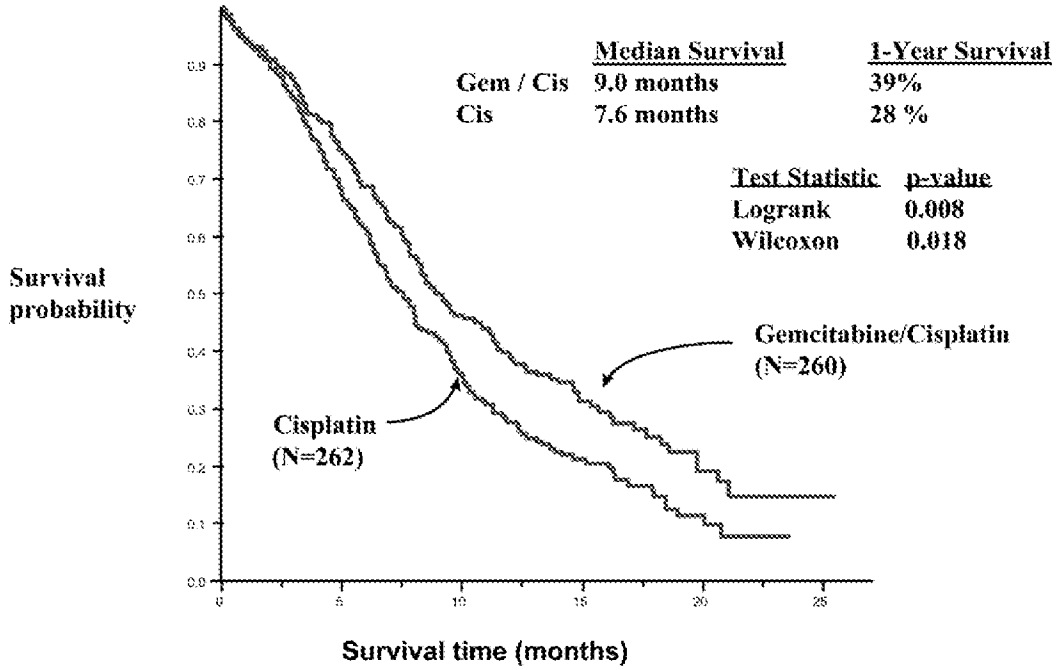


Figure 3: Kaplan-Meier Survival Curve in Gemzar plus Cisplatin versus Cisplatin in Patients with NSCLC Study (N=522)

Table 14: Randomized Trials of Gemzar plus Cisplatin in Patients with NSCLC

Trial	28-day Schedule[a]		21-day Schedule[b]	
Treatment Arm	Gemzar plus Cisplatin	Cisplatin	Gemzar plus Cisplatin	Etoposide plus Cisplatin
Number of patients	260	262	69	66
Demographic/Entry Characteristics				
Male	70%	71%	93%	92%
Median age, years	62	63	58	60
Range	36 to 88	35 to 79	33 to 76	35 to 75
Stage IIIA	7%	7%	N/A[c]	N/A[c]
Stage IIIB	26%	23%	48%	52%
Stage IV	67%	70%	52%	49%
Baseline KPS[d] 70 to 80	41%	44%	45%	52%

Baseline KPS[d] 90 to 100	57%	55%	55%	49%
Efficacy Outcomes				
Survival				
Median in months	9.0	7.6	8.7	7.0
(95% CI[e]) months	8.2, 11.0	6.6, 8.8	7.8, 10.1	6.0, 9.7
p-value[f]	p=0.008		p=0.18	
Time to Disease Progression				
Median in months	5.2	3.7	5.0	4.1
(95% CI[e]) months	4.2, 5.7	3.0, 4.3	4.2, 6.4	2.4, 4.5
p-value[f]	p=0.009		p=0.015	
Tumor Response	26%	10%	33%	14%
p-value[f]	p<0.0001		p=0.01	

[a] 28-day schedule — Gemzar plus cisplatin: Gemzar 1000 mg/m^2 on Days 1, 8, and 15 and cisplatin 100 mg/m^2 on Day 1 every 28 days; Single-agent cisplatin: cisplatin 100 mg/m^2 on Day 1 every 28 days.

[b] 21-day schedule — Gemzar plus cisplatin: Gemzar 1250 mg/m^2 on Days 1 and 8 and cisplatin 100 mg/m^2 on Day 1 every 21 days; Etoposide plus Cisplatin: cisplatin 100 mg/m^2 on Day 1 and intravenous etoposide 100 mg/m^2 on Days 1, 2, and 3 every 21 days.

[c] N/A Not applicable.

[d] Karnofsky Performance Status.

[e] CI=confidence intervals

[f] p-value two-sided Fisher's Exact test for difference in binomial proportions; log rank test for time-to-event analyses.

14.4 Pancreatic Cancer

The safety and efficacy of Gemzar was evaluated in two trials, a randomized, single-blind, two-arm, active-controlled trial conducted in patients with locally advanced or metastatic pancreatic cancer who had received no prior chemotherapy and in a single-arm, open-label, multicenter trial conducted in patients with locally advanced or metastatic pancreatic cancer previously treated with 5-FU or a 5-FU-containing regimen. The first trial randomized patients to receive Gemzar 1000 mg/m^2 intravenously over 30 minutes once weekly for 7 weeks followed by a one-week rest, then once weekly dosing for 3 consecutive weeks every 28-days in subsequent cycles (n=63) or to 5-fluorouracil (5-FU) 600 mg/m^2 intravenously over 30 minutes once weekly (n=63). In the second trial, all patients received Gemzar 1000 mg/m^2 intravenously over 30 minutes once weekly for 7 weeks followed by a one-week rest, then once weekly dosing for 3 consecutive weeks every 28-days in subsequent cycles.

The primary efficacy outcome measure in both trials was "clinical benefit response". A patient was considered to have had a clinical benefit response if either occurred:

- The patient achieved a ≥50% reduction in pain intensity (Memorial Pain Assessment Card) or analgesic consumption, or a 20-point or greater improvement in performance status (Karnofsky Performance Status) for a period of at least 4 consecutive weeks, without showing any sustained worsening in any of the other parameters. Sustained worsening was defined as 4 consecutive weeks with either any increase in pain intensity or analgesic consumption or a 20-point decrease in performance status occurring during the first 12 weeks of therapy.

OR

- The patient was stable on all of the aforementioned parameters, and showed a marked, sustained weight gain (≥7% increase maintained for ≥4 weeks) not due to fluid accumulation.

The randomized trial enrolled 126 patients across 17 sites in the US and Canada. The demographic and entry characteristics were similar between the arms (Table 15). The efficacy outcome results are shown in Table 15 and for overall survival in Figure 4. Patients treated with Gemzar had statistically significant increases in clinical benefit response, survival, and time to disease progression compared to those randomized to receive 5-FU. No confirmed objective tumor responses were observed in either treatment arm.

Table 15: Randomized Trial of Gemzar versus 5-Fluorouracil in Pancreatic Cancer

	Gemzar	5-FU
Number of patients	63	63
Demographic/Entry Characteristics		
Male	54%	54%
Median age	62 years	61 years
Range	37 to 79	36 to 77
Stage IV disease	71%	76%
Baseline KPS[a] ≤70	70%	68%
Efficacy Outcomes		
Clinical benefit response	22.2%	4.8%
p-value[b]	p=0.004	

Survival		
Median	5.7 months	4.2 months
(95% CI)	(4.7, 6.9)	(3.1, 5.1)
p-value[b]	p=0.0009	
Time to Disease Progression		
Median	2.1 months	0.9 months
(95% CI)	(1.9, 3.4)	(0.9, 1.1)
p-value[b]	p=0.0013	

[a] Karnofsky Performance Status.

[b] p-value for clinical benefit response calculated using the two-sided test for difference in binomial proportions. All other p-values are calculated using log rank test.

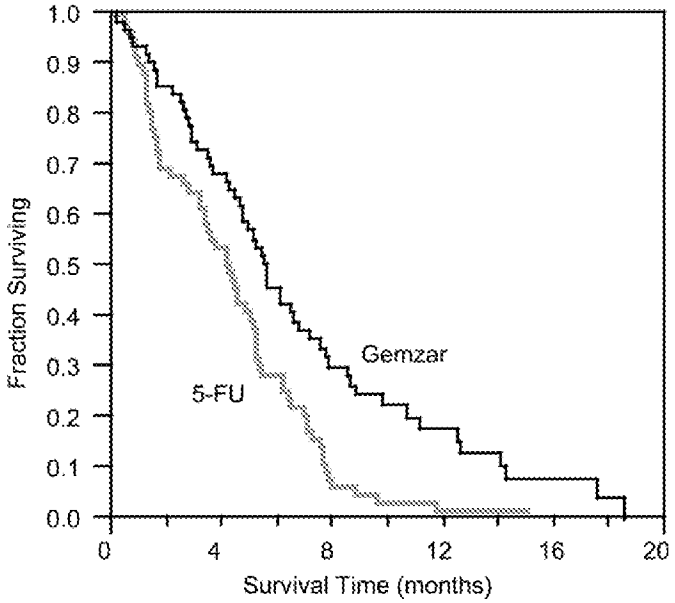


Figure 4: Kaplan-Meier Survival Curve

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Gemzar (gemcitabine for injection, USP), is available in sterile single-use vials individually packaged in a carton containing:
200 mg white to off-white, lyophilized powder in a 10-mL size sterile single-use vial – NDC 0002-7501-01 (No. 7501)
1 g white to off-white, lyophilized powder in a 50-mL size sterile single-use vial – NDC 0002-7502-01 (No. 7502)

16.2 Storage and Handling

Unopened vials of Gemzar are stable until the expiration date indicated on the package when stored at controlled room temperature 20° to 25°C (68° to 77°F) and that allows for excursions between 15° and 30°C (59° and 86°F) [See USP Controlled Room Temperature] *[see Dosage and Administration (2.5 and 2.6)]*.

17 PATIENT COUNSELING INFORMATION

- Advise patients of the risks of low blood cell counts and the potential need for blood transfusions and increased susceptibility to infections. Instruct patients to immediately contact their healthcare provided for development of signs or symptoms of infection, fever, prolonged or unexpected bleeding, bruising, or shortness of breath *[see Warnings and Precautions (5.2)]*
- Advise patients of the risks of pulmonary toxicity including respiratory failure and death. Instruct patients to immediately contact their healthcare provider for development of shortness of breath, wheezing, or cough *[see Warnings and Precautions (5.3)]*
- Advise patients of the risks of hemolytic-uremic syndrome and associated renal failure. Instruct patients to immediately contact their healthcare provider for changes in the color or volume of urine output or for increased bruising or bleeding *[see Warnings and Precautions (5.4)]*
- Advise patients of the risks of hepatic toxicity including liver failure and death. Instruct patients to immediately contact their healthcare provider for signs of jaundice or for pain/tenderness in the right upper abdominal quadrant *[see Warnings and Precautions (5.5)]*

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Anti-HER2 Immunoliposomes for Targeted Drug Delivery

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ABSTRACT

No Abstract

Anti-HER2 Immunoliposomes for Targeted Drug Delivery

K. HONG,[a,c] D.B. KIRPOTIN,[a] J.W. PARK,[b] Y. SHAO,[a] R. SHALABY,[a] G. COLBERN,[a] C.C. BENZ,[b] AND D. PAPAHADJOPOULOS[a]

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The pharmaceutical application of liposomes has been greatly advanced over the last decade by the introduction of long-circulating liposomes,[1,2] refinement of liposome preparation techniques,[3] and efficient loading of drug into liposomes.[4] The development of stable, long circulating liposomes has led to a new era in liposome drug delivery. For example, sterically stabilized liposomes (Doxil, Sequus Pharmaceuticals, Inc.) were generated by grafting polyethyleneglycol (PEG) onto the liposome surface. These liposomes display prolonged drug circulation and extravasate in solid tumors. However, these liposomes do not interact directly with tumor cells *in vitro* or *in vivo,* and instead they release drug for eventual diffusion into tumor cells.

Similarly, progress in monoclonal antibody (mAb)-based therapy of cancer has led to clinical validation after two decades of research (reviewed in ref. 5). A leading example has been the development of mAb directed against the p185HER2 (HER2) receptor tyrosine kinase, the product of the HER2 (erbB2, *neu*). HER2 is highly overexpressed in a significant proportion of cancers and HER2 overexpression is clearly associated with poor prognosis in breast cancer.[6]

Anti–HER2 immunoliposomes were developed to combine the tumor-targeting properties of mAbs such as rhuMAbHER2 (recombinant humanized anti-HER2 monoclonal antibody) with the drug delivery properties of sterically stabilized liposomes. We previously showed that anti-HER2 immunoliposomes efficiently bind to and internalize in HER2-overexpressing cells *in vitro*, resulting in intracellular drug delivery.[7,8] Here we show the therapeutic properties of anti-HER2 immunoliposomes containing doxorubicin (dox) in animal models (FIG.1) and the novel mechanism of intracellular drug delivery of anti-HER2 immunoliposomes in these models (FIG. 2).

The antitumor efficacy of anti-HER2 immunoliposome–dox against human breast cancer xenografts was compared with control groups: saline and nontargeted liposome-dox + free rhuMAbHER2 (FIG. 1). Anti-HER2 immunoliposome-dox tested produced marked antitumor effects, including tumor growth inhibition, tumor regressions, and cures of mice and showed superior activity to the other treatment conditions.

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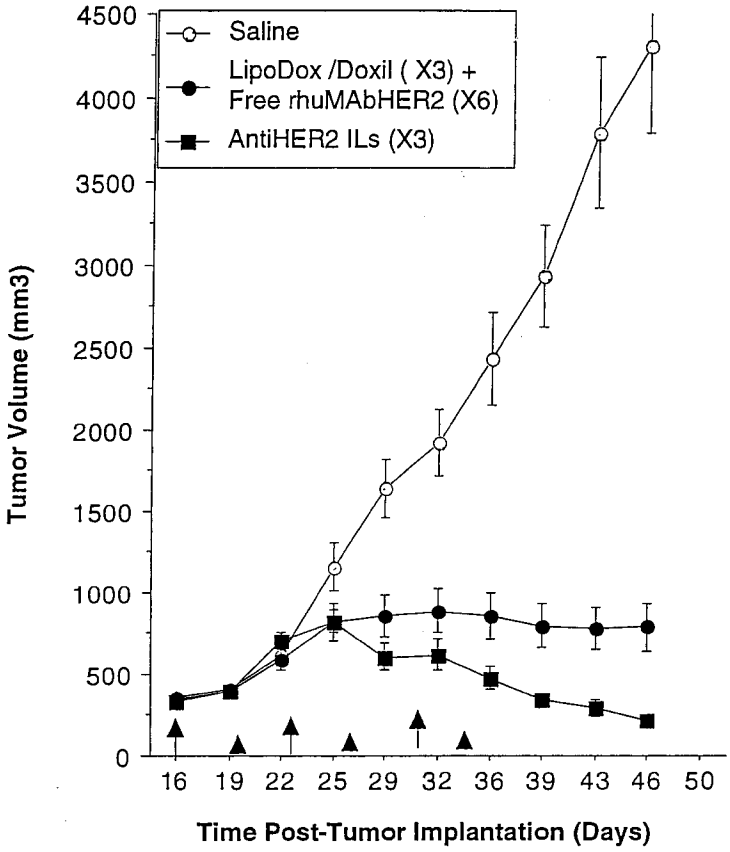

FIGURE 1. Efficacy of anti-HER2 immunoliposome-dox versus combination therapy in the BT-474 (HER2-overexpressing human breast cancer cells) tumor xenograft model. Dox-loaded anti-HER2 immunoliposomes containing rhuMAbHER2 Fab' covalently conjugated on the distal end of PEG-phosphatidylethanolamine (*square*) versus combination therapy (*circles*) of liposomal dox (Doxil) + free rhuMAbHER2 (Herceptin). Immunoliposomes and liposomal dox were administered iv at a total dox dose of 15 mg/kg on the indicated days posttumor implantation (*arrows*), and rhuMAbHER2 was administered at 0.3 mg/kg twice weekly over 6 doses (*arrows and arrowheads*).

Examination of tumors following iv treatment with gold-labeled immunoliposomes or liposomes revealed dramatic differences in intratumoral distribution and mechanism of delivery (FIG. 2). Immunoliposomes were observed dispersed throughout the tumor, and on higher magnification, they were predominantly seen within the cytoplasm of tumor cells. By contrast, liposomes accumulated extracellularly or within resident macrophages. These results have now confirmed that immunoliposomes, unlike nontargeted liposomes, achieved intracellular drug delivery *in vivo*. It is likely that this mechanism accounts for the significantly enhanced efficacy of immunoliposomes against HER2-overexpressing tumors.

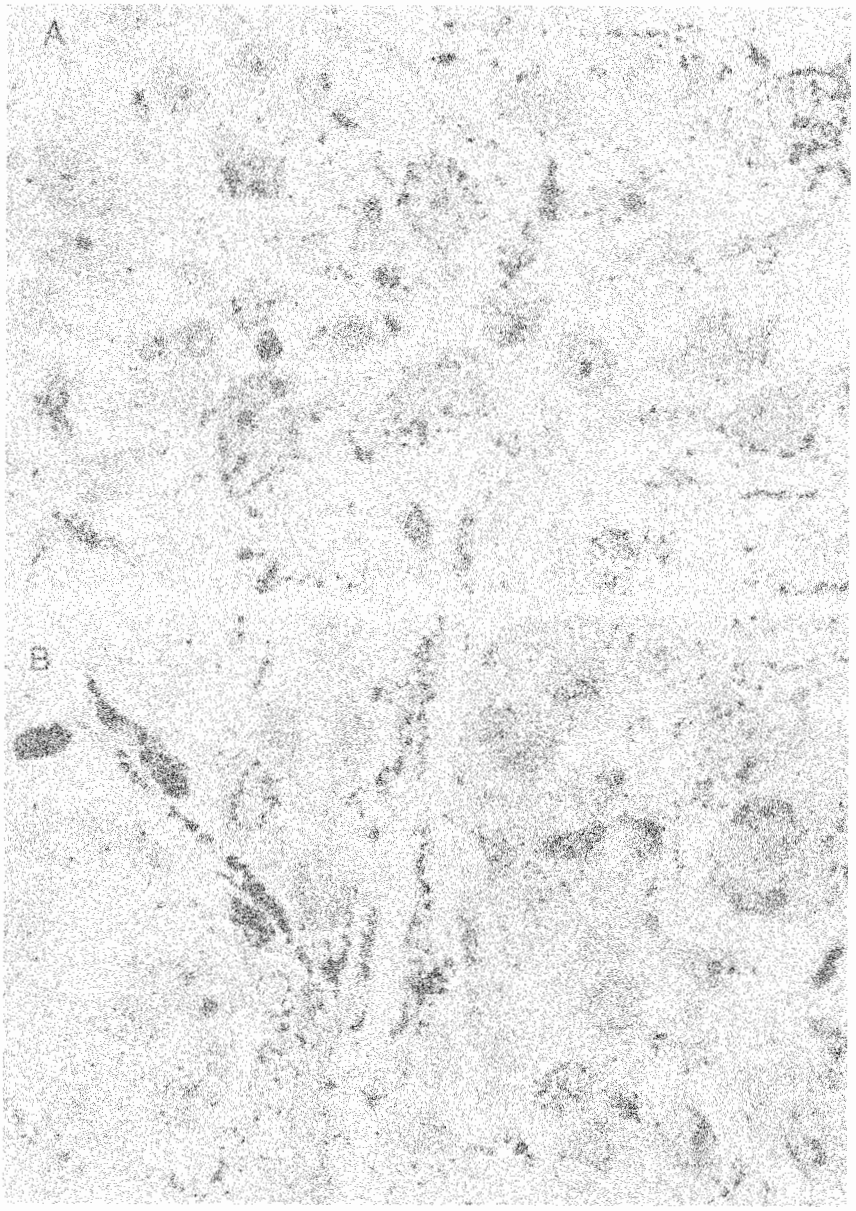

FIGURE 2. Localization of anti-HER2 immunoliposomes versus liposomes in breast cancer xenografts. Liposomes were loaded with colloidal gold and injected iv in nude mice bearing established sc BT-474 tumor xenografts. Twenty-four hours following the injection, mice were sacrificed and tumor was excised. Tissues were fixed and embedded and sections cut for silver enhancement. **(A)** Immunoliposomes were distributed diffusely throughout tumor tissue and had accumulated predominantly within tumor cells. **(B)** Control liposomes were predominantly concentrated in interstitial regions and especially within tissue macrophages.

We conclude that tumor-targeted drug delivery using anti-HER2 immunoliposomes enhances the therapeutic index of doxorubicin chemotherapy and therefore may be a potent and useful therapy for cancers with HER2 overexpression. In addition, the strategy of immunoliposome delivery may have broad utility for targeted delivery of other anticancer agents, such as those with narrow therapeutic indices, pharmacokinetic limitations, or a requirement for intracellular delivery.

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ORIGINAL ARTICLE

Phase I study of irinotecan by 24-h intravenous infusion in combination with 5-fluorouracil in metastatic colorectal cancer

Mariko Kambe · Hiroaki Kikuchi · Makio Gamo · Takashi Yoshioka · Yasuo Ohashi · Ryunosuke Kanamaru

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Abstract
Background This study was intended to ascertain the feasibility of a combination therapy with irinotecan by 24-h intravenous infusion (24-h CPT-11) and 5-fluorouracil (5-FU) for patients with metastatic colorectal cancer, to estimate the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD), to determine the recommended dose (RD) for the Phase II study, and to evaluate the efficacy of the combination therapy.
Methods The dosage regimen was as follows: CPT-11 was given by 24-h CPT-11 on day 1, followed by 24-h intravenous infusion of 5-FU on day 2. This regimen was repeated every 2 weeks. The dose of CPT-11 was escalated in five steps from 50 to 75, 100, 125, or 150 mg/m^2 (levels 1–5), whereas the dose of 5-FU was fixed at 800 mg/m^2.
Results Twenty-six patients were recruited for this study, and 25 of the 26 patients were eligible for the assessment. The DLTs of 24-h CPT-11/5-FU therapy included grade 3 diarrhea in 1 patient treated at level 1, and grade 3 neutropenia in 1 patient and grade 4 neutropenia in 1 patient at level 4. In level 5, in 3 cases the next administration could not be done for 22 days or more as a consequence of anorexia. Thus, the level 5 was made a MTD and the level 4 was made a RD. The main side effects of grade 3 or higher, although nausea/vomiting occurred, were mild and tolerable in severity overall. The overall response rate was 24.0% (6PR/25).
Conclusion This study suggests that 24-h CPT-11/5-FU therapy is feasible and effective for treatment of metastatic colorectal cancer.

Keywords Colorectal cancer · Irinotecan (CPT-11) · 5-Fluorouracil (5-FU)

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Introduction

5-Fluorouracil (5-FU), which was introduced in 1958, has kept its position as a key drug for chemotherapy of colorectal cancer for about 40 years. 5-FU alone has been investigated for the dosage regimen by bolus injection, intravenous infusion, or other methods of administration primarily in the United States and Europe. In the late 1980s, combination chemotherapy with leucovorin (LV) was studied based on the biochemical modulation theory. This combination chemotherapy has been established as a standard treatment of colorectal cancer. Irinotecan (CPT-11) is

a camptothecin derivative extracted from *Camptotheca acuminata*. It has been recognized that CPT-11 exerts potent tumor-reducing activity by inhibiting DNA topoisomerase I (topo-I) [1]. A synergetic effect is observed between CPT-11 and 5-FU when they are administrated sequentially, and CPT-11 followed by 5-FU shows a better effect [2]. In addition, an attempt has been made to use irinotecan by weekly 24-h infusion as the second-line therapy for metastatic colorectal cancer, and the usefulness of this regimen has been suggested [3]. Especially, a Phase III study conducted mainly in the United States and Europe demonstrated that CPT-11/5-FU/LV combination therapy results in a survival benefit in patients with colorectal cancer. Currently, CPT-11/5-FU/LV has been established as the standard first-line therapy for colorectal cancer [4, 5].

A preclinical study suggested that a higher antitumor activity of CPT-11 is produced by long-term exposure with continuous intravenous infusion at a low dose to tumors than by exposure by short infusion with high dose intensity because the activity of CPT-11 is schedule dependent, although not markedly so [6]. Thus, a new approach by 24-h intravenous infusion of CPT-11 has been investigated for treatment of colorectal cancer [3, 7, 8].

We conducted a Phase I study to ascertain the feasibility of a combination therapy with CPT-11 by 24-h intravenous infusion and 5-FU for patients with metastatic colorectal cancer, to estimate the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD), to determine the recommended dose for the Phase II study, and to evaluate the efficacy of this combination therapy.

Patients and method

Patient eligibility

Inclusion criteria were as follows: (1) patients with histologically proven colorectal cancer; (2) patients with measurable or assessable lesions; (3) patients whose major organ functions were maintained adequately (white blood cells $\geq$ 4,000/mm^3; neutrophils $\geq$ 2,000/mm^3; platelets $\geq$ 100,000/mm^3; hemoglobin $\geq$ 9.5 g/dl; AST/ALT $\leq$ 2.5 $\times$ institutional upper limit of normal AST/ALT; total serum bilirubin $\leq$ 2.0 mg/dl; BUN $\leq$ 25 mg/dl; serum creatinine $\leq$ 1.5 mg/dl; creatinine clearance $\geq$ 50 ml/min; and normal ECG, excluding cardiac arrhythmias and ischemic changes); (4) patients whose performance status (ECOG) was 0–2; (5) patients who were free from carryover effects or adverse reactions from prior treatment; (6) life expectancy $\geq$ 3 months; (7) age $\geq$ 15 years and $\leq$ 75 years; and (8) patients who gave written informed consent.

Exclusion criteria were as follows: (1) severe fluid retention (pleural effusion or ascites); (2) metastasis to the central nervous system (CNS); (3) fresh bleeding from gastrointestinal tract; (4) diarrhea (watery stool); (5) infections; (6) intestinal paralysis or intestinal obstruction; (7) interstitial pneumonia or pulmonary fibrosis; (8) uncontrolled diabetes; (9) cardiac failure, renal failure, or hepatic failure; (10) active double cancer; (11) active psychiatric disorder; (12) previous abdominal irradiation; (13) pregnant women, nursing mothers, or women of childbearing potential; and (14) any patients who were judged to be inappropriate for the study by the investigator.

Treatment and dose escalation schedule

CPT-11 was administered by 24-h intravenous infusion on day 1, followed by 24-h intravenous infusion of 5-FU on day 2 every 2 weeks. For the dose-finding study, the dose levels were determined for three patients at each level, as a rule, a modified Fibonacci scheme [9]. Although the dose of 5-FU was fixed at 800 mg/m^2, dose levels of CPT-11 were escalated in five steps (levels 1–5) from 50 mg/m^2 as the starting dose to 75, 100, 125, and 150 mg/m^2. Each dose level was assessed for DLTs developing until the second course of treatment. Based on the assessment of DLT developing at the dose level, it was determined whether inclusion of additional patients and escalation to the next level were acceptable.

Dose-limiting toxicity (DLT) and maximum tolerated dose (MTD)

Dose-limiting toxicity (DLT) was defined as follows: (1) grade 3 or 4 hematological toxicity, (2) grade 3 or 4 leukopenia or neutropenia accompanied with a fever >38.0°C, (3) grade 3 or 4 nonhematological toxicity (excluding nausea/vomiting, anorexia, and alopecia), and (4) an event such that the next infusion was not carried out within 22 days after the previous infusion.

To determine the maximum tolerated dose (MTD), three patients were enrolled at each level. If none of the three patients developed any DLT, the dose of CPT-11 was escalated to the next level. If one or two of three patients developed a DLT, then three additional patients were enrolled at the same dose level. If three of six patients developed a DLT, the current level was considered as the MTD. If not more than two of the six patients developed a DLT, the dose of CPT-11 was escalated to the next level. If all three patients developed a DLT, the current level was considered as the MTD.

Assessment

Adverse reactions were evaluated according to the WHO Common Toxicity Criteria. The antitumor effect was

evaluated according to the Efficacy Evaluation Criteria in Solid Cancer of the Japan Society of Clinical Oncology.

Pharmacokinetics

Plasma concentrations of CPT-11 and its metabolite SN-38 during combination therapy with 24-h CPT-11 and 5-FU were examined. Blood samples were collected at the following time points: 1, 6, 12, 24 (equal to end of CPT-11 infusion), 25, 27, 30, 36, and 48 h after start of CPT-11 infusion. The volume of blood collected was 2 ml each, and at least 1 ml plasma was collected by centrifuge. The analytes were determined by high-performance liquid chromatography.

Results

Patient population

Twenty-six patients were recruited for this study, and 25 of the 26 patients were eligible for the assessment, excluding 1 patient who had diarrhea before the start of infusion. The demographic and baseline characteristics of the 25 patients are shown in Table 1.

Dose-limiting toxicity and other toxicities

Major adverse reactions reported during the study are shown in Table 2. DLTs included grade 3 diarrhea in one patient at level 1, grade 3 neutropenia in one patient at level 4, grade 3 leukopenia and grade 4 neutropenia in one patient at level 4. In level 5 (CPT-11 150 mg/m^2), in three cases the next administration could not be done for 22 days or more as a consequence of anorexia. In addition, hematological toxicities including grade 1–2 anemia in seven patients were observed. Nonhematological toxicities included nausea/vomiting. Generally, all toxicities were mild or moderate and tolerable.

Maximum tolerated dose and recommended dose

In this study, with level 5 (CPT-11 150 mg/m^2, 5-FU 800 mg/m^2), because there were three of six cases in which the next administration was delayed for 22 days or more because of toxicity, this level was made the MTD. As a result, level 4 (CPT-11 125 mg/m^2, 5-FU 800 mg/m^2) was made the recommended dose (RD) of 24 h CPT-11/5-FU therapy.

Antitumor activity

The antitumor effect was not used as the primary endpoint. The antitumor effect in 25 evaluable patients was 6 partial response (PRs), 9 no change (NCs), and 10 progressive disease (PDs): the response rate was 24.0% (95% CI, 7.3–40.7%) (colon cancer, 16.7%; rectal cancer, 30.8%). According to dose levels, 3 PRs, 1 NC, and 2 PDs in 6 patients occurred at the recommended dose, level 4: the response rate was 50.0% (95% CI, 10.0–90.0%].

The median time to response was 28 days (range, 7–74 days), and the duration of response (median) was 90 days (range, 48–165 days).

Pharmacokinetics

Changes in the plasma concentration of CPT-11 showed almost the same pattern at all levels. The plasma concentration increased until 12–24 h after the start of infusion. After the completion of infusion, it decreased quickly, and reached approximately the quantitation limit 24 h after the completion of infusion. As the dose of CPT-11 at each

Table 1 Patient characteristics

	Level 1	Level 2	Level 3	Level 4	Level 5	Total
No. of patients	6	4	3	6	6	25
Gender						
Male/female	4/2	4/0	3/0	3/3	5/1	19/6
Age						
Median (range)	62 (57–70)	61 (55–61)	56 (55–61)	51 (36–60)	53 (43–65)	58 (34–70)
PS (ECOG) 0/1/2	1/3/2	1/3/0	1/2/0	3/2/1	2/4/0	8/14/3
Primary colon/rectum	3/3	3/1	2/1	3/3	1/5	12/13
Metastatic site						
Liver	1	1	2	0	3	8
Lung	4	3	1	3	4	15
Lymph nods	3	0	0	4	1	8
Others	1	1	0	1	0	3

Table 2 Toxicity

Level (CPT-11 dose)	No. of patients	Leukopenia			Neutropenia			Anemia			Diarrhea			Nausea/vomiting			Anorexia		
		Grade			Grade			Grade			Grade			Grade			Grade		
		3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)	3	4	≥Gr 3 (%)
1 (50 mg/m^2)	6	0	0	0	0	0	0	0	0	0	1	0	16.7	0	0	0	1	0	0
2 (75 mg/m^2)	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3 (100 mg/m^2)	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4 (125 mg/m^2)	6	1	0	16.7	1	1	33.3	0	0	0	0	0	0	1	0	16.7	1	0	0
5 (150 mg/m^2)	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0

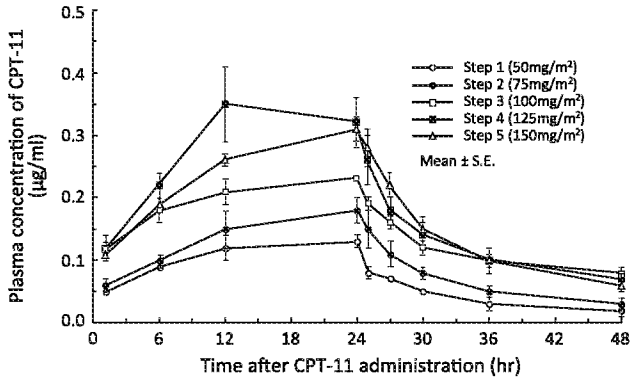

Fig. 1 Mean plasma concentrations of irinotecan (CPT-11) after drip infusion of CPT-11 and 5-fluorouracil (5-FU) (800 mg/m^2) in humans

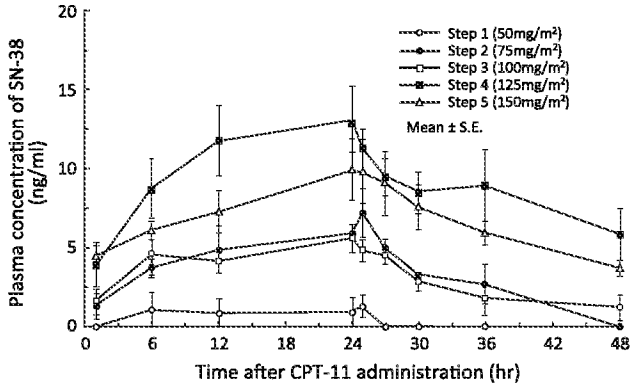

Fig. 2 Mean plasma concentrations of SN-38 after drip infusion of CPT-11 and 5-FU (800 mg/m^2) in humans

level increased, the plasma concentration increased (Fig. 1). The concentration of SN-38 reached a peak 24–25 h after the start of infusion. However, a consistent pattern of changes in plasma concentrations of SN-38 was not observed among levels, and both increase and decrease in the plasma concentration occurred more slowly than those of CPT-11. No dose-dependent pattern was observed for the plasma concentration of SN-38 (Fig. 2).

Discussion

We conducted a Phase I study of combination therapy with CPT-11 by 24-h intravenous infusion with 5-FU at the Institute of Development, Aging and Cancer, Tohoku University and two other institutions. The study confirmed that this therapy was feasible for the treatment of patients with metastatic colorectal cancer. Major adverse reactions were grade 3 diarrhea, grade 3/4 neutropenia, and delayed administration for more than 22 days because of adverse reactions; these were dose-limiting toxicities (DLTs). Most

other adverse reactions were mild or moderate and well tolerable. The doses of 24-h CPT-11/5-FU therapy up to level 5 were below the MTD. Level 4 (CPT-11 125 mg/m^2 on day 1 and 5-FU 800 mg/m^2 on day 2) was regarded as the RD.

In the analysis for overall response, six patients achieved PR with a response rate of 24%. Among the other patients, ten had NC and none had PD. Among six patients in level 4, which is the RD, three achieved PR with a response rate of 50%; of the others, one had NC and two had PD.

Nowadays, the regimen adding a molecular targeted agent such as bevacizumab and cetuximab to infusional 5-FU/LV/CPT-11 (FOLFIRI) and infusional FU/LV/L-OHP (FOLFOX) is widely used as the standard therapy in metastatic colorectal cancer [10, 11]. Especially, CPT-11 is recommended for the second treatment or later. In that case, several administration methods that alleviate adverse reactions are necessary in consideration of the impact from previous treatments.

Furthermore, our study was designed on the assumption that 24-h intravenous infusion would be an appropriate dosing method based on its drug profile because CPT-11 has a schedule-dependent mechanism of action, although not markedly so.

The recommended dose of CPT-11 with 5-FU at a fixed dose of 800 mg/m^2 was determined by reference to the schedule in JCOG9703 in which LV was not included [12]. As a result, this 24-h CPT-11/5-FU therapy showed a better effect with lower incidence of adverse events than FOLFIRI, previously reported as the second-line treatment [13, 14].

Mild toxicity in this 24-h CPT-11/5-FU therapy is similar to that reported by other studies which examined 24-h CPT-11 with UFT or UFT/LV [7, 8].

In the analysis of drug disposition, the CPT-11 to SN-38 conversion seems to decrease. Our study suggested that 24-h CPT-11/5-FU therapy is effective for treatment of metastatic colorectal cancer because the high safety of the therapy was demonstrated in patients with metastatic colorectal cancer, although grade 3 or 4 hematological toxicities, which could be resolved by supportive treatment, were seen, and the response rate was 50% at the recommended dose (level 4). In addition, a biweekly treatment schedule is suitable for ambulatory chemotherapy. A biweekly treatment schedule might be useful to complete the treatment program because the drug-free period of about 2 weeks would allow recovery from adverse reactions occurring during the treatment.

In conclusion, 24-h CPT-11/5-FU combination therapy for metastatic colorectal cancer may be a worthy regimen to evaluate endpoints including progression-free survival and overall survival in a Phase II study.

Conflict of interest Y. Ohashi received lecture fees and manuscript fee from Daiichi Sankyo. The other authors have no conflict of interest.

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Ion-Selective Electrode for Transmembrane pH Difference Measurements

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A triethylammonium-sensitive electrode was constructed using sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate as an ion-exchanger and benzyl 2-nitrophenyl ether as a solvent mediator in a poly(vinyl chloride) membrane matrix and was used to determine the pH difference across a cell membrane. The method is based on monitoring of the pH gradient-induced uptake of triethylammonium in situ. The triethylammonium electrode exhibited a near-Nernstian response to triethylammonium in the concentration range of 5×10^{-6}–1×10^{-2} M with a slope of 58.5 mV per concentration decade in a buffer solution composed of 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5). The limit of detection was 1 μM. In experiments using liposomes, the uptake of triethylammonium into liposomes was quantitatively induced according to the pH difference across the liposomal membrane. The transmembrane pH differences in *Escherichia coli* cells and the light-induced pH differences across the envelope vesicles of *Halobacterium halobium* were successfully determined by the present method.

Ion-selective electrodes are now commonly used to analyze cellular membrane functions for cation–sugar cotransport[1,2] and membrane potential.[3,4] These uses of ion-selective electrodes are attractive, because these methods are capable of monitoring a reaction process in turbid cell suspension on a continuous basis without any sampling or separation. We are interested in applying the ion-selective electrode to determine the pH difference across a cell membrane (ΔpH) and recently developed a methylammonium-selective electrode for this purpose.[5] This method seems to be superior to a tracer-labeled methylamine method,[6–9] because no radiolabeling is needed. However, the electrode suffered significant interference from K^+ and required pretreatment of cells to measure ΔpH.[5]

A prerequisite for applicability of an organic amine for ΔpH measurement is that it must be permeable in its neutral form but impermeable in its charged form.[6–9] Thus, all amines, except for the quaternary amines that have no neutral form and the lipophilic amines that can permeate through membrane even in their charged form, can basically be used as probes for ΔpH measurement. We chose triethylamine in the present study, because the triethylammonium electrode, which shows little interference by inorganic cations such as Na^+ and K^+, was expected to be constructed with a combination of a lipophilic ion-exchanger, sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (NaHFPB), and an appropriate solvent mediator, mimicking several organic ammonium-sensitive electrodes developed previously.[10,11] Furthermore, the charged triethylammonium form was thought to be unable to pass through the cell membrane, because the more lipophilic tetraethylammonium ion with one more methyl group than the triethylammonium ion has been reported to be impermeable through liposomal and biological membranes.[12,13]

Using the triethylammonium electrode, we succeeded in determining ΔpH formed in artificial liposomes, the transmembrane pH differences in *Escherichia coli* cells, and the light-induced pH differences across the envelope vesicles of *Halobacterium halobium*.

THEORY

The principle of ΔpH measurement is that, at equilibrium, the concentration of the neutral form of triethylamine becomes identical on both sides of the membrane, leading to the following relationship:

$$[H^+]_{in}/[H^+]_{out} = [(C_2H_5)_3NH^+]_{in}/[(C_2H_5)_3NH^+]_{out} \quad (1)$$

where the subscripts "in" and "out" mean inside and outside the membrane, respectively. Thus, the pH difference across the membrane (ΔpH), defined as $pH_{in} - pH_{out}$, can be expressed as

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follows:

$$\Delta pH = -\log \frac{[(C_2H_5)_3NH^+]_{in}}{[(C_2H_5)_3NH^+]_{out}} \quad (2)$$

This equation means that, under conditions where pH_{out} is higher than pH_{in}, triethylamine in the external medium is concentrated into the cell until the triethylammonium concentration ratio inside and outside the cell reaches ΔpH. Thus, ΔpH can be estimated by measuring the triethylammonium concentration ratio inside and outside the cell. The dissociation constant, pK_a, of triethylammonium (10.7 at 25 °C)[14] is much higher than physiological pH (around 7.5), and therefore, most of the amine is in the charged form and the total triethylamine concentration is for all practical purposes equivalent to that of the charged amine concentration, which can be measured using the triethylammonium electrode. The triethylammonium concentration inside cells was estimated from the decrease in the extracellular triethylammonium concentration. Thus, ΔpH was calculated from the following equation proposed previously:[5]

$$\Delta pH = -\log \left[\frac{V+v}{v} 10^{(E_1 - E_2)/S} - \frac{V}{v}\right] \quad (3)$$

where V and v represent outer medium volume and intracellular volume, respectively; E_1 is initial potential at 100 μM triethylammonium setting in this experiment; E_2 is electric potential after pH gradient was formed; and S is the slope of the triethylammonium electrode.

EXPERIMENTAL SECTION

Materials. The reagents were obtained from the following sources: triethylamine hydrochloride was from Tokyo Kasei (Tokyo, Japan); NaHFPB was from Dojindo Laboratories (Kumamoto, Japan); benzyl 2-nitrophenyl ether was from Fluka (Buchs, Switzerland); poly(vinyl chloride) (PVC; degree of polymerization, 1020) was from Nacalai Tesque (Kyoto, Japan); egg phosphatidylcholine (PC), egg phosphatidylethanolamine (PE), and egg phosphatidylglycerol (PG) were from Lipid Products (Red Hill, Surrey, U.K.); cholesterol was from Sigma (St. Louis, MO); 2,2,6,6-tetramethylpiperidone-*N*-oxyl (TEMPONE) was from Molecular Probes (Eugene, OR); and potassium tris(oxalate)chromate trihydrate was from Aldrich (Milwaukee, WI). All other chemicals were of analytical reagent grade.

Electrode System. A triethylammonium-sensitive electrode was constructed using a PVC-based membrane.[10,11] The components of the membrane were 0.1 mg of NaHFPB, 60 μL (~70 mg) of benzyl 2-nitrophenyl ether, and 30 mg of PVC. The materials were dissolved in tetrahydrofuran (~1 mL) and poured into a flat Petri dish (28-mm diameter). Then, the solvent was evaporated off at room temperature. The resulting membrane was excised and attached to a PVC tube (4-mm o.d., 3-mm i.d.) with tetrahydrofuran adhesive. PVC membranes containing other solvent mediators were similarly prepared using 0.1 mg of NaHFPB, 60 μL of solvent mediator, and 30 mg of PVC. Each PVC tube was filled with an internal solution composed of 1 mM triethylamine hydrochloride and 10 mM NaCl, and the sensor membrane was conditioned overnight. The electrochemical cell arrangement was Ag,AgCl/internal solution/sensor membrane/sample solution/1 M NH_4NO_3 (salt bridge)/10 mM KCl/Ag,AgCl. The electromotive force (emf) between the silver/silver chloride electrodes was measured using a voltmeter with high input impedance produced by a field-effect transistor operational amplifier (LF356; National Semiconductor, Sunnyvale, CA; input resistance $>10^{12}$ Ω) and recorded. The detection limit was defined as the intersection of the extrapolated linear regions of the calibration graph.[15] The selectivity coefficients of the electrode, K_{ij}^{Pot}, were determined by the separate solution method[15,16] using the respective chloride salts at 10 mM and calculated from the equation

$$\log K_{ij}^{Pot} = (E_j - E_i)/S + \log c_i - \log c_j^{1/z_j}$$

where E_i and E_j represent the emf readings measured for triethylammonium and the interfering ion, respectively; S is the theoretical slope of the electrode for triethylammonium (59.2 mV at 25 °C); c_i and c_j are the concentrations of triethylammonium and the interfering ion, respectively; and z_j is the charge of the interfering ion. The electrode was stored in 1 mM triethylamine hydrochloride and 10 mM NaCl when not in use. All measurements were performed at room temperature (~25 °C).

Preparation of Liposomes and ΔpH Measurements. Liposomes were prepared using the reversed-phase evaporation method[5] as follows. Aliquots of lipid stock solutions containing egg PC (10 μmol, 7.7 mg) and cholesterol (7.5 μmol, 2.9 mg) dissolved in chloroform/methanol (1:2, v/v) were placed in a centrifuge test tube (10 mL; Nichiden-Rika, Kobe, Japan). The solvent was evaporated using a centrifugal evaporator (RD400; Yamato, Tokyo, Japan), and the residual lipid was dried under vacuum for several hours. The lipid was then dissolved in 1.5 mL of diethyl ether, followed by the addition of 1 mL of an aqueous solution composed of 50 mM NaCl and 100 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5). The mixture was sonicated (5201; Ohtake Works, Tokyo, Japan) at 50 W for 1 min at 0 °C to obtain a homogeneous emulsion. Immediately, it was transferred to a round-bottom flask (20 mL), and the diethyl ether solvent was removed using a conventional rotary evaporator under reduced pressure (using an aspirator) at 25 °C. After the diethyl ether was completely removed by passing nitrogen gas through the mixture, a homogeneous suspension of liposomes was formed. The liposomes were centrifuged (105000*g*, 20 min) and washed once with 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) to lower the buffer capacity of the outer medium of the liposomes. The final pellet was suspended in 1 mL of the above washing solution. The osmotic pressures of the inner and outer aqueous solutions were measured with an OS osmometer (Fiske, Needham, MA); both were ~300 mOsm. Liposomes made from egg PE (24 μmol, 17.5 mg)/egg PG (6 μmol, 4.7 mg), and the lipid extracts from *E. coli* cells (8 mg) were similarly prepared. *E. coli* lipid was extracted according to the method described previously.[17]

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The procedure used to evaluate ΔpH depending on the external pH was as follows. The appropriate volume of the liposome suspension prepared above was pipetted and diluted in an assay solution containing 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) to make a volume of 490 μL. Then, 10 μL of 5 mM triethylamine hydrochloride was added to adjust the initial triethylammonium concentration of the liposome suspension to 100 μM. The final volume was 500 μL. The final lipid concentrations of liposome suspensions containing egg PC/cholesterol, egg PE/egg PG, and the lipid extracts from *E. coli* cells were 3.8, 13.3, and 4.9 mg of lipid/mL, respectively. The triethylammonium and reference electrodes were immersed in each liposome suspension, along with a miniaturized pH glass electrode (1826A-06T; Horiba, Kyoto, Japan), to simultaneously monitor the external pH of the solution. The suspension was constantly stirred with a stir bar. The present electrode system, including the reference electrode,[18] was compact, and therefore, an assay solution volume as low as 500 μL could be examined. The pH of the outer medium was changed by addition of a small amount of 160 mM sodium hydroxide or 160 mM hydrochloric acid.

The internal volumes of liposomes were evaluated by the spin label method using a combination of the membrane-permeable spin label TEMPONE and an impermeable broadening agent (potassium tris(oxalate)chromate) as described previously.[5,19] The intravesicular volumes of liposomes made from egg PC/cholesterol, egg PE/egg PG, and the lipid extracts from *E. coli* cells were 11.8, 3.4, and 9.0 μL/mg of lipid, respectively.

Preparation of *E. coli* Cells and ΔpH Measurements. *E. coli* W3133-2, a derivative of K-12, was used. Cells were grown in minimal salt medium, supplemented with 1% polypeptone, at 37 °C under aerobic conditions.[17] Cells were harvested during the exponential phase of growth, washed twice with buffer (150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.6), and suspended in the same buffer at 40 mg of cell protein/mL. The protein content was determined by the method of Lowry et al.[20] The cell suspension was diluted in an assay solution containing 150 mM NaCl, 10 mM sodium lactate, and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.6), and then triethylamine hydrochloride was added to adjust the initial triethylammonium concentration in the cell suspension to 100 μM, to create conditions similar to those in the liposome experiments. The final protein concentration of *E. coli* cells was 12.8 mg of cell protein/mL. An initial external pH of 7.6 was regarded as the internal pH of *E. coli* cells.[21-23] An internal volume of the *E. coli* cells of 3.7 μL/mg of cell protein[24] was used in this study.

Preparation of *H. halobium* Envelope Vesicles and Light-Induced ΔpH Measurements. The strains of *H. halobium* used were S_9 and KY-4. S_9 contains bacteriorhodopsin and halorhodopsin,[25] while KY-4 isolated from strain S_9 has halorhodopsin alone.[26] The cells were grown in peptone medium, and envelope vesicles were prepared by sonication as described by Lanyi and MacDonald.[27] The sidedness of the vesicles was checked by NADH-menadione reductase activity, and all preparations showed 85–90% right-side-out vesicles.[27]

The vesicles prepared from S_9 and KY-4 were suspended in test tubes (5 mL; Nichiden-Rika, Kobe, Japan) containing an assay solution composed of 4 M NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5), and then triethylamine hydrochloride was added to adjust the initial triethylammonium concentration in the vesicle suspension to 100 μM. The final volume was 500 μL. The final protein concentrations of S_9 and KY-4 vesicles were 14.6 and 13.6 mg of protein/mL, respectively. The test tube was put in a special glass vessel constructed for the thermostating of test solution. Illumination was provided through a cutoff filter (>520 nm) (Y-52; Toshiba, Tokyo, Japan) with a 1-kW tungsten projector lamp (Master Hilux H-130; Rikagaku Seiki, Tokyo, Japan). Light intensity at the front of the test tube was 750 W/m^2, which was measured with a Kettering radiant power meter (model 4090; Yellow Springs, OH). An internal volume of *H. halobium* envelope vesicles of 2.9 μL/mg of protein[28] was used in this study.

RESULTS AND DISCUSSION

Response Characteristics of the Electrodes. To obtain the most suitable electrode, we examined the effects of solvent mediators on response to triethylammonium, because solvent mediators have been shown to markedly affect the response characteristics of the electrodes based on NaHFPB.[10,11] Calibration graphs were obtained by measuring known amounts of triethylamine hydrochloride added to a solution containing 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) and plotting the concentrations against the obtained corresponding emf values. The solvent mediators tested were benzyl 2-nitrophenyl ether, 2-fluoro-2′-nitrodiphenyl ether, *o*-nitrophenyl octyl ether, dioctyl phthalate, bis(2-ethylhexyl) sebacate, tris(2-ethylhexyl) phosphate, and tricresyl phosphate. Among them, both benzyl 2-nitrophenyl ether and 2-fluoro-2′-nitrodiphenyl ether afforded higher degrees of sensitivity to triethylammonium; however, the potential stability of benzyl 2-nitrophenyl ether was significantly superior to that of 2-fluoro-2′-nitrodiphenyl ether. Thus, we used benzyl 2-nitrophenyl ether in the present experiments. The electrode using benzyl 2-nitrophenyl ether exhibited a near-Nernstian response to triethylammonium in the concentration range of 5×10^{-6}–1×10^{-2} M with a slope of 58.5 mV per concentration decade (Figure 1a). The lower limit of detection was 1 μM. We further made a calibration graph in a solution containing 4 M NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) (Figure 1b). It should be emphasized that the electrode still showed a high degree of sensitivity toward triethylammonium even in the presence of an extremely high concentration of Na^+ and gave a slope of 58.0 mV per concentration decade with a detection limit of 6 μM. Thus, the electrode

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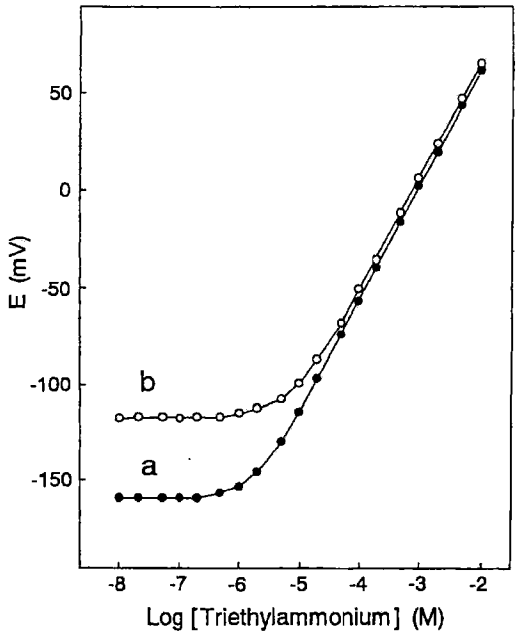

Figure 1. Calibration graphs for the electrode with triethylammonium in buffer solution containing (a) 150 mM NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) and (b) 4 M NaCl and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5).

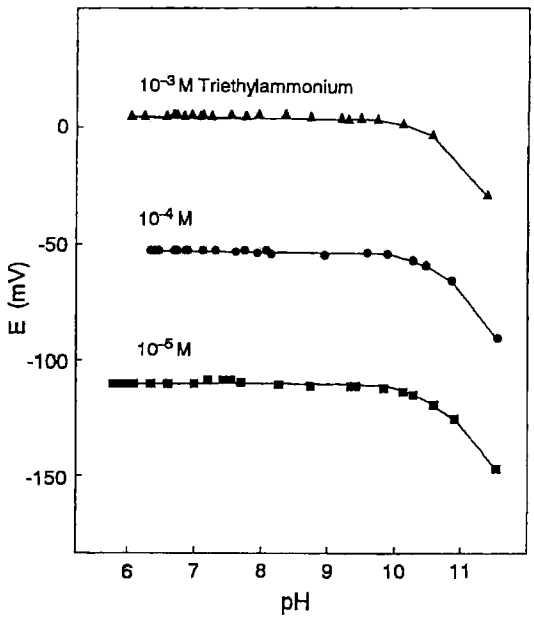

Figure 2. Effects of pH on electrode emf response at three triethylammonium concentrations in the presence of 150 mM NaCl. The pH of the solution was changed by adding an appropriate amount of dilute hydrochloric acid or sodium hydroxide solution.

Table 1. Selectivity Coefficients, log $k_{i,j}^{Pot}$[a]

interfering ion (j)	log $k_{i,j}^{Pot}$	interfering ion (j)	log $k_{i,j}^{Pot}$
Mg^{2+}	−5.4	NH_4^+	−3.5
Ca^{2+}	−5.2	$CH_3NH_3^+$	−2.8
Li^+	−4.1	choline	−1.0
Na^+	−3.8	$(CH_3)_4N^+$	−0.2
K^+	−3.4	$(C_2H_5)_4N^+$	1.3

[a] i is triethylammonium and j is the interfering ion.

can also be used even in the presence of such high Na^+ concentrations. The response time (90% final signal) of the electrode was less than 10 s when the concentration of triethylammonium was changed from 50 to 100 μM in both solutions containing 150 mM NaCl and 4 M NaCl buffered with 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5).

The selectivity coefficients of the electrode are given in Table 1. The electrode showed no significant interference from inorganic cations such as Na^+ and K^+. However, the response to lipophilic quaternary ammonium ions such as $(C_2H_5)_4N^+$ was greater than that to triethylammonium, because the selectivity of the electrode using the ion-exchanger was determined by the order of the lipophilicity of the organic ammonium ions.[10,11] The pH dependence of the electrode at three triethylammonium concentrations is shown in Figure 2. The pH dependence was measured in 150 mM NaCl, and the pH of the solution was changed by adding an appropriate amount of dilute hydrochloric acid or sodium hydroxide solution. The response of the electrode was independent of pH in the range of 6−10. The decrease in the potential above pH 10 was attributable to an increase in the concentration of the unprotonated form of triethylamine, because the pK_a value of triethylammonium was reported to be 10.7.[14] Thus, the electrode was applicable over a wide pH range including the present range of 7.5−9.6.

ΔpH Measurements for Liposomal Membranes. First, we examined whether the triethylammonium electrode could be applied to determine the pH difference across liposomal membranes composed of egg PC and cholesterol, which was successfully determined by the methylammonium electrode reported previously.[5] As the spontaneous diffusion rates of H^+ and/or OH^- across liposomal membranes are rather low,[29-31] it was assumed that the inner pH of liposomes would be constant during the short period of the experiments. Thus, it was expected that when the outer pH was made more alkaline than the inner liposomal pH, the uptake of triethylammonium in liposomes would be induced. As shown in Figure 3, when sodium hydroxide was added to the liposome suspension to make $pH_{in} < pH_{out}$, a significant decrease in electric potential and a corresponding decrease in triethylammonium concentration in the outer medium were observed, and further addition of sodium hydroxide caused further accumulation of triethylammonium inside the liposomes. The accumulated triethylammonium in the liposomes was released when the outside pH was returned to the initial value. This result clearly showed that the triethylammonium electrode can monitor changes in triethylammonium concentration caused by transmembrane pH differences. It is reasonable to consider that the accumulation of triethylamine inside liposomes did not affected inner pH, because the buffer capacity of the inner medium of the liposomes was high and the initial triethylamine concentration was low.

We calculated the ΔpH from eq 3 and examined its dependence on the external pH. The intravesicular volume of liposomes used in this study was estimated as 11.8 μL/mg of lipid by a spin label

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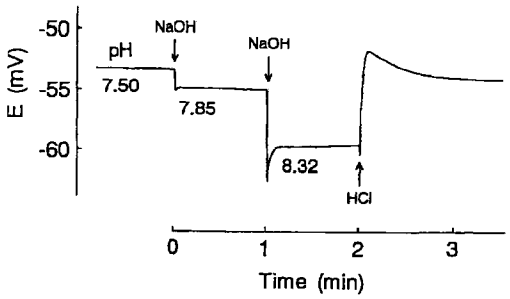

Figure 3. Monitoring of changes in emf with variations in the external pH. Liposomes composed of egg PC/cholesterol were suspended in a solution (500 μL) containing 100 μM triethylamine hydrochloride, 150 mM NaCl, and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) at 3.8 mg of lipid/mL. At the times indicated by the first and second arrows, 3 and 2 μL, respectively, of 160 mM NaOH were added. The third arrow indicates the time at which 5 μL of 160 mM HCl was added to reestablish the initial pH. The pH values of the solution after addition of NaOH and HCl were monitored simultaneously using a miniaturized pH glass electrode and are shown on the curves.

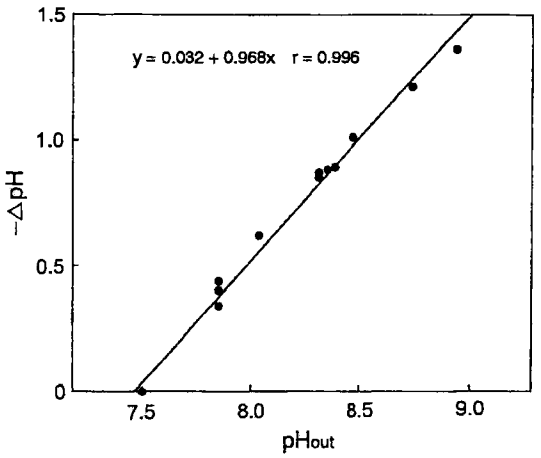

Figure 4. Dependence of ΔpH across the liposomal membrane on external pH. The sign of ΔpH on the ordinate was changed to negative to illustrate the differences ($pH_{out} - pH_{in}$). The external pH shown on the abscissa was measured using a glass pH electrode.

method. This corresponded to an inner volume (v) of liposomes and an outer medium volume (V) of 22.5 and 477.5 μL, respectively. Using these values and electric potential changes, we calculated ΔpH and found a strong linear correlation between ΔpH and external pH, as shown in Figure 4. We plotted −ΔpH as the ordinate, to highlight differences ($pH_{out} - pH_{in}$) and represent increasing external pH as a positive value. A slope of 1 means that any change in pH_{out} yields an equal change in −ΔpH. Linear regression analysis revealed that the slope and the intercept of the line were 0.968 and 0.032, respectively ($r = 0.996$; $n = 11$). We further analyzed the correlation between −ΔpH and external pH using two liposomes prepared from egg PE/egg PG and lipid extracted from *E. coli*. Linear regression analysis using liposomes composed of egg PE/egg PG measured in the external pH range (7.5−9.1) showed a slope of 0.968 and intercept of 0.050 ($r = 0.996$; $n = 11$), while that using liposomes composed of *E. coli* lipid measured in the external pH range (7.5−9.3) showed a slope of 0.989 and intercept of 0.060 ($r = 0.996$; $n = 11$). These results indicated that the triethylammonium electrode was quite suitable

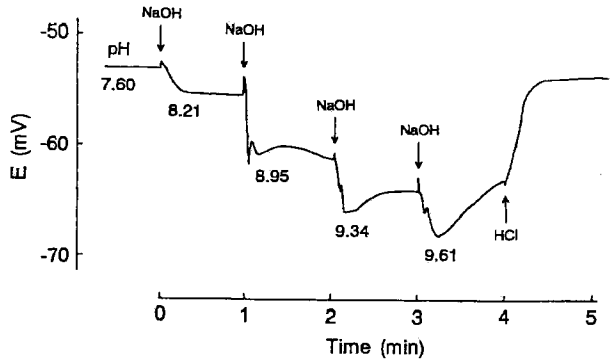

Figure 5. Monitoring of changes in emf with variations in the external pH. *E. coli* cells were suspended in a solution (500 μL) containing 100 μM triethylamine hydrochloride, 150 mM NaCl, 10 mM sodium lactate, and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.6) at 12.8 mg of cell protein/mL. At the times indicated by the first to fourth arrows, 1 μL of 1 M NaOH was added. The fifth arrow indicates the time at which 4 μL of 1 M HCl was added to reestablish the initial pH. The pH values of the solution after addition of NaOH and HCl were monitored simultaneously using a miniaturized pH glass electrode and are shown on the curves.

for determining ΔpH across liposomal membranes irrespective of the kind of liposomes and will be effective as an alternative to tracer-labeled methylamine, which has been used extensively to date,[6−9] to determine the internal pH of cells (pH_{in}) through the measurement of ΔpH and the external pH (pH_{out}) of the medium using a pH electrode.

ΔpH Measurements for *E. coli* Cells. Then, we applied the method to examine the external pH dependence of ΔpH of *E. coli* cells. Previously, we used a methylammonium electrode for this measurement.[5] However, the uptake of methylammonium into *E. coli* cells could not be measured directly, because a large amount of K^+ efflux was induced from *E. coli* cells when the pH of the medium was made alkaline,[32] and this K^+ efflux seriously interfered with the electrode response.[5] The selectivity coefficient of the methylammonium electrode toward K^+ ($\log k_{i,j}^{Pot} = -1.1$) was not sufficient to allow measurement of methylammonium uptake in the presence of a large amount of K^+.[5] In contrast, the present triethylammonium electrode showed remarkably high selectivity against K^+ ($\log k_{i,j}^{Pot} = -3.4$), and this seemed to be sufficient to measure the external pH dependence of ΔpH of *E. coli* cells even in the presence of a large amount of K^+. As shown in Figure 5, when sodium hydroxide was added to the *E. coli* cell suspension to make $pH_{in} < pH_{out}$, a decrease in electric potential and a corresponding decrease in triethylammonium concentration in the outer medium were clearly observed. However, further addition of large amounts of sodium hydroxide caused the gradual efflux of triethylammonium accumulated in the *E. coli* cells, leading to an increase in triethylammonium concentration in the outer medium. When the outside pH was returned to the initial pH by adding hydrochloric acid, the electric potential returned to the initial value.

To discuss these external pH-dependent ΔpH changes in *E. coli* cells with regard to cellular membrane function, changes in the internal pH of the cells (pH_{in}) with variations in the external

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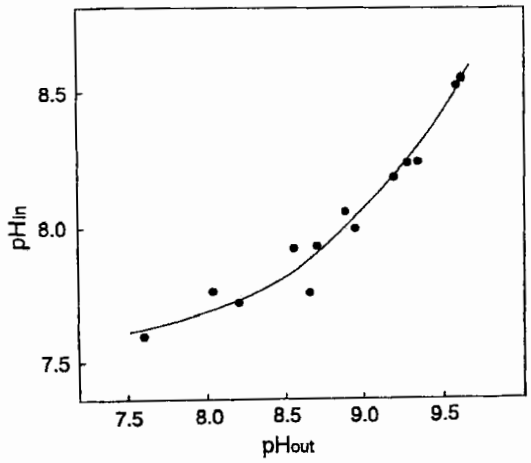

Figure 6. Changes in the internal pH of *E. coli* cells (pH_{in}) with variations in the external pH (pH_{out}).

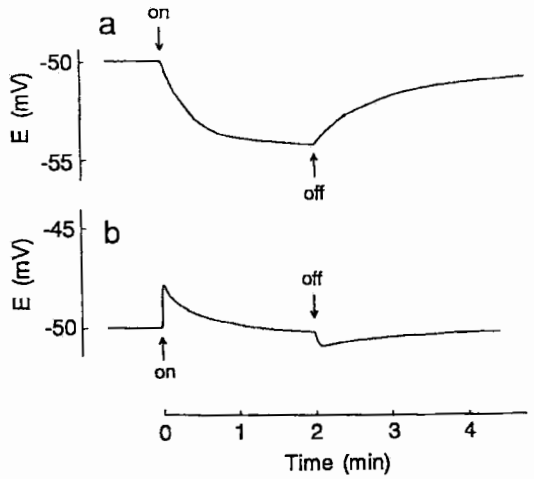

Figure 7. Light-induced ΔpH changes in the envelope vesicles of the strains KY-4 (a) and S_9 (b) of *H. halobium* in a solution (500 μL) containing 100 μM triethylamine hydrochloride, 4 M NaCl, and 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.5) at concentrations of 13.6 and 14.6 mg of protein/mL, respectively. The light intensity was 750 W/m^2.

pH of medium (pH_{out}) were plotted (Figure 6). The pH_{in} values were calculated using the relation of $pH_{in} = \Delta pH + pH_{out}$. As can be clearly seen in Figure 6, the intracellular pH of the cells was rather constant at around 7.6−7.8 over the range of extracellular pH from 7.6 to 8.5. When the extracellular pH was above 9, however, the intracellular pH increased rapidly and exceeded 8. This profile of the pH dependence of *E. coli* cells was in accordance with that observed previously with tracer-labeled methylamine.[21,22] The increase in pH_{in} under higher pH_{out} suggested that a significant amount of H^+ permeates through *E. coli* membranes, in contrast to artificial liposomal membranes. As discussed previously,[5] in biological membranes there are many ion pathways via membrane proteins. Ion movement through the membrane proteins is important for the function of cell membranes. This membrane "leakage" must be successfully controlled to keep the intracellular pH neutral even in alkaline environments. The present results showed that *E. coli* intracellular pH regulation is successful up to around pH 8.5, and then it gradually diminishes at more alkaline pH, probably due to retarded membrane functions.

Light-Induced ΔpH Changes in *H. halobium* Envelope Vesicles. Since the present electrode was highly sensitive even in 4 M NaCl, we applied the method to measure light-induced ΔpH changes in the envelope vesicles of *H. halobium*. The envelope vesicles of *H. halobium* have two representative light-reactive pigments: halorhodopsin, which is an inward-directed light-driven Cl^- pump, creating in turn passive H^+ uptake, and bacteriorhodopsin, which expels protons from inside to outside upon illumination.[33−35] First, we used the strain KY-4, which has halorhodopsin alone.[26,28] Halorhodopsin elicits subsequent H^+ uptake into the cells upon illumination, resulting in $pH_{in} < pH_{out}$.[28] Thus, it was expected that the uptake of triethylammonium inside cells would occur upon illumination. Indeed, potential changes corresponding to the uptake of triethylammonium were clearly observed as shown in Figure 7a. We calculated the light-induced ΔpH from eq 3. Using the intravesicular volume of KY-4 estimated as 2.9 μL/mg of protein[28] and electrical potential change, we calculated ΔpH as 0.8 under the present conditions, which agreed well with that reported previously.[28] Then, we used strain S_9, which has both halorhodopsin and bacteriorhodopsin.[26,28] It has been experimentally established that when S_9 vesicles are illuminated in 4 M NaCl at around neutral pH, rapid acidification (proton extrusion due to the action of bacteriorhodopsin) occurs in the outer medium, which is followed by prolonged alkalinization (proton uptake elicited by the action of halorhodopsin) in the medium.[28] We confirmed this complicated pH change using the triethylammonium electrode. As shown in Figure 7b, the initial rapid acidification (due to the action of bacteriorhodopsin) in the outer medium caused a small increase in the concentration of triethylammonium released from vesicles initially containing 100 μM triethylammonium. Then, a gradual decrease in the concentration of triethylammonium, which corresponded to the uptake of triethylammonium inside cells induced by alkalinization in the outer medium (due to the action of halorhodopsin and the succeeding passive H^+ uptake), was clearly observed. This profile was in good accordance with the results obtained previously with a pH glass electrode[3] and the spin probe 4-amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl.[28]

CONCLUSIONS

The present approach can be applied to ΔpH measurements in various cell membranes, because the triethylammonium electrode showed no significant interference from Na^+ and K^+ present in large amounts in biological systems. This method is a new fundamental technique for estimating ΔpH in artificial and biological membranes.

ACKNOWLEDGMENT

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A multinational phase II study of PEP02 (liposome irinotecan) for patients with gemcitabine-refractory metastatic pancreatic cancer.

Meeting:
2011 ASCO Annual Meeting

Category:
Gastrointestinal (Noncolorectal) Cancer

Subcategory:
Pancreatic Cancer

Session Type and Session Title:
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Background: PEP02 is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved pharmacokinetics and tumor biodistribution of both CPT-11 and its active metabolite-SN38 compared to the free form drug. PEP02 has showed encouraging safety and efficacy in various tumor types, including significant antitumor activity in a human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model. In previous phase I studies, PEP02 either alone or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (PC). This phase II study aims to evaluate PEP02 monotherapy as second-line treatment in pts with metastatic, GEM-refractory PC. **Methods:** Pts were eligible if they had metastatic pancreatic adenocarcinoma, KPS $\geq$ 70, and progressed following one line of GEM-based therapy. Treatment consisted of PEP02 120 mg/m^2 administered as a 90-minute infusion every 3 weeks. A Simon's 2-stage design was used with 16 pts in the first stage and 39 pts in total; primary objective was 3-month survival rate ($OS_{3\text{-month}}$). **Results:** Between March 2009 and September 2010, 41 pts were enrolled at 3 centers in the U.S. and Taiwan. Characteristics for the 40 ITT pts: 19 M/21 F; age 39-82 yrs; 25 Asian/15 Caucasian, KPS 100/90/80/70: 7/16/7/10. Mean number of treatment cycles is 5.25 (range, 1-26). Objective response rate is 5% and disease control rate (minor response + stable disease > 2 cycles) is 50%. 10 (30.3%) of 33 pts with elevated baseline

CA19-9 have had > 50% biomarker decline. The $OS_{3\text{-month}}$ is 75%, with median progression free survival (PFS) and OS of 9 and 21.6 weeks, respectively. There was no correlation between duration of prior GEM-based therapy and survival after PEP02 treatment. The most common G3/4 adverse events are: leucopenia/neutropenia, anemia, diarrhea, and fatigue. **Conclusions:** This study has already met its primary endpoint (predicted OS3-month ≥ 65%). PEP02 appears to have both activity and tolerable side effects for pts with metastatic, GEM-refractory PC, and represents a promising option for this pt population with few options.

A Multinational Phase II Study of PEP02 (MM-398), Liposome Irinotecan, for Patients with Gemcitabine-refractory Metastatic Pancreatic Cancer

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University of California, San Francisco Comprehensive Cancer Center, San Francisco, CA[1]; National Cheng-Kung University Hospital, Tainan, Taiwan[2]; National Taiwan University Hospital, Taipei, Taiwan[3]; Pharma Engine, Inc., Taipei, Taiwan[4]; National Health Research Institutes, Tainan, Taiwan[5]

Abstract:

Background: PEP02 (MM-398) is a novel nanoparticle liposome formulation of irinotecan (CPT-11) that has improved pharmacokinetics and tumor biodistribution of both CPT-11 and its active metabolite-SN38 compared to the free form drug. PEP02 has shown encouraging preclinical activity in various tumor types, including significant antitumor activity in a human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model. In previous phase I studies, PEP02 either alone or in combination with 5-FU/LV demonstrated prolonged disease control in 5 of 7 (71%) patients (pts) with gemcitabine (GEM)-refractory advanced pancreatic cancer (APC). This phase II study aims to evaluate PEP02 monotherapy as 2nd-line treatment in pts with metastatic, GEM-refractory APC.

Methods: Pts were eligible if they had metastatic pancreatic adenocarcinoma, KPS ≥ 70, and progressed following one line of GEM-based therapy. Treatment consisted of intravenous injection of PEP02 120 mg/m^2 over 90 minutes every 3 weeks. A Simon's 2-stage design was used with 16 pts in the 1st stage and 39 pts in total; primary objective was 3-month survival rate ($OS_{3\text{-month}}$).

Results: Between March 2009 and September 2010, 41 pts were enrolled at 3 centers in the U.S. and Taiwan. Characteristics for the 40 ITT pts: 19 M/21 F; age 39-82 yrs; 25 Asian/15 Caucasian; KPS 100/90/80/70: 7/17/6/10. Until end of May, 2 pts are still undergoing PEP02 treatment and 7 pts are still alive. Mean number of treatment cycles is 5.4 (range, 1-26). Objective response rate is 7.5% and disease control rate is 47.5%. Of the 25 pts who were evaluable for clinical benefit response (CBR), 5(20%) achieved CBR. Eleven (34.4%) of 32 pts with elevated baseline CA19-9 had > 50% biomarker decline. The $OS_{3\text{-month}}$ is 75%, with median progression free survival (PFS) and OS of 9.6 and 22.4 weeks, respectively. There was no correlation between duration of prior GEM-based therapy and survival after PEP02 treatment. The most common G3/4 toxicities are: neutropenia (30%), leucopenia (22.5%), anemia (15%), diarrhea (7.5%), and fatigue (7.5%).

Conclusion: This study has met its primary endpoint (predicted $OS_{3\text{-month}} \geq 65\%$). PEP02 appears to have both activity and tolerable side effects for pts with metastatic, GEM-refractory APC, and represents a promising option for this pt population with few treatment options.

INTRODUCTION

Advanced Pancreatic Cancer (APC)

- Gemcitabine-based therapy has represented the standard of care for the 1st-line treatment for APC since 1996. Selected patients with good performance status, may benefit from combination regimens. Recent data also support the use of FOLFIRINOX in the 1st-line setting.
- At present, there is no standard 2nd-line chemotherapy for patients who have progressed on front-line therapy.

PEP02

- PEP02 is irinotecan hydrochloride (also known as CPT-11), a topoisomerase I inhibitor, encapsulated in a liposome drug delivery system.
- In the human pancreatic cancer L3.6pl orthotopic nude mouse xenograft model, PEP02 showed potent antitumor activity, including durable tumor regressions, and were markedly superior to the equivalent dose of CPT-11.
- 7 patients with APC received PEP02 as part of previous phase I studies. One patient had PR (PEP02 180 mg/m^2 q3w) and 4 patients had SD (PEP02 at 60, 80, 120 mg/m^2 q3w in combination with 5FU/LV). All patients had received 1-5 prior regimens.

OBJECTIVES

- **Primary**
  - To measure 3-month survival rate
- **Secondary**
  - To assess the objective tumor response, PFS, duration of response, overall survival, tumor marker response of CA19-9
  - To evaluate the effect on the clinical benefit parameters
  - To evaluate the toxicities

METHODS

- **Study Design**
  - Open-label, single arm, multicenter study
  - An optimal Simon's 2-stage was adopted.
    - H_0 of $OS_{3\text{-month}}$ = 40% vs. H_1 of $OS_{3\text{-month}}$ = 65%
    - If ≥ 8 of the first 16 pts in stage 1 survive > 3 months, proceed onto stage 2 with additional 23 pts. Positive result defined as ≥ 21/39 pts achieving $OS_{3\text{-month}}$ endpoint.
- **Study Regimen**
  - 120 mg/m^2 q3w; subsequent allowance made to start at 100 mg/m^2 d/t poor tolerance in U.S. pts
  - Premedicated with dexamethasone and 5-HT_3 antagonists
  - Dose escalation up to 150 mg/m^2 if no > G1 treatment-related toxicity during first cycle
- **Clinical Benefit Evaluation**
  - Primary parameters: pain (visual analog scale and morphine consumption) and performance status
  - Secondary parameter: weight
  - Responders require a marked and ≥ 4 weeks improvement in at least one parameter without worsening in the other.

PATIENTS

- **Key Inclusion Criteria**
  - Histologically or cytologically confirmed adenocarcinoma of exocrine pancreas

- **Key Inclusion Criteria (continue)**
  - Metastatic disease (including locally recurrent disease with regional lymph node involvement)
  - Documented disease progression after prior gemcitabine-based therapy
  - Total bilirubin within normal range
  - Karnofsky performance status ≥ 70
- **Key Exclusion Criteria**
  - Prior irinotecan treatment

RESULTS

- 41 patients were registered with 40 patients receiving PEP02 treatment from March 2009 to September 2010.
- Until May 2011, 2 patients are still undergoing treatment and 7 patients are still alive.

Table 1. Patient demographics and baseline characteristics

Characteristic	n=40
Sex, n (%)	
Male / Female	19 (47.5) / 21 (52.5)
Age, mean (range) years	58.8 (39-82)
Study site, n (%)	
Taiwan / USA	22 (55) / 18 (45)
Ethnicity, n (%)	
Asian / Caucasian	25 (62.5) / 15 (37.5)
Karnofsky performance status, n (%)	
100	7 (17.5)
90	17 (42.5)
80	6 (15.0)
70	10 (25.0)
Prior treatment to cancer disease, n (%)	
Chemotherapy	40 (100)
Radiotherapy	10 (25.0)
Surgery	16 (40.0)
1st line chemotherapy and duration in months	
Gem-monotherapy, n (%) / median (range)	9 (22.5) / 2 (1.5-24)
Gem-based combination, n (%) / median (range)	31 (77.5) / 5 (1-16)
With elevated CA19-9, n (%)	32 (80)
Baseline clinical benefit parameters, n (%)	
pain intensity ≥ 20 (out of 100)	16 (40.0)
morphine consumption ≥ 10 mg/day	13 (32.5)

Table 2. Study medication

Treatment cycles, mean (range)	5.4 (1-26)
≧ 8 cycles, n (%)	11 (27.5)
≧ 12 cycles, n (%)	5 (12.5)
Dose modification, n (%)	
increased to 150 mg/m^2	2 (5)
maintained 120 mg/m^2	28 (70)
maintained or decreased to 100 mg/m^2	8 (20)
decreased to 80 mg/m^2	2 (5)

Table 3. Efficacy data

Survival					
Median PFS			Median survival		
9.6 weeks			22.4 weeks		
3-month		6-month		1-year	
75%		42.5%		25%	
Best Tumor Response					
PR	Minor response (shrinkage >10%)	SD	PD	Not evaluable	
3 (7.5%)	8 (20%)	8 (20%)			
	Disease control		11 (27.5%)	10 (25%)	
	19 (47.5%)				
CA19-9 Tumor Marker Response (decline > 50%) among 32 pts with elevated baseline			**Clinical Benefit Response** among 25 CBR-evaluable pts		
11 (34.4%)			5 (20%)		

Figure 1. Kaplan-Meier survival curve

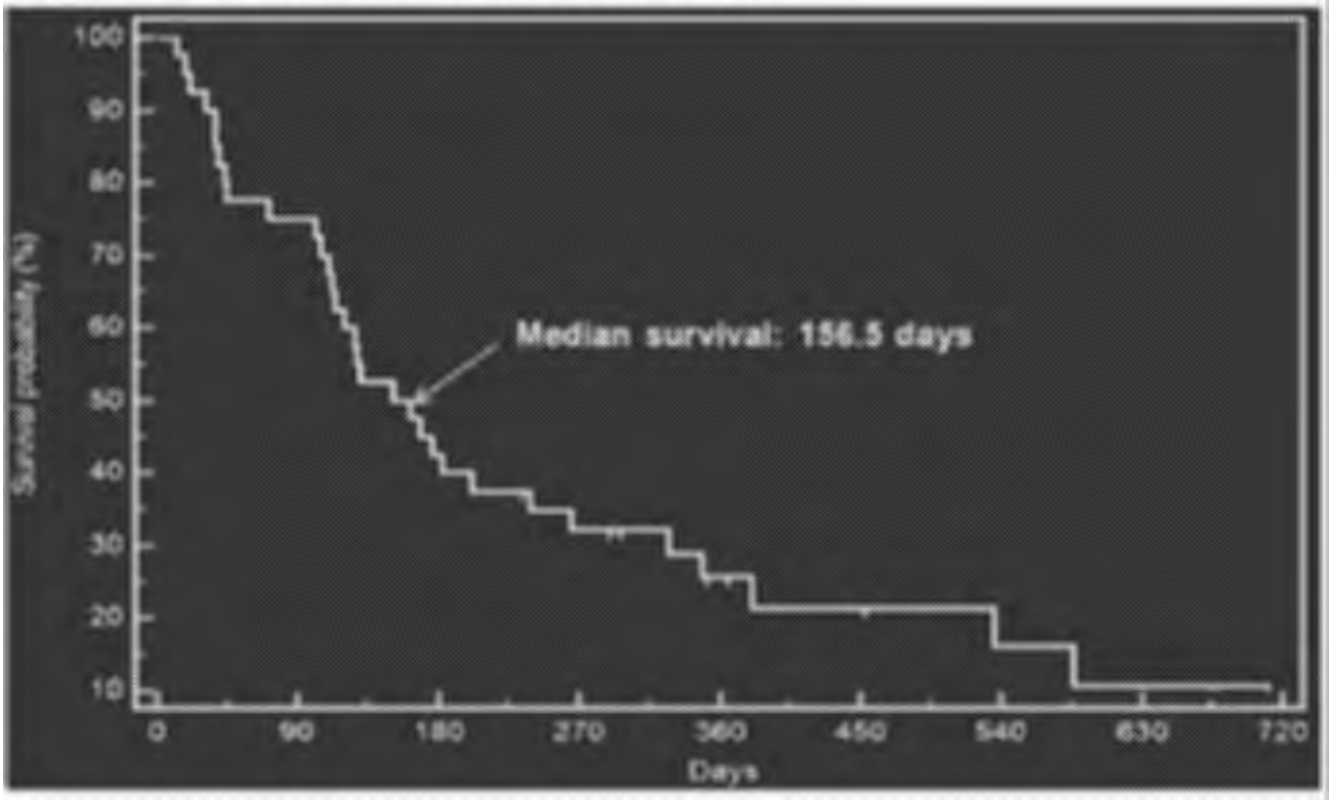


Figure 2. Prior therapy and outcome of PEP02 treatment

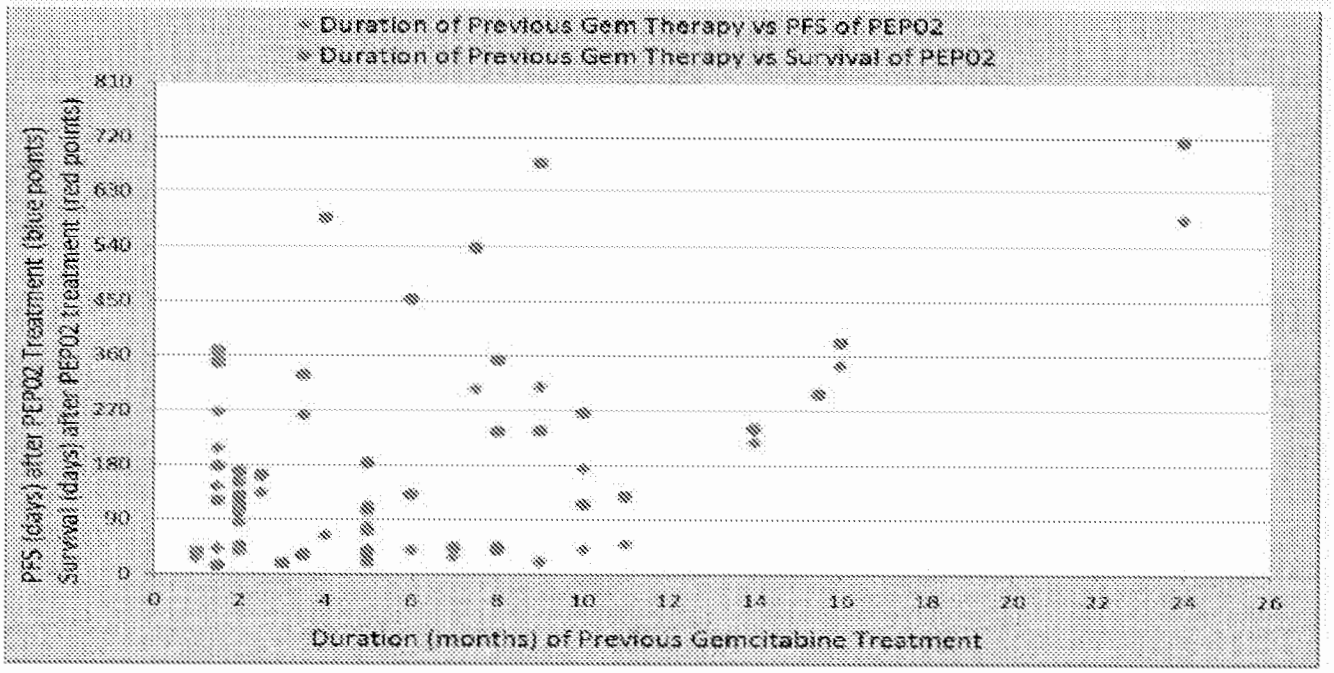


Figure 3. Waterfall plot of tumor response

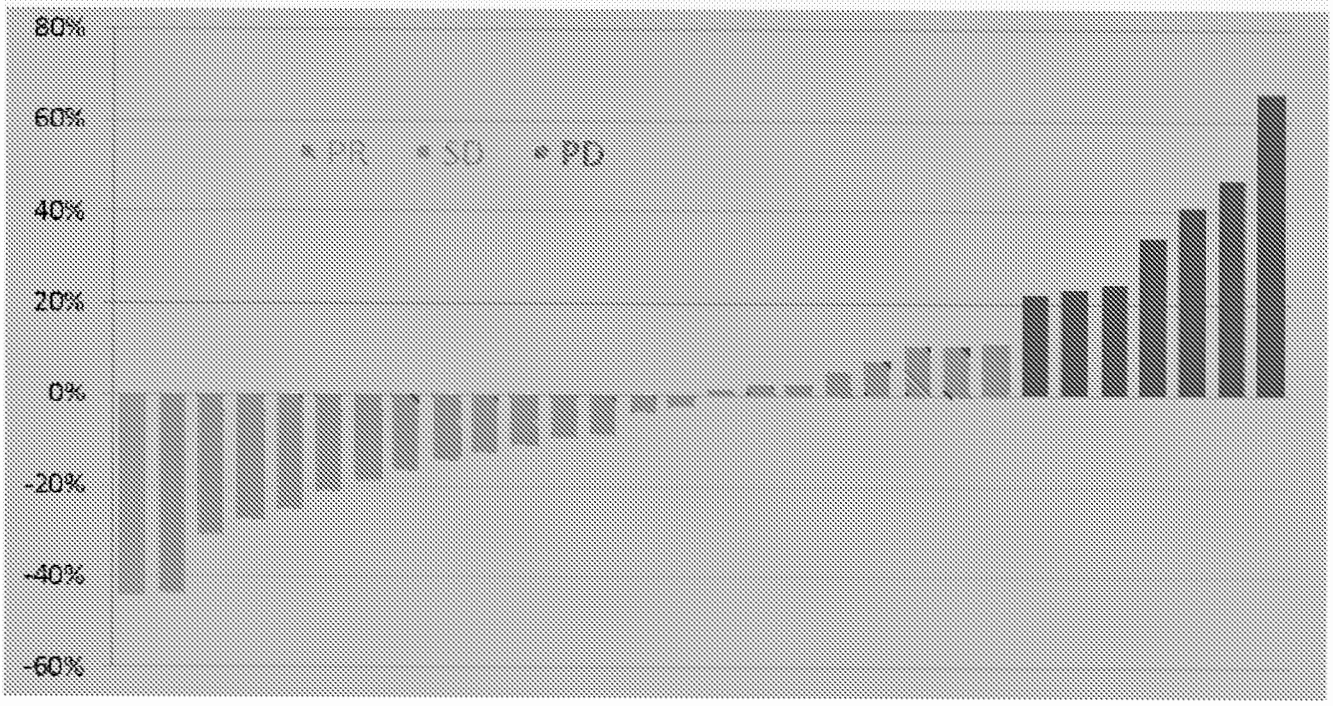
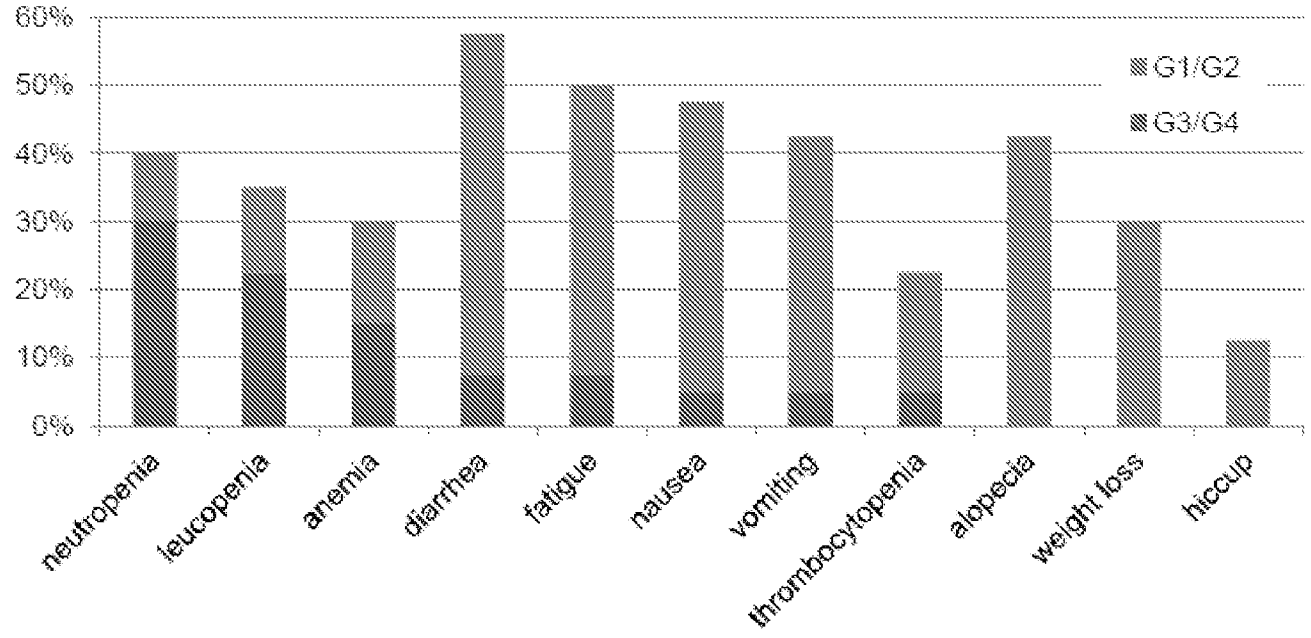


Figure 4. Toxicities



CONCLUSIONS

Efficacy

- PEP02 achieved the primary endpoint of 3-month survival rate of 75% in metastatic pancreatic cancer patients who failed prior gemcitabine therapy.
- Median survival after PEP02 treatment was approximately 5.2 months, and more than 20% patients had significantly extended survival.
- Almost 30% of patients received PEP02 for 6 months or more.
- Significant tumor shrinkage and CA19-9 decline, as well as sustained clinical benefit, were noted in a number of patients.

Safety

- Grade 3/4 toxicities were primarily hematologic in nature, with non-hematologic toxicities (GI, fatigue) occurring in less than 10% of patients. Acute cholinergic symptoms were rarely reported.

Conclusion

- Based on these results, PEP02 deserves consideration for evaluation in phase III study designed for the treatment of refractory metastatic pancreatic cancer.

A Multinational Phase II Study of PEP02 (MM-398), Liposome Irinotecan, for Patients with Gemcitabine-refractory Metastatic Pancreatic Cancer

A. Ko, M. A. Tempero, Y. S. Shan, W. C. Su, Y. L. Lin, E. Dito, X. Dito, Y. W. Wang, G. Yuh, L. T. Chen

Figure 1. Kaplan Meier survival curve

Figure 2. Price Toxicity and Outcome of PEP02 Treatment

Figure 3. Waterfall plot of tumor response

Figure 4. Toxicities

Randomized Phase III Study of High-Dose Fluorouracil Given As a Weekly 24-Hour Infusion With or Without Leucovorin Versus Bolus Fluorouracil Plus Leucovorin in Advanced Colorectal Cancer: European Organization of Research and Treatment of Cancer Gastrointestinal Group Study 40952

By C-H. Köhne, J. Wils, M. Lorenz†, P. Schöffski, R. Voigtmann, C. Bokemeyer, M. Lutz, U. Kleeberg, K. Ridwelski, R. Souchon, M. El-Serafi, U. Weiss, O. Burkhard, H. Rückle, M. Lichinitser, T. Langenbuch, W. Scheithauer, B. Baron, M.L. Couvreur, and H.J. Schmoll

Purpose: **This trial was conducted to determine whether high-dose fluorouracil (FU) given as a weekly 24-hour infusion is more active than bolus FU + leucovorin (LV), and whether high-dose infusional FU can be modulated by LV.**

Patients and Methods: **A total of 497 patients with previously untreated metastatic colorectal cancer were randomly assigned to receive bolus FU 425 mg/m^2 intravenously + LV 20 mg/m^2 on days 1 to 5 and repeated on day 28 (FU + LV), or FU 2,600 mg/m^2 as a 24-hour infusion alone (FU_{24h}) or in combination with 500 mg/m^2 LV (FU_{24h} + LV)—all given weekly ×6 followed by a 2-week rest period. Survival was the major study end point.**

Results: **With a median follow-up of more than 3 years, survival did not differ among the treatment groups (median FU + LV, 11.1 months [95% CI, 10.2 to 15.0 months]; FU_{24h}, 13.0 months [95% CI, 10.4 to 15.4 months]; FU_{24h} + LV, 13.7 months [95% CI, 12.0 to 16.4 months]; P = .724). Progression-free survival (PFS) was significantly longer for FU_{24h} + LV (median FU + LV, 4.0 months [95% CI, 3.4 to 4.9]; FU_{24h}, 4.1 months [95% CI, 3.4 to 5.0]; FU_{24h} + LV 5.6 months [95% CI, 4.4 to 6.7]; P = .029). The response rates in the subgroup of patients with measurable disease were 12%, 10%, and 17% for FU + LV, FU_{24h}, and FU_{24h} + LV, respectively (not significant). Occurrence of grade 3 and 4 diarrhea was higher in the FU_{24h} + LV arm (22%) compared with the FU_{24h} (6%) or FU + LV (9%) arms; however, stomatitis (11% in FU + LV v 3% in FU_{24h} v 5% in FU_{24h} + LV arms) and hematologic toxicity were higher in the bolus FU + LV arm. Global quality of life did not differ within the three arms.**

Conclusion: **Neither FU_{24h} + LV nor FU_{24h} prolong survival, relative to bolus FU + LV. Leucovorin increases PFS if added to FU_{24h}, but increases toxicity.**

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FLUOROURACIL (FU) is one of the major cytotoxic agents[1] for the treatment of metastatic colorectal cancer. When used as an intravenous bolus application, leucovorin (LV) is usually added as a biochemical modulator to improve the efficacy of FU.[2] This concept is based on the preclinical observation that 5-fluorodesoxyuridine monophosphate forms a ternary complex in the presence of reduced folates, mainly 5,10-methylenetetrahydrofolate, with thymidilate synthase, a key enzyme in DNA synthesis.[3] Randomized trials and a meta-analysis have indicated a doubling of the response rate for modulated FU compared with FU alone; however, no meaningful improvement for median survival has been achieved.[2]

Also, according to randomized trials and a meta-analysis, administration of FU as a continuous infusion has been considered more efficacious compared with bolus application.[4] Furthermore, continuous infusion of FU differs from bolus injection, with a more favorable toxicity profile. A lower incidence of gastrointestinal and hematologic toxicity is observed, but skin toxicity described as hand-and-foot syndrome may appear. Infusional FU has been used as a weekly 24-hour or 48-hour infusion, as well as an indefinite infusion continuing throughout weeks and months. Weekly or biweekly infusional regimens are gaining acceptance. The high FU dose-intensity hereby achieved may be an important factor contributing to the activity of these schedules.[5] The recent results of deGramont et al[6] demonstrated superior activity of a regimen given as a bolus followed by a continuous infusion on 2 consecutive days and repeated every 2 weeks. This regimen demonstrated higher response rates and prolongation of the progression-free interval, but failed to improve survival compared with the bolus Mayo Clinic regimen. In a phase I study, Ardalan et al[7] defined the maximum tolerated dose (MTD) for FU as a 24-hour infusion of 2.6 g/m^2 when

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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given as weekly therapy, which was confirmed by Haas et al.[8] Interestingly, when LV[9] was combined with such a regimen, the MTD remained at 2.6 g/m^2.

In a three-arm randomized study of the Arbeitsgemeinschaft Internistische Onkologie (AIO), a weekly 24-hour infusion of FU was combined with LV (FU_{24h} + LV), interferon alfa-2b (IFN-α-2b; FU_{24h} + IFN), or both LV + IFN-α-2b (FU_{24h} + LV + IFN-α-2b).[10,11] To identify the most suitable regimen for further phase III evaluation to determine progression-free survival (PFS) and overall survival (OS) in comparison with a standard FU bolus regimen, the major study end point of this trial was the response rate. Gastrointestinal toxicity of FU_{24h} + LV + IFN-α-2b was increased, and no difference in the response rate was observed when compared with FU_{24h} + LV. However, FU_{24h} + LV resulted in a significantly higher response rate, as well as a significantly longer PFS and OS (12.7 *v* 16.6 months, $P = .03$) compared with FU_{24h} + IFN-α-2b.

Encouraged by these promising results for FU_{24h} + LV, we chose survival as the primary end point for this study. The objectives were to evaluate whether FU_{24h} alone prolongs survival relative to the Mayo Clinic regimen, and whether biochemical modulation by high-dose LV can improve the efficacy of FU_{24h}.

PATIENTS AND METHODS

In this prospective, multicenter, randomized trial, patients were included if they had histologically verified adenocarcinoma of the colon or rectum beyond a curative option by surgery. Patients were required to have an Eastern Cooperative Oncology Group performance status of 2 or less. No previous chemotherapy for metastatic disease was allowed, with the exception of previous adjuvant treatment if it was completed at least 6 months before inclusion. In case of prior radiation, metastases had to be outside the radiation field. Patients had to have measurable and/or assessable disease according to WHO assessment methods. The initial evaluation of metastases had to be done within 2 weeks before inclusion. Patients were required to have leukocyte levels above 3,000/mm^3 and platelet levels greater than 100,000/mm^3. The creatinine levels were required to be below 2× the upper limit of normal (ULN). Patients 18 to 75 years of age had to give informed consent, and regular follow-up should have been possible for them. Patients were excluded in cases of CNS-metastasis or second malignancy, with the exception of adequately treated in situ carcinoma of the cervix or nonmelanoma skin cancer; patients with severe cardiac or lung failure, those with uncontrolled angina, and pregnant or nursing women were also excluded.

Pretreatment evaluation consisted of a medical history, physical examination, chest x-ray, abdominal ultrasound, and computed tomography scans of abdomen and thorax according to localization of assessable lesions. Magnetic resonance imaging was recommended in case of a pelvic mass. Routine blood test and biochemistry were also performed. Before each cycle for infusional FU, or after 2 cycles for the bolus regimen, we repeated these examinations, as well as tumor evaluation for response for all measurable lesions.

Before random assignment, patients were stratified by institution, Eastern Cooperative Oncology Group performance status (0, 1 *v* 2), tumor assessability (measurable *v* nonmeasurable), and prior adjuvant pretreatment (yes *v* no).

The study was performed according to the Declaration of Helsinki and approved by the Protocol Review Committee of the European Organization of Research and Treatment of Cancer (EORTC) and the AIO, and local biomedical ethics committees. Plausibility of the data was checked for all centers by the study coordinators. Regular site visits were not performed.

Protocol Treatment

Patients randomized to the standard Mayo Clinic regimen received LV 20 mg/m^2 as intravenous (IV) bolus, followed by FU 425 mg/m^2 as IV bolus, given on days 1 to 5 and repeated at weeks 4, 8, and every 5 weeks thereafter. Those 4- or 5-week periods represented one cycle of chemotherapy.

Patients randomly assigned to the two experimental arms received 2,600 mg/m^2 of IV FU as a 24-hour infusion on days 1, 8, 15, 22, 29, and 36 with (FU_{24h} + LV) or without (FU_{24h}) LV 500 mg/m^2 IV as a 2-hour infusion before each FU administration. This cycle was repeated on day 50.

In all arms, the treatment was given until disease progression or occurrence of unacceptable toxicity, or it was stopped on request by the patient. A minimum of one cycle for the infusional treatment or two cycles in case of the Mayo regimen was foreseen, unless this was not in the patient's benefit. In case of complete response, the treatment was to be discontinued after 1 year.

Patients receiving the infusional regimens did not receive therapy on the scheduled day unless gastrointestinal toxicity (diarrhea, mucositis) was completely resolved, leukocyte levels were greater than 3×10^9/L, and platelet levels were greater than 100×10^9/L using the National Cancer Institute common toxicity criteria scale. FU was reduced by 20% for all succeeding administrations if patients experienced grade 4 leukopenia, or thrombocytopenia, diarrhea, mucositis, or skin toxicity greater than grade 2 at any time during therapy. If at any time during therapy with bolus FU, a patient experienced grade 4 leukopenia, thrombocytopenia ≥ grade 3, diarrhea ≥ grade 3, stomatitis ≥ grade 3, skin toxicity ≥ grade 3, or neurologic toxicity ≥ grade 3, the dose of FU was to be reduced by 20% in subsequent applications.

Antiemetics and other symptomatic therapies were allowed. The choice of the antiemetic regimen was left to the discretion of the responsible physician. In case of severe diarrhea, the administration of loperamide or octreotide 100 μg subcutaneously three times daily in combination with rigorous fluid and electrolyte replacement was recommended until diarrhea ceased.

Criteria of Evaluation

OS was defined as the interval from the date of random assignment to the date of death. PFS was the interval from the date of random assignment to the date of progression or death.

Tumor response was only assessed in patients with measurable lesions of at least 2 cm in diameter according to WHO criteria. Complete response was defined as disappearance of all known disease (measurable, assessable, and nonmeasurable) for at least 4 weeks. Partial response required a reduction of 50% or greater in the sum of the cross-product of the maximum perpendicular diameters of all measurable indicator lesions lasting for at least 4 weeks. Patients were considered to have disease progression if the measurable tumor lesions increased more than 25% according to the initial staging, or if a new lesion appeared. Patients not meeting the criteria for response or progression were considered to have stable disease. The response duration was measured from the day of random assignment until disease progression.

The EORTC quality-of-life questionnaire (QLQ C30, version 2.0) was to be handed out to the patients at baseline, after each cycle, at 1 month after the end of treatment, and at 2 monthly follow-up examinations thereafter.

Statistical Methods

The main study end point was OS. Pair-wise comparisons between the three groups of patients were performed. Assuming a median duration of survival in the control group (bolus FU + LV) of approximately 12 months, a total of 369 deaths was necessary to detect a presumed targeted increase of 6 months (ie, from 12 to 18 months) with a two-sided type I error of 0.02 to keep an overall type I error of 0.05 and a power of 80%. Assuming a 4-year duration of recruitment, and a follow-up of 12 months after closing the trial to patient entry, a total of 477 patients (159 in each arm) were to be randomly assigned.

The duration of survival and PFS curves were estimated using the Kaplan-Meier technique. Pair-wise comparisons between the three arms were done with a two-sided unstratified log-rank test. To adjust for

confounding variables, retrospective stratification and the Cox proportional hazards model were used. Comparisons of time-to-event criteria were performed using the intention-to-treat principle (ie, all randomly assigned patients are taken into account and analyzed in the treatment group to which they were assigned at random assignment). Only patients with measurable disease entered the analysis for response. Comparisons were performed using a two-sided χ^2 test. All patients who started the assigned treatment were included in the safety analysis. Comparisons of grade 3 to 4 rates between two arms were performed using a two-sided Fisher's exact test.

RESULTS

From December 1995 to September 1998, 497 patients from 59 institutions were randomly assigned. A total of 166 patients was allocated to the FU_{24h} arm, 164 to the FU_{24h} + LV arm, and 167 to the standard Mayo Clinic arm. Seventeen patients were considered ineligible (7 in the FU_{24h} arm and 5 each in the FU_{24h} + LV and bolus FU + LV arms). Reasons for ineligibility were as follows: no measurable or assessable cancer (8 patients), prior adjuvant treatment within 6 months of random assignment (2 patients), no informed consent (4 patients), high bilirubin levels above 2× ULN (2 patients), and non–small-cell lung cancer as prior malignant disease (1 patient).

Patient characteristics are listed in Table 1. More females were in the FU_{24h} arm, and fewer patients in this arm had weight loss at baseline. Rectal cancer was more common in patients assigned to FU_{24h} + LV. Slightly more patients assigned to the Mayo Clinic regimen seemed to have a normal lacate dehydrogenase level, and had more than one tumor site involved. Other known prognostic factors, such as initial WBC count and serum alkaline phosphatase, were equally distributed among the treatment arms.

Table 1. Patient Characteristics

Variable	Treatment Arm		
	FU_{24h} (n = 166)	FU_{24h} + LV (n = 164)	Bolus FU + LV (n = 167)
Age, years			
Median	61	62	61
Range	25-76	23-76	32-76
Sex, male, % of patients	54	62	63
WHO, performance status, % of patients			
WHO 0	54	52	53
WHO 1	41	41	41
WHO 2	5	7	7
Weight loss, % of patients			
None	57	51	49
≤5%	20	26	26
6–10%	13	14	14
>10%	7	4	9
Missing	2	5	2
Site of primary tumor, % of patients			
Colon	55	45	57
Rectum + rectosigmoid	44	54	42
Unknown	1	2	1
No adjuvant chemotherapy, % of patients	84	86	85
Measurable tumor, % of patients	83	81	83
No. of sites involved,* % of patients			
1	62	59	55
2	26	28	34
> 2	10	9	10
Unknown	2	5	1
Alkaline phosphatase ≥ 2.5 × ULN, % of patients	14	10	15
LDH ≥ 2.5 × ULN, % of patients	16	10	7
WBC ≥ 10 × 10^9/L, % of patients	20	20	20

Abbreviations: FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil; ULN, upper limit of normal; LDH, lactate dehydrogenase.

*The number of sites is accounting for the involvement of primary, lymph node, lung, liver, skin, soft tissue, bone, brain, and an other category.

Toxicity and Drug Administration

Fourteen patients were excluded from the toxicity analysis — six had incomplete files, and eight did not get the assigned treatment. The median number of administered cycles were 2, 2, and 4 in the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively. The cycle duration was different between the arms; thus, the treatment duration was in fact similar in all arms. The median cumulative FU doses were 30.9 g/m^2, 30.8 g/m^2, and 8.3 g/m^2 in the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively. The median duration of all cycles for the infusional schedules was 50 days. In the bolus arm, the median duration was 28 days for the first 2 cycles and 35 days for the following cycles, as scheduled in the protocol. However, in more than one third of patients, cycles were delayed by 1 week or more in both infusional regimens, while they were shortened by 1 week ore more in more than one third of patients in the bolus arm. The relative FU dose-intensity compared with the theoretical dose to be administered was 91%, 89%, and 101% for the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively. However, approximately 50% of patients in the infusional FU arms received less than 90% of the relative FU dose-intensity, compared with only 12% in the FU bolus arm. A total of 19% of patients in the FU_{24h} arm, 20% in the bolus FU + LV arm, but 34% in the FU_{24h} + LV arm, required (at least once) an FU dose reduction at any time during the course of their treatment. The main reason for FU dose reductions was gastrointestinal toxicity in 24% of patients in the FU_{24h} + LV arm, but in only 7.6% and 14.5% of patients receiving FU_{24h} or bolus FU + LV, respectively. Approximately 70% of patients in the infusional FU arms, but only 40% in the bolus FU + LV arm, had at least one treatment delay. Gastrointestinal toxicity was also the reason for treatment delays in 11% and 22% of patients in the FU_{24h} or FU_{24h} + LV arms, and in only 4% of patients in the bolus FU + LV arm.

Toxicities observed per patient are listed in Table 2. Two patients each in the FU_{24h} arm and the bolus FU + LV arm, and 1 patient in the FU_{24h} + LV arm, died of treatment-related septicemia following severe gastrointestinal toxicity. More patients stopped treatment for toxicity reasons in the FU_{24h} + LV arm; 4.2% did so in the bolus FU + LV arm; 5.6% in the FU_{24h} arm; and 10.5% in the FU_{24h} + LV arm.

Leukopenia and stomatitis were more frequent in patients receiving bolus FU + LV, while patients receiving FU_{24h} + LV experienced more diarrhea and hand-and-foot syndrome. The FU_{24h} regimen was the least toxic regarding all toxicity parameters.

Cardiovascular events (grade 1 to 4) at any time during treatment, whether or not related to therapy, were observed in

Table 2. Maximum Toxicity per Patient

Variable	Treatment		
	FU_{24h} (n = 158; % of patients)	FU_{24h} + LV (n = 154; % of patients)	Bolus FU + LV (n = 159; % of patients)
WBC (FU_{24h} + LV *v* bolus FU + LV, $P = .053$)			
Grade 1	17	17	14
Grade 2	3	3	18
Grade 3	2	1	6
Grade 4	2	0.7	0.7
Platelets			
Grade 1	13	19	21
Grade 2	0.7	0.7	3
Grade 3	0	0	0.7
Grade 4	1	0	0
Hemoglobin (FU_{24h} + LV *v* bolus FU + LV, $P = .01$)			
Grade 1	56	61	55
Grade 2	19	16	22
Grade 3	0.7	0	4
Grade 4	1	0	0.7
Diarrhea (FU_{24h} + LV *v* FU_{24h}, $P < .01$; FU_{24h} + LV *v* bolus FU + LV, $P = .015$)			
Grade 1	20	12	18
Grade 2	8	16	14
Grade 3	4	16	8
Grade 4	2	6	1
Nausea			
Grade 1	27	33	31
Grade 2	17	16	15
Grade 3	4	4	2
Grade 4	0	0	0.7
Vomiting			
Grade 1	14	14	14
Grade 2	12	9	9
Grade 3	3	5	1
Grade 4	0	0.7	0.7
Stomatitis (bolus FU + LV *v* FU_{24h}, $P = .03$ bolus FU + LV *v* FU_{24h} + LV, $P = .065$)			
Grade 1	15	15	24
Grade 2	4	10	14
Grade 3	3	3	10
Grade 4	0	2	1
Hand and foot syndrome (FU_{24h} + LV *v* FU_{24h}, $P = .014$)			
Grade 1	8	8	7
Grade 2	4	8	3
Grade 3	0	4	1
Infection			
Grade 1	3	5	2
Grade 2	5	5	4
Grade 3	2	0.7	1
Grade 4	0	0.7	2
Alopecia			
Grade 1	11	14	14
Grade 2	0.7	2	3
Grade 3	0.7	0.7	0

NOTE. Significant or nearly significant differences are shown (Fisher's exact test).

Abbreviations: FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil.

7.5% and 5.7% of patients receiving FU_{24h} or FU_{24h} + LV, and in 4.4% of patients receiving bolus FU + LV. Chest pain at any time during treatment was reported in 4 patients receiving the bolus schedule and two and seven patients receiving FU_{24h} or FU_{24h} + LV, respectively. During the first four weeks of treatment angina-like chest pain occurred in one patient each in the bolus or FU_{24h} regimen and in 2 patients in the FU_{24h} + LV arm, suggesting a likely FU associated cardiotoxicity. Other cardiac events were arrhythmias, hypo- or hypertension, and heart failure. In total, the observed frequencies in cardiovascular side effects were not significantly different.

Quality of Life

Patient compliance with filling out quality-of-life questionnaires was low. At baseline, forms were collected from 54%, 56%, and 70% of patients in the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively, and gradually decreased over time to 21%, 21%, and 28% at year 1. As shown in Figure 1, the global quality-of-life score did not differ significantly among the three treatment arms and did not differ over time. No differences were seen in the domains for functioning (physical, role, cognitive, and social) and symptoms (fatigue, nausea/vomiting, pain, dyspnea, insomnia, appetite, constipation, diarrhea).

Response

Clinical response could be assessed in 409 patients with measurable disease (Table 3). The overall response rates were 10%, 17%, and 12% in the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively. The response rates did not differ significantly between treatment arms. Patients responding to the bolus regimen had a median response duration of 8.1 months (95% CI, 4.4 to 10.4 months); those responding to FU_{24h} had a median response duration of 9.2 months (95% CI, 6.3 to 16.4 months); and responders in the FU_{24h} + LV arm had a median response duration of 5.6 months (95% CI, 3.9 to 7.9 months). With small numbers, these differences were not statistically different.

PFS and Survival

PFS was significantly different among the treatment arms, with a median of 4.0 months (95% CI, 3.4 to 4.9 months) for the bolus FU + LV arm, 4.1 months (95% CI, 3.4 to 5.0 months) for the FU_{24h} arm, and 5.6 months (95% CI, 4.4 to 6.7 months) for the FU_{24h} + LV arm. The PFS durations were compared between pairs of treatment arms. The corresponding P values were in favor of FU_{24h} + LV ($P = .03$) when compared with bolus FU + LV, and $P = .02$ for the comparison with FU_{24h}. No difference was observed between bolus FU + LV and FU_{24h} ($P = .8$). The overall significance for difference between treatments was $P = .03$. The combination of FU_{24h} + LV showed an approximate 22% reduction in the instantaneous progression rate compared with bolus FU + LV, and 24% compared with FU_{24h} (Fig 2).

With a median follow-up of more than 3 years at the time of this analysis, 86% of patients have died. The survival did not differ significantly among treatment groups (Fig 3), with an overall P value of .7. Patients receiving bolus FU + LV had a median survival of 11.1 months (95% CI, 10.2 to 15.0 months). Patients receiving FU_{24h} had a median survival of 13.0 months (95% CI, 10.4 to 15.4 months), and patients on the FU_{24h} + LV arm a median survival of 13.7 months (95% CI, 12.0 to 16.4 months). A total of 63%, 68%, and 68% of patients received a

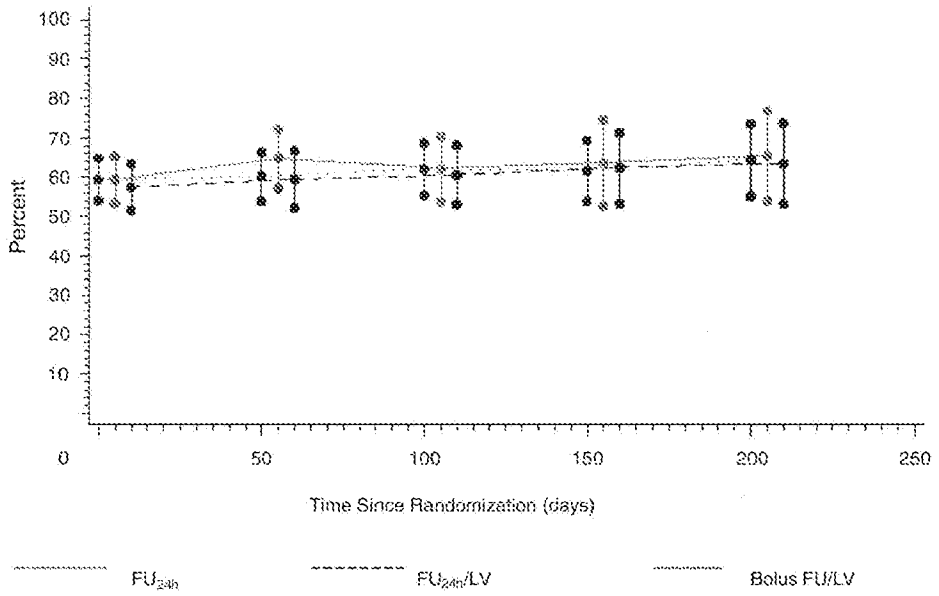


Fig 1. Global quality of life and 99% CIs. FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil.

second-line treatment that contained oxaliplatin (22%, 21%, and 22%) or irinotecan (4%, 8%, and 4%) in the FU_{24h}, FU_{24h} + LV, and bolus FU + LV arms, respectively. The most frequent second-line therapy was infusional FU ± LV.

DISCUSSION

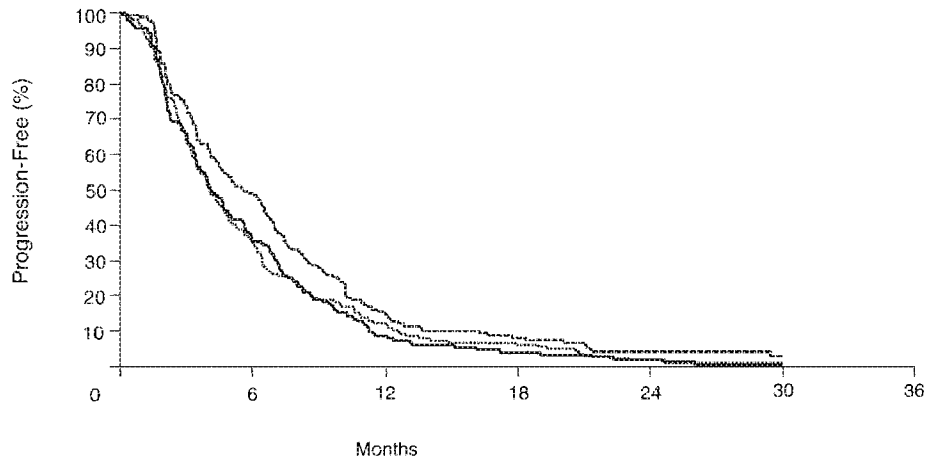
This study failed to demonstrate a 6-month difference in survival for weekly high-dose infusional FU with or without LV compared with the Mayo Clinic regimen. This rather ambitious hypothesis was based on earlier findings of a randomized trial investigating the role of LV, IFN-α-2b, or both as modulators of weekly high-dose infusional FU, in which a statistically significant superior survival over infusional FU + IFN-α-2b (12.7 months) was observed for LV modulation (16.2 months) in this schedule.[11] It is unlikely that the use of second-line treatment may have counterbalanced any potential survival difference induced by first-line treatment with infusional FU, as it was equally distributed among the treatment arms.

However, this trial demonstrates that 500 mg/m^2 LV, when given in combination with FU_{24h}, significantly prolongs the median PFS compared with the Mayo regimen or FU_{24h} alone. Single-agent FU_{24h} seemed to have a similar efficacy, but lower toxicity relative to the modulated bolus regimen. However, the differences in the length of cycles may have potentially biased the calculation of PFS. The first two cycles of infusional regimens were expected to last for 100 days, while the first 4 cycles in the bolus arm were expected to last for 126 days, which may be equivalent to 1 month. However, more than one-third of patients had a treatment delay for at least 1 week in the infusional regimens (totaling at least 2 weeks after two cycles), while more than one third of patients in the bolus arm had a third or fourth cycle that was shorter by at least one week. At least in these patients, the length of treatment was 114 or 112 days for two infusional or four bolus cycles, respectively. Secondly, when the two infusional regimens were compared, there was a clear difference in the PFS times in the two arms, rendering it unlikely

Table 3. Best Response to Treatment

Response	FU_{24h} (n = 138)		FU_{24h} + LV (n = 132)		Bolus FU + LV (n = 139)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
CR	3	2	3	2	0	0
PR	11	8	20	15	16	12
NC	68	49	59	45	69	50
PD	38	28	34	26	36	26
Not assessable	11	8	12	9	11	8
Early death	7	5	4	3	7	5
CR/PR	14	10	23	17	16	12
95% CI	5% to 15%		11% to 24%		6% to 17%	
Duration of response, months						
Median	9.2		5.6		8.1	
95% CI	6.3 to 16.4		3.9 to 7.9		4.4 to 10.4	

Abbreviations: FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil; CR, complete response; PR, partial response; NC, no change; PD, progressive disease.



O	N	Number of patients at risk:				
162	166	58	14	6	3	1
152	164	78	22	12	6	3
165	167	59	20	10	3	1

1. FU_{24h}
2. FU_{24h}/LV
3. Bolus FU/LV

Median(months)[98% CI]: overall p=0.029

FU_{24h} 4.1 [3.4-5.0];
FU_{24h}/LV 5.6 [4.4-6.7];
Bolus FU/LV 4.0 [3.4-4.9];

FU_{24h}/LV vs FU_{24h}	logrank p-value= 0.016	HR: 0.76 95%CI[0.59-0.99]
FU_{24h}/LV vs Bolus FU/LV_{24h}	logrank p-value= 0.029	HR: 0.78 95%CI[0.6-1.02]
Bolus FU/LV vs FU_{24h}	logrank p-value= 0.79	HR: 0.97 95%CI[0.75-1.26]

Fig 2. Progression-free survivol. FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil; HR, hazard ratio.

that the difference in the length of cycles was the major reason for the difference seen between the bolus FU + LV and the FU_{24h} + LV arms. The results of our study at least indicate that LV is effective in prolonging the progession-free survival of weekly high-dose infusional FU_{24h}, which has not been demonstrated before. It is for this reason that in the EORTC study 40986, FU_{24h} + LV is used as the reference treatment to be compared with irinotecan[12] added to this regimen, as PFS is the major study end point.

The Southwest Oncology Group[13] performed a large randomized phase II trial with several infusional FU regimens. Continuous infusion of 200 mg/m^2 FU per day was used alone or combined with 20 mg/m^2 of weekly IV LV. No difference in response rate or median survival was achieved with low-dose LV. The weekly administration of 250 mg/m^2 *N*-phosphonacetyl-L-aspartate was also unable to increase the antineoplastic activity of high-dose FU_{24h} in two randomized trials.[13,14] Successful biochemical modulation of high-dose infusional FU was reported from an earlier EORTC trial for low-dose methotrexate.[15]

The results of our trial support the findings of a French study,[6] in which a biweekly schedule of bolus FU followed by FU infusion in combination with LV given on 2 consecutive days was compared with the monthly (bolus FU) Mayo Clinic schedule. The infusional regimen resulted in a significantly higher response rate and longer PFS compared with the IV bolus Mayo Clinic regimen, without a difference in survival, all consistent with our findings.

The results of our trial confirm a lower rate of severe leukopenia for infusional regimens compared with the IV bolus Mayo Clinic regimen. However, severe diarrhea occurred in 22% of patients receiving FU_{24h} + LV, which was significantly higher as compared with the Mayo Clinic schedule as well as with the FU_{24h} regimen. This was, however, expected, considering our earlier randomized trial. Rather than indicating increased gastrointestinal toxicity of the modulated infusional regimen, the difference in diarrhea probably is due more to an unusually low incidence of grade 3 or 4 diarrhea (9%) and mucositis (11%) observed in patients receiving the Mayo Clinic regimen, as we would have expected a higher rate for both diarrhea and mucositis, and more frequent treatment delays in the bolus regimen.[16]

Another interesting observation is that occurrence of hand-and-foot syndrome was higher in the modulated high-dose infusional arm as compared with high-dose infusional FU alone. Thus, LV is probably increasing the incidence of hand-and-foot syndrome with infusional FU.

The high rate of FU dose reduction and treatment delays in the FU_{24h} + LV arm indicates that the FU dose used in the modulated infusional regimen is probably at the upper limit of tolerance, and should be reduced[17] to 2,000 mg/m^2 when combined with irinotecan and/or oxaliplatin, though full-dose has been suggested by a phase I trial.[18]

In conclusion, FU_{24h} seems to be as efficacious as the Mayo regimen, but it is less toxic. FU_{24h} + LV is associated with a significantly increased PFS compared with both the Mayo

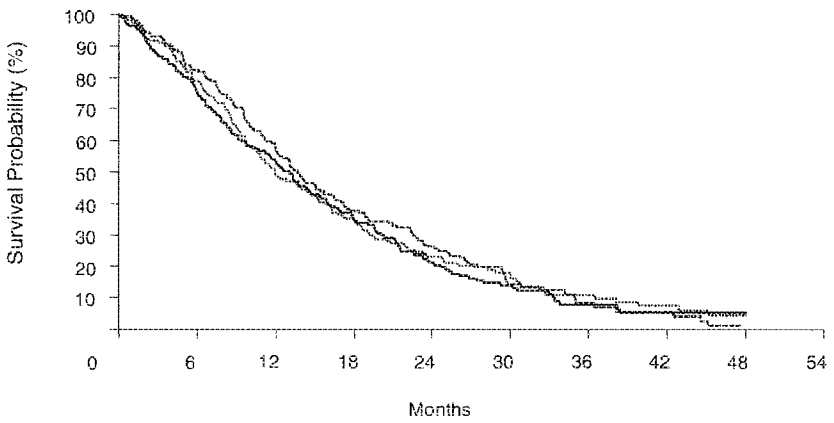


Fig 3. Overall survival. FU_{24h}, fluorouracil as a weekly 24-hour infusion; LV, leucovorin; FU, fluorouracil; HR, hazard ratio.

O	N	Number of patients at risk:								
142	166	120	83	52	31	18	7	2	1	1. FU_{24h}
139	164	131	85	56	39	17	6	4	0	2. FU_{24h}/LV
145	167	132	77	53	34	18	10	6	2	3.Bolus FU/LV

median (months)[98%CI]: overall p=0.724

FU_{24h}	13.0	[10.4-15.4];
FU_{24h}/LV	13.7	[12.0-16.4];
BolusFU/LV	11.9	[10.2-15.0];

FU_{24h}/LV vs FU_{24h}	logrank p-value= 0.38 HR: 0.90 95%CI [0.68-1.19]
FU_{24h}/LV vs Bolus FU/LV	logrank p-value= 0.70 HR: 0.95 95%CI [0.72-1.26]
Bolus FU/LV vs FU_{24h}	logrank p-value= 0.76 HR: 0.96 95%CI [0.73-1.27]

regimen and FU_{24h}, but it does not improve survival, and it increases gastrointestinal toxicity. Although reducing the disease progression rate by 22% may have important palliative implications, other treatment options may also be reasonable alternatives for first-line treatment of metastatic colorectal cancer if the use of irinotecan or oxaliplatin combination is not considered. The slightly improved antineoplastic efficacy must be weighed against higher costs associated with the use of Port-a-Caths and pumps, which are necessary to ensure outpatient treatment.

However, modulated infusional regimes, either the LV5FU2[6] or this AIO regimen, are probably the more optimal FU schedules for combination treatment with oxaliplatin or irinotecan. In trials in which PFS is the main study end point, LV-modulated infusional FU may be the preferred mode of FU administration when new treatment options such as oral fluoropyrimidines alone or in combination with irinotecan or oxaliplatin are studied. Interestingly, some FU bolus regimes seem to be too toxic to be combined with irinotecan or oxaliplatin,[19] as has been shown recently.

APPENDIX

The appendix is included in the full-text version of this article, available on-line at www.jco.org. It is not included in the PDF (via Adobe® Acrobat Reader®) version.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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[CANCER RESEARCH 62, 4282–4288, August 1, 2002]

Novel Chondroitin Sulfate-binding Cationic Liposomes Loaded with Cisplatin Efficiently Suppress the Local Growth and Liver Metastasis of Tumor Cells *in Vivo*[1]

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ABSTRACT

An increased level of chondroitin sulfate (CS) expression on the cell surface is often associated with malignant transformation and the progression of tumor cells. In this study, CSs expressed on highly metastatic tumor cells were used as a target for the selective delivery of anticancer drugs by polyethylene glycol (PEG)-coated liposomes that contained a new cationic lipid 3,5-dipentadecycloxybenzamidine hydrochloride (TRX-20). We found that PEG-coated TRX-20 liposomes (TRX-20 liposomes) bound preferentially to certain CSs, such as CS B, CS D, and CS E, whereas PEG-coated liposomes lacking TRX-20 showed no significant binding to any of the glycosaminoglycans tested. *In vitro*, TRX-20 liposomes, but not plain PEG liposomes, avidly bound to and were readily internalized by highly metastatic tumor cells such as LM8G5 and ACHN cells, which express large amounts of CS on the cell surface. When TRX-20 liposomes were loaded with cisplatin, they effectively killed the CS-expressing tumor cells *in vitro*, whereas cisplatin-PEG liposomes lacking TRX-20 were totally ineffective. When injected systemically, TRX-20 liposomes preferentially accumulated in the liver and in solid s.c. LM8G5 tumors. Therapeutic experiments in mice bearing a s.c. LM8G5 tumor revealed that cisplatin-loaded TRX-20 liposomes were significantly more effective in reducing the local tumor growth than cisplatin-loaded plain PEG liposomes or free cisplatin. Furthermore, the cisplatin-loaded TRX-20 liposomes markedly suppressed metastatic spreading of LM8G5 tumor cells to the liver, significantly increasing the survival time of the tumor-bearing mice. These results demonstrate that the CS-targeted delivery of anticancer drugs by novel cationic liposomes represents a potentially useful strategy to prevent the local growth and metastasis, particularly to the liver, of tumor cells that have enhanced expression of CS.

INTRODUCTION

GAGs[3] exist in tissues mainly as proteoglycans (1). They include heparin, heparan sulfates, dermatan sulfates, keratan sulfates, hyaluronic acid, and CSs. GAG chains are highly anionic and hence have been implicated in numerous cellular functions, including cell adhesion (2, 3), cellular growth (4), tumor cell invasion (5), and viral infection (6).

Altered levels of production of and structural changes in GAGs have been reported in many neoplastic tissues (7, 8). In particular, an increased production of CSs has been found in transformed fibroblasts (9), mammary carcinoma cells (10, 11), and melanoma cells (12, 13). The selective and enhanced expression of CSs in certain types of malignant cells raises the possibility that the tumor-associated CSs may be a suitable molecular target for the selective delivery of anticancer drugs to malignant cells.

Liposomes have been used as carriers for the targeted delivery of anticancer drugs (14). Recent technical advances have established that liposomes containing PEG-conjugated lipids show reduced uptake by phagocytic cells and hence remain in the blood circulation for a long period of time (15). The sustained residence in the circulation enhances the liposomal accumulation in solid tumors (16–18) through tumor vessels where the permeability of endothelial barriers is much greater than it is in normal tissues. Experimentally, the unique tumoritropic accumulation of PEG liposomes has allowed the successful delivery of liposome-encapsulated drugs to solid tumors *in vivo* (19, 20). On the basis of these results, several liposomal formulations loaded with chemotherapeutic drugs are already being used clinically (21).

We recently developed a new formulation for long-circulating PEG liposomes that contain a new cationic lipid, TRX-20. We reported previously that TRX-20 liposomes preferentially bound to subendothelial cells and mesangial cells *in vitro* by recognizing CSs on the surface of these cells (22). When administered to mice with glomerulonephritis, the TRX-20 liposomes selectively accumulated in glomerular mesangial lesions where vascular permeability was increased and CSs were abundantly expressed (23). Furthermore, prednisolone encapsulated into TRX-20 liposomes showed an increased therapeutic efficacy, compared with the free drug (23). These results prompted us to investigate whether the TRX-20 liposomes could be used to selectively deliver anticancer drugs to tumor cells *in vivo* that have enhanced expression of CSs.

In this study, we examined *in vitro* and *in vivo* the applicability of TRX-20 liposomes to the targeted delivery of cisplatin to highly invasive LM8G5 and ACHN tumor cells expressing large amounts of CSs. We demonstrated that TRX-20 liposomes loaded with cisplatin successfully killed these tumor cells *in vitro* and effectively suppressed their local growth in s.c. sites, with a marked suppression of liver metastasis *in vivo*. Our results suggest that TRX-20 liposomes represent a novel strategy for the targeted delivery of anticancer drugs to CS-expressing tumor cells *in vivo*.

MATERIALS AND METHODS

Mice. Female C3H/HeN mice and ICR nude mice, 10–14 weeks old, were obtained from Charles River Japan (Kanagawa, Japan) and kept in standard housing. All animal experiments were performed under the experimental protocol approved by the Ethics Review Committee for Animal Experimentation of Osaka University Graduate School of Medicine.

Cell Lines. The ACHN human renal adenocarcinoma cell line was kindly provided by Dr. Tae Takeda (National Children's Medical Research Center, Tokyo, Japan). The LM8G5 murine osteosarcoma cell line, which has a high

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[3] The abbreviations used are: GAG, glycosaminoglycan; CS, chondroitin sulfate; PEG, polyethylene glycol; PEG liposomes, PEG-modified liposomes; TRX-20, 3,5-dipentadecycloxybenzamidine hydrochloride; TRX-20 liposomes, TRX-20-containing PEG liposomes; HSPC, hydrogenated soybean phosphatidylcholine; Chol, cholesterol; PEG-PE, distearoylphosphatidylethanolamine PEG; rhodamine-PE, rhodamine B-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt; mAb, monoclonal antibody; *Cis*-TRX20L, cisplatin encapsulated in TRX-20 liposome; *Cis*-PEGL, cisplatin encapsulated in plain PEG liposome; CSPG, chondroitin sulfate proteoglycan.

potential for metastasis to the liver, was isolated from LM8 cells (Riken Cell Bank, Tsukuba, Japan) after five successive cycles of *in vivo* selection procedures (24). The HT-29 human colon adenocarcinoma cell line was obtained from American Type Culture Collection. The ACHN and HT-29 cells were maintained in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum, 1% (v/v) 100× nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomycin. The LM8G5 cells were maintained in DMEM (Sigma-Aldrich) containing the same additives as above.

Reagents. The lipids used in this study were as follows: HSPC (Lipoid, Ludwigshafen, Germany), Chol (Solvay Pharmaceuticals B.V., Weesp, The Netherlands), PEG-PE (M_r = 5,000; Genzyme Pharmaceuticals, Liestal, Switzerland), and Lissamine rhodamine-PE (Molecular Probes, Inc., Eugene, OR). TRX-20 was synthesized as reported previously (22). CS A, CS B, CS C, CS D, CS E, hyaluronic acid, heparan sulfate, keratan sulfate, chondroitin, mAb CS-56 (mouse IgM, specific for CS; Refs. 25, 26), chondroitinase ABC, and heparitinase I were all obtained from Seikagaku Corporation (Tokyo, Japan). Heparin and mouse IgM were obtained from Sigma-Aldrich.

Liposome and Liposomal Cisplatin Preparation. Liposomes were prepared as described (22) with some modifications. The lipid mixtures HSPC:Chol = 54:46 or HSPC:Chol:TRX-20 = 50:42:8, which include 0.2 mol % of rhodamine-PE, were dissolved in *t*-butyl alcohol and lyophilized. After hydration at 65°C in 0.9% NaCl for empty liposomes or in 7.1 mg/ml cisplatin (Heraeus, Hanau, Germany) in 0.9% NaCl for cisplatin-containing liposomes, the liposomes were prepared by vigorous vortexing, sonication, and extrusion through double-stacked polycarbonate membranes (Nucleopore Whatman, Inc., Clifton, NJ) to obtain sized liposomes (~100 nm in diameter). Unentrapped, precipitated cisplatin was removed by successive filtering through a 0.45-μm pore filter and gel filtration (Sepharose 4FF; Amersham Pharmacia Biotech AB, Uppsala, Sweden). A PEG-PE solution was added to the liposomes and incubated at 60°C for 30 min to incorporate 0.75 mol % of PEG-PE into the liposomal outer membrane. The lipid concentration was determined using a phospholipid determination kit (Wako Pure Chemical Industries). The cisplatin concentration was determined by high-performance liquid chromatography.

***In Vitro* Liposome Binding to GAGs.** GAGs (5 μg/well) were immobilized on Sumilon Amino-type 96-well plates (Sumitomo Bakelite, Tokyo, Japan) overnight at 4°C and treated with 50 milliunits/ml of chondroitinase ABC or 20 milliunits/ml of heparitinase I for 1 h at 37°C or left untreated. After the plates were washed with PBS, rhodamine-labeled TRX-20 liposomes (50 nmol of lipid) were added to them, and the plates were incubated for 1 h at 37°C. After being washed with PBS, the liposomes were solubilized by adding lysis buffer [20 mM Tris-HCl (pH 7.4), 0.1% SDS, 1% Triton-X100, and 1% sodium deoxycholate]. The fluorescence intensity was measured by a fluorescence plate reader, Fluoroskan II (Labsystems, Helsinki, Finland).

Cell ELISA. Tumor cells were seeded (3×10^3 cells/well) into 96-well plates and incubated for 2 days. The cells were rinsed twice with PBS and fixed with 3% paraformaldehyde for 30 min. After the cells were washed with the washing buffer (PBS containing 0.1% BSA), they were blocked with 3% BSA in PBS and treated with or without 50 milliunits/ml of chondroitinase ABC or 20 milliunits/ml of heparitinase I for 1 h at 37°C. The cells were washed again in washing buffer, then incubated with diluted CS-56 (1:100 diluted ascites) mAb for 1 h, followed by peroxidase-conjugated goat antimouse IgG+M (Biosource International, Camarillo, CA; 1:1000). To quantify the reaction, *o*-phenylenediamine was used as a substrate.

***In Vitro* Liposome Binding to Tumor Cells.** The tumor cell monolayer was treated with chondroitinase ABC or heparitinase I as above or left untreated, then the rhodamine-labeled TRX-20 liposomes or PEG liposomes (50 nmol of lipid) were added, and the monolayers were incubated for 1 h at 37°C. The monolayers were washed with PBS, bound liposomes were solubilized by adding lysis buffer, and rhodamine fluorescence was measured as above.

***In Vitro* Uptake of Liposomes.** Tumor cells (8×10^3/well) were cultured for 48 h in 8-well Lab-Tek chambers (Nalge Nunc International), then rhodamine-labeled TRX-20 liposomes or control PEG liposomes (50 nmol of lipid) were added for 1 h at 37°C. After extensive washing with PBS, the tumor cells were treated with chondroitinase ABC (50 milliunits/ml) for 1 h at 37°C or left untreated to evaluate whether the TRX-20 liposomes were surface-associated or internalized by the tumor cells. In some experiments, the tumor cells were incubated for 24 h before the chondroitinase ABC treatment to allow intracellular uptake of surface-bound liposomes. Subsequently, the tumor cells were incubated with 10 μM Hoechst 33342 (Molecular Probes, Inc.) for nuclear staining, fixed in 5% phosphate-buffered formalin, and examined with a fluorescence microscope BX50 (Olympus, Tokyo, Japan).

***In Vitro* Cytotoxicity Studies.** Tumor cells (1.5×10^3 cells/well) were first incubated for 24 h to make a monolayer, then treated with free cisplatin or cisplatin entrapped in TRX-20 liposomes or PEG liposomes for 24 h. After the drugs were removed by washing the cells with PBS, the cells were further incubated for 60 h at 37°C. Cell proliferation was determined by water-soluble tetrazolium salt assay as described previously (27).

Biodistribution of TRX-20 Liposomes in s.c. Tumor-bearing Mice. C3H/HeN and ICR nude mice were inoculated s.c. with LM8G5 (2×10^6) and HT-29 (2.5×10^6) cells, respectively, on day 0. On day 21, rhodamine-labeled TRX-20 liposomes or control PEG liposomes (0.5 mol/kg) were given to mice by intracardiac injection. The mice were sacrificed 24 h after the treatment, and peripheral blood samples were collected. Various tissues, including s.c. tumors, were removed after whole-body perfusion with heparinized 0.9% NaCl solution. Rhodamine-PE was extracted from tissue homogenates using the M-Per mammalian protein extraction reagent (Pierce Chemical Co., Rockford, IL) or from blood samples (diluted five times with 0.9% NaCl solution) by methanol and chloroform as described previously (28). The biodistribution of the liposomes was determined by measuring the rhodamine fluorescence as described above.

***In Vivo* Therapeutic Experiments.** For the s.c. tumor models, C3H/HeN and ICR nude mice were inoculated s.c. with LM8G5 (2×10^6 cells) and HT-29 (2.5×10^6 cells), respectively, on day 0. On days 7 and 14, the mice were given free cisplatin or *Cis*-TRX20L or *Cis*-PEGL (3.5 mg/kg) by intracardiac injection (100 μl). Control mice received injections of sterile 0.9% NaCl solution. The s.c. tumor cell growth was monitored by measuring the three diameters of the tumor nodules, and the tumor volume was calculated using the following formula: volume = $1/6 \times \pi \times d1 \times d2 \times d3$. In the LM8G5 liver metastasis model, C3H/HeN mice received an intracardiac injection of LM8G5 cells (1×10^6 cells in 200 μl of PBS) on day 0. On day 3, 5 mg/kg of free cisplatin or cisplatin entrapped in liposomes were given as above. For the evaluation of liver metastasis, mice were sacrificed on day 14, the number of tumor nodules was counted macroscopically, and the liver weight was measured. In separate experiments, mice were monitored for their survival after treatment. To study the acute toxicity of different cisplatin formulations, ICR nude mice were given free cisplatin or cisplatin entrapped in TRX-20 liposomes or PEG liposomes (10 mg/kg) by intracardiac injection and monitored for survival.

Statistical Analysis. The statistical differences observed between different groups regarding s.c. tumor cell growth and liver tumor burden were deter-

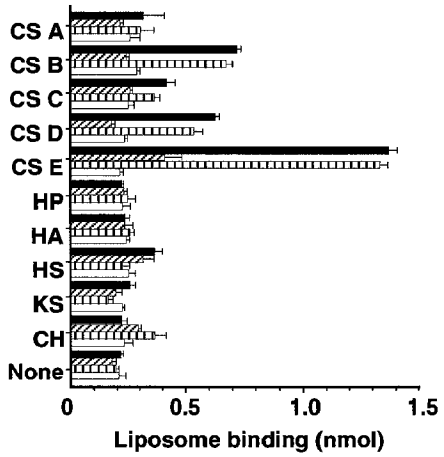

Fig. 1. TRX-20 liposomes preferentially bind to certain CSs *in vitro*. Each GAG (5 μg/well) was immobilized onto 96-well plates. The amount of binding of rhodamine-labeled liposomes to the immobilized GAGs (■) or to GAGs treated with chondroitinase ABC (50 milliunits/ml; ▨) or heparitinase I (20 milliunits/ml; ▥) was determined. Binding of rhodamine-labeled PEG liposomes to untreated GAGs (□) is shown as the control. CS, chondroitin sulfate; HP, Heparin; HA, hyaluronic acid; HS, heparan sulfate; KS, keratan sulfate; CH, chondroitin.

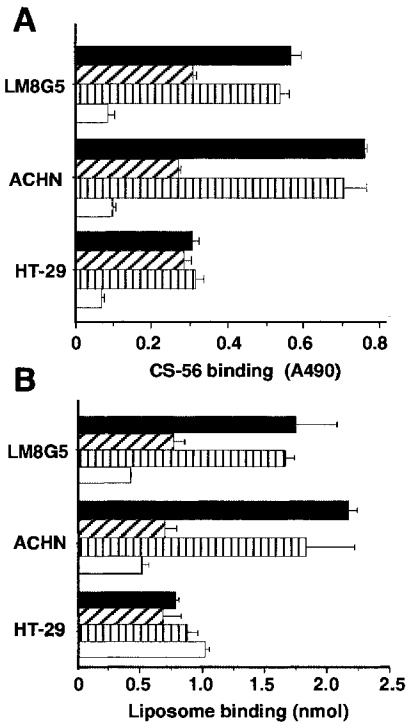

Fig. 2. TRX-20 liposomes bind to tumor cell lines expressing large amounts of CS. *A*, expression of CS on LM8G5, ACHN, and HT-29 cells. Cells were untreated (■) or treated with chondroitinase ABC (50 milliunits/ml; ▨), or heparitinase I (20 milliunits/ml; ▥) and examined for CS expression using the anti-CS mAb CS-56. Normal mouse IgM was used as the negative control (□). *B*, TRX-20 liposome binding to various tumor cells. Tumor cells were left untreated (■) or treated with chondroitinase ABC (50 milliunits/ml; ▨) or heparitinase I (20 milliunits/ml; ▥), and the amount of binding of rhodamine-labeled TRX-20 liposomes was determined. Rhodamine-labeled PEG liposomes were used as the control (□).

mined using the standard Student's *t* test. The significance of the differences between the groups in the survival experiment was determined using the Mantel-Cox log-rank test.

RESULTS

TRX-20 Liposomes Bind to Certain Types of CSs. To examine the binding ability of TRX-20 liposomes to GAGs, rhodamine-labeled TRX-20 liposomes were added to various GAG species that had been immobilized on plastic plates. As shown in Fig. 1, TRX-20 liposomes showed strong binding to CS E, moderate binding to CS B and CS D, and almost no binding to the other GAG chains tested. Control PEG liposomes did not show significant binding to GAGs. The TRX-20 liposome binding to the CSs was abolished by pretreatment of the GAGs with chondroitinase ABC but not heparitinase I. These results confirmed our previous observations (22) and extended them by showing that TRX-20 liposomes preferentially recognize certain CS chains such as CS E, CSB, and CS D.

TRX-20 Liposomes Bind to Tumor Cells that Express Large Amounts of CS. To examine whether TRX-20 liposomes could selectively bind tumor cells expressing high levels of CS on the cell surface, three tumor cell lines expressing different levels of CS were used. As shown in Fig. 2*A*, the murine osteosarcoma LM8G5 and human renal adenocarcinoma ACHN cell lines showed strong reactivity to the anti-CS mAb CS-56, indicating that these cell lines express large amounts of CS on the cell surface. In contrast, the human colon adenocarcinoma HT-29 cells showed only weak reactivity to the anti-CS mAb, indicating that they express little CS on the cell surface. Interestingly, the binding of TRX-20 liposomes to these cell lines almost completely reiterated the study with mAb CS-56 in that, as shown in Fig. 2*B*, the TRX-20 liposomes bound strongly to both LM8G5 and ACHN but very little bound to HT-29. The TRX-20 liposome binding was completely abrogated by chondroitinase but not heparitinase treatment, indicating that the TRX-20 liposomes preferentially bind to tumor cells that express large amounts of CS.

TRX-20 Liposomes Are Internalized by CS-expressing Tumor Cells. To determine whether TRX-20 liposomes could be internalized by tumor cells, the rhodamine-labeled TRX-20 liposomes and control PEG liposomes were examined for uptake. After 1 h of incubation followed by washing, the LM8G5 cells showed strong fluorescence from rhodamine-labeled TRX-20 liposomes (Fig. 3*A*), which, however, almost completely disappeared after the chondroitinase ABC treatment (Fig. 3*B*), indicating that the cell-associated fluorescence was mainly attributable to surface-bound liposomes. When LM8G5 cells were incubated for 24 h before the chondroitinase ABC treatment, they showed strong fluorescence that localized mainly to the cytoplasm; the cell-associated fluorescence remained unaltered even after the enzyme treatment (Fig. 3, *C* and *D*), indicating that TRX-20 liposomes were actually internalized by the tumor cells. When the same cells were incubated with rhodamine-labeled plain PEG liposomes, they showed little fluorescence (Fig. 3*E*). In addition, when HT-29 cells, which show low binding of TRX-20 liposomes, were incubated with the liposomes, they also showed almost no cytoplasmic fluorescence (Fig. 3*F*). These results indicate that TRX-20 liposomes are efficiently internalized by LM8G5 cells upon binding to the CSs expressed on the cell surface. Avid uptake of TRX-20 liposomes was also observed in ACHN cells (data not shown).

Cisplatin Entrapped in TRX-20 Liposomes but not in Plain PEG Liposomes Kills CS-expressing Tumor Cells *in Vitro*. To evaluate the efficacy of TRX-20 liposomes for drug delivery to tumor cells, the *in vitro* cytotoxicity of *Cis*-TRX20L was examined in

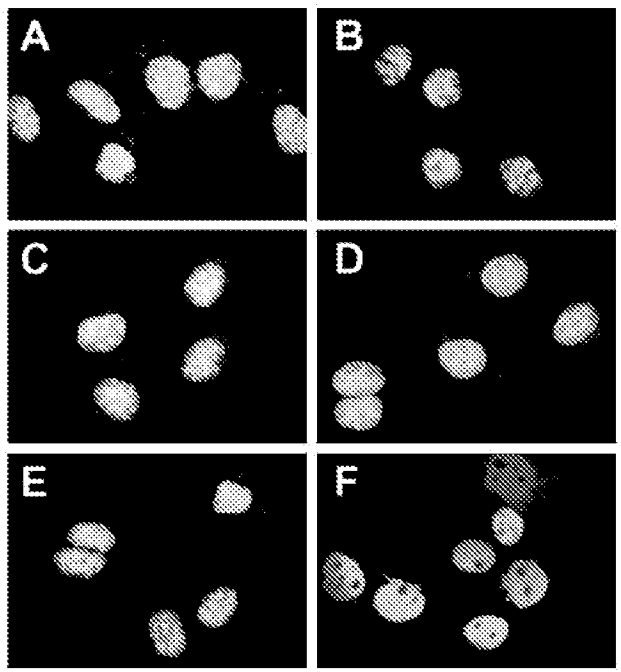

Fig. 3. Uptake of TRX-20 liposomes by tumor cells. LM8G5 cells were incubated with rhodamine-labeled TRX-20 liposomes for 1 h at 37°C. After extensive washing with PBS to remove unbound liposomes, the cells were either left untreated (*A*) or were treated immediately (*B*) with chondroitinase ABC (50 milliunits/ml) for 1 h at 37°C. In a parallel experiment, LM8G5 cells were incubated for 24 h, then left untreated (*C*) or were treated (*D*) with chondroitinase ABC to allow liposome uptake. The cells were then stained with Hoechst 33342 (10 mM) for 30 min and observed under a fluorescence microscope. LM8G5 cells incubated with rhodamine-labeled PEG liposomes (*E*) and HT-29 cells incubated with rhodamine-labeled TRX-20 liposomes (*F*) are shown as controls.

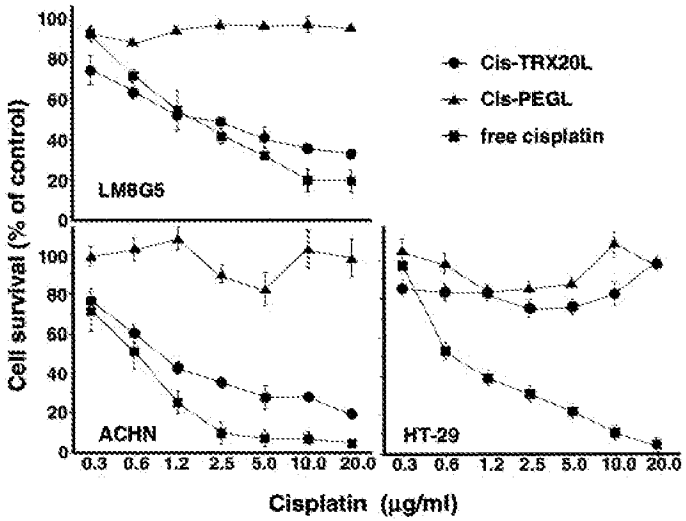

Fig. 4. Selective antitumor effects of *Cis*-TRX20L on tumor cells expressing CSs. LM8G5, ACHN, and HT-29 cells were incubated with various concentrations of free cisplatin (■), *Cis*-TRX20L (●), or *Cis*-PEGL (▲) for 24 h. After the drugs were removed, the tumor cells were further incubated for 60 h. Cellular proliferation was determined by colorimetric assay as described in "Materials and Methods."

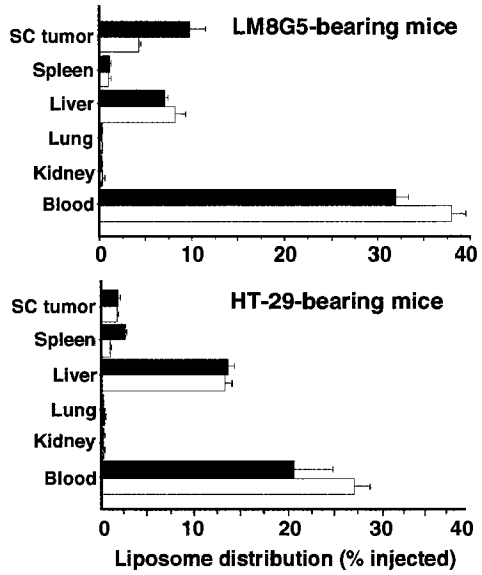

Fig. 5. Biodistribution of TRX-20 liposomes in s.c. tumor-bearing mice. Mice bearing LM8G5 or HT-29 cells were given rhodamine-labeled TRX-20 liposomes (■) or PEG liposomes (□) by intracardiac injection (lipid; 0.5 mol lipid/kg). After 24 h, the liposome distribution to solid tumors and various tissues was determined.

comparison with the cytotoxicity of *Cis*-PEGL and free cisplatin. As shown in Fig. 4, *Cis*-TRX20L exhibited a dose-dependent cytotoxicity against LM8G5 and ACHN cells, which was comparable with that observed with free cisplatin. In contrast, *Cis*-TRX20L showed only a low-grade cytotoxicity against HT-29 cells, which express only marginal levels of CS. Because free cisplatin efficiently killed HT-29 cells, these results suggest that the low cytotoxicity of *Cis*-TRX20L against HT-29 cells was not because of insensitivity of the HT-29 cells to cisplatin but rather to low liposomal binding of the tumor cells. In addition, *Cis*-PEGL, which showed little tumor cell binding, exhibited almost no cytotoxicity against any of the tumor cells examined. Taken together, these results demonstrate that *Cis*-TRX20L has selective and potent cytotoxic activities against tumor cells expressing large amounts of CS.

TRX-20 Liposomes Efficiently Accumulate in Solid Tumors *in Vivo*. High and selective accumulation of drug carriers at the tumor site is essential for the success of drug targeting *in vivo*. To examine whether TRX-20 liposomes would selectively accumulate in solid tumors expressing CS *in vivo*, rhodamine-labeled TRX-20 liposomes, or rhodamine-labeled control PEG liposomes were administered by intracardiac injection to mice bearing a s.c. LM8G5 or HT-29 tumor, and liposome distribution was determined 24 h after the injection. As shown in Fig. 5, in animals bearing an LM8G5 tumor, TRX-20 liposomes accumulated in s.c. LM8G5 tumors at twice the levels of the PEG liposomes, whereas both liposomal preparations were retained in the blood circulation and accumulated in the liver equally well. Interestingly, in mice bearing the HT-29 tumor, both the TRX-20 liposomes and PEG liposomes showed low accumulation in the tumor but comparably high accumulation in the blood and liver. In both groups of tumor-bearing mice, TRX-20 and PEG liposomes accumulated in the lung and kidney only marginally. Collectively, these results demonstrate that TRX-20 liposomes but not PEG liposomes can selectively accumulate in a s.c. tumor expressing large amounts of CS, whereas both TRX-20 and PEG liposomes have long circulation times and accumulate preferentially in the liver.

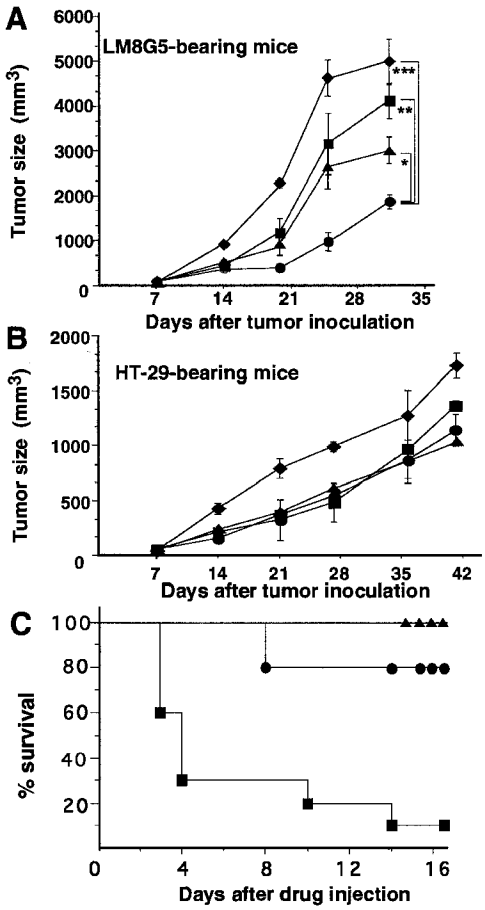

Fig. 6. Antitumor efficacy of free and liposomal cisplatin to s.c. tumor cells in mice. In *A* and *B*, antitumor effects of free and liposomal cisplatin. Mice were inoculated s.c. with 5×10^6 LM8G5 (*A*) or HT-29 (*B*) cells on day 0. Free cisplatin (■), *Cis*-TRX20L (●), *Cis*-PEGL (▲; 3.5 mg cisplatin/kg), or 0.9% NaCl solution as the control (◆) was administered by intracardiac injection on days 7 and 14 to evaluate antitumor effects *in vivo*. The antitumor effects of the different cisplatin formulations were determined by measuring the size of the s.c. tumor nodules. *, $P < 0.05$ compared with free cisplatin and *Cis*-PEGL. **, $P < 0.05$ compared with *Cis*-PEGL. ***, $P < 0.01$ compared with free cisplatin. *C*, acute toxicity of free and liposomal cisplatin in ICR nude mice. Mice were given free cisplatin (■), *Cis*-TRX20L (●), or *Cis*-PEGL (▲; 10 mg cisplatin/kg) by a single intracardiac injection and their survival was monitored. The mean survival time of mice that received treatment with *Cis*-TRX20L or *Cis*-PEGL was prolonged significantly ($P < 0.05$) in comparison with that of mice treated with free cisplatin.

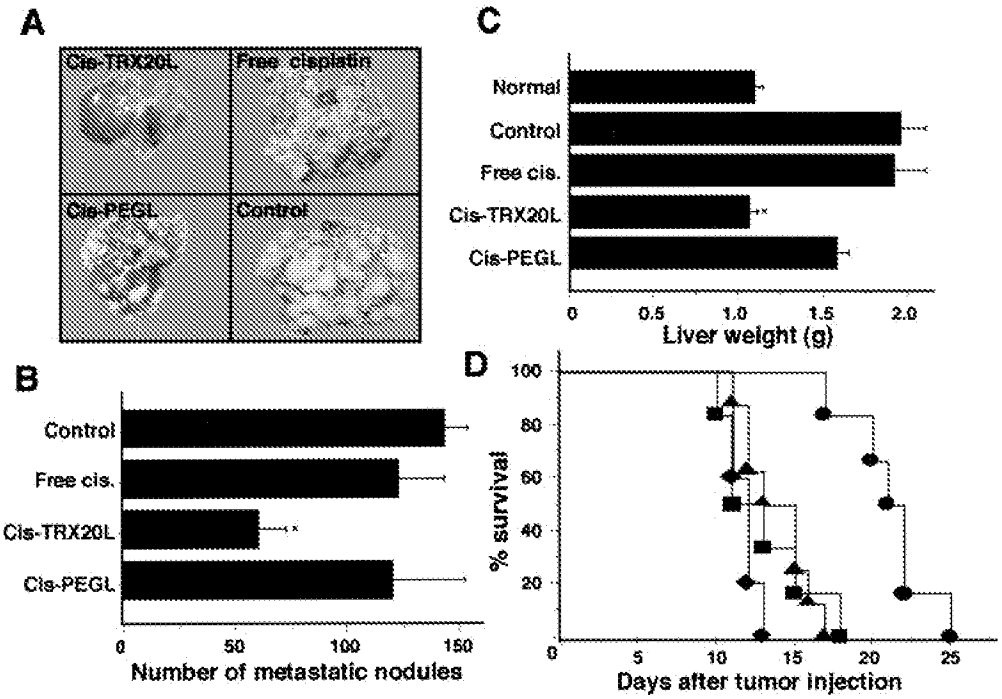

Fig. 7. Antitumor efficacy of free and liposomal cisplatin in LM8G5 liver metastasis models. C3H/HeN mice were given an intracardiac injection of LM8G5 cells (1×10^6) on day 0. On day 3, mice were given the different formulations of cisplatin (5 mg/kg) or 0.9% NaCl solution as the control by a single cardiac injection. Mice were sacrificed on day 14 to determine the efficacy of the cisplatin preparations for experimental tumor metastasis. *A*, metastatic nodules in the liver of mice injected with LM8G5 cells. *B*, number of liver metastatic nodules in mice. *C*, liver weight. Data are represented as the mean ± SD of 4 mice in each group. *, $P < 0.05$ compared with free cisplatin and *Cis*-PEGL. *D*, survival of mice treated with free cisplatin (■), *Cis*-TRX20L (●), *Cis*-PEGL (▲), or 0.9% NaCl solution (◆). The mean survival time of mice that received the *Cis*-TRX20L treatment was prolonged significantly ($P < 0.005$) in comparison with other groups ($n = 6$).

Cisplatin Entrapped in TRX-20 Liposomes Inhibits the Growth of s.c. Tumors in Mice. We then examined the therapeutic efficacy of TRX-20 liposomes loaded with chemotherapeutic drugs. C3H/He and ICR nude mice were given a s.c. inoculation of LM8G5 cells and HT-29 cells, respectively, and were then treated with *Cis*-TRX20L, *Cis*-PEGL, or free cisplatin on days 7 and 14 (3.5 mg/kg). In mice inoculated with LM8G5 cells, *Cis*-TRX20L was quite effective in suppressing the s.c. tumor growth compared with free cisplatin and *Cis*-PEGL (Fig. 6*A*). By contrast, in mice given HT-29 cells, *Cis*-TRX20L, *Cis*-PEGL, and free cisplatin were all only marginally effective with no significant differences in their efficacies of growth suppression (Fig. 6*B*). It is interesting to note that although *Cis*-PEGL was much inferior to free cisplatin in *in vitro* cytotoxicity (Fig. 4), its *in vivo* effect was equivalent to that of free cisplatin, which may be attributable to a long half-life of *Cis*-PEGL in the blood circulation. As shown in Fig. 6*C*, acute toxicity experiments with a large-dose bolus administration of various cisplatin preparations indicated that *Cis*-TRX20L and *Cis*-PEGL had much reduced toxicity compared with free cisplatin. Collectively, these data show that *Cis*-TRX20L has selective cytotoxicity against tumor cells that express large amounts of CS *in vivo* and may provide an efficient and safe means to deliver anticancer drugs to tumor tissues.

Cisplatin Entrapped in TRX-20 Liposomes Prevents Liver Metastasis in Mice. Another remarkable property of *Cis*-TRX20L was shown in its potent activity of inhibiting experimental metastasis to the liver. Mice given an intracardiac injection of LM8G5 cells develop numerous metastatic nodules in the liver within 14 days. When *Cis*-TRX20L was given to the tumor-bearing mice on day 3 after the tumor inoculation, it markedly reduced the number and size of metastatic nodules in the liver (Fig. 7, *A* and *B*). In contrast, *Cis*-PEGL was much less effective than *Cis*-TRX20L in reducing the number of metastatic nodules, although it decreased the size of individual tumor nodules moderately (Fig. 7, *A* and *B*). The free form of cisplatin was almost completely ineffective (Fig. 7, *A–C*). Measurement of the liver weight, which reflects tumor load, gave similar results, indicating that *Cis*-TRX20L almost completely suppressed the increase in liver weight, whereas *Cis*-PEGL was moderately effective, and free cisplatin was nearly ineffective (Fig. 7*C*). Consistently, *Cis*-TRX20L was most effective in increasing the mean survival time of the tumor-bearing mice compared with *Cis*-PEGL and free cisplatin ($P < 0.005$; Fig. 7*D*). These results clearly show that *Cis*-TRX20L is much more potent in inhibiting liver metastasis than either *Cis*-PEGL or free cisplatin.

DISCUSSION

In this study, we have demonstrated that TRX-20 liposomes preferentially recognize CS E, CS B, and CS D, that they selectively accumulate in s.c. solid tumors that express high levels of CS, and that they significantly suppress tumor growth when cisplatin is encapsulated within them. We also showed that *Cis*-TRX20L was far superior to *Cis*-PEGL or free cisplatin in suppressing the liver metastasis of a high CS-expressing tumor, LM8G5.

Previous *in vitro* and *in vivo* studies demonstrated that long-circulating liposomes, such as those sterically stabilized by conjugating PEG to the lipid bilayers, significantly increase the therapeutic efficacy of antitumor drugs (19, 20). In addition, coupling-specific ligands such as mAbs (17, 29, 30) or surface-bound, site-specific molecules (31) to the PEG terminus are even more beneficial in improving the therapeutic efficacy of these drugs. In this study, we used a new cationic lipid TRX-20 (22) as a targeting device for such PEG-coated liposomes. TRX-20 liposomes can selectively recognize certain types of CS chains, including CS E, CS B, and CS D, but not other CSs, although all CSs are composed of disulfated disaccharides and have highly anionic properties. It should be stressed in this regard that the TRX-20 liposomes interacted only poorly with non-CS GAGs, including common extracellular matrix components such as heparan sulfate and hyaluronic acid, which may at least partly account for their low uptake by the lung and kidney, which abundantly express these extracellular matrix components (7, 32). Currently, the precise structural requirement for TRX-20 liposomes to bind specific CS oligosaccharide chains remains unclear.

CSs exist on the cell surface or in the extracellular space as CSPGs (1). Cell surface CSPGs have been implicated in cell adhesion and

motility (33). In particular, CD44-related CSPG (34) and melanoma CSPG (5) have been reported to regulate melanoma cell motility and invasive behavior on extracellular matrix components such as collagen I (5, 34) and fibrinogen (2). Although we do not know what kind of CSPGs are expressed on the surface of the LM8G5 or ACHN cells that were used in this study, CSs are likely to provide a suitable target for liposome binding and internalization by tumor cells. Further study to identify the CSPGs involved in the TRX-20 liposome binding will help increase the accuracy of drug targeting to tumor tissues.

Although the present results show a significantly increased efficacy of *Cis*-TRX20L to suppress the growth and metastasis of tumor cells *in vivo* compared with other cisplatin preparations, it should be pointed out that the treatment of mice with *Cis*-TRX20L did not result in the complete eradication of the tumor cells with the dose and schedules used in this study. Although *Cis*-TRX20L significantly suppressed liver metastasis and prolonged the mean survival time of the mice, all of the animals died within 25 days after inoculation with the LM8G5 cells in our model; death was mainly because of metastasis to tissues other than liver. This may be partly because *Cis*-TRX20L was given only once on day 3, partly to a limited penetration of these long-circulating liposomes into the interior of established solid tumors *in vivo* (35) and partly to their low accumulation in tissues other than liver. Clearly, additional development of these liposomes should be pursued to rectify these deficiencies. Detailed pharmacokinetic and pharmacodynamic studies are in progress in our laboratory to improve efficacy and safety of *Cis*-TRX20L.

In humans, significant increases in CS content have been reported in a variety of epithelial and mesenchymal neoplasms, including pancreatic carcinoma (36), colon carcinoma (37), rectum carcinoma (38), hepatocellular carcinoma (39), and prostate carcinoma (40). In addition, the enhanced expression of CS often correlates with the metastatic ability of tumor cells (41, 42). Hence, these observations point to the usefulness of tumor-associated CSs for the targeted delivery of anticancer drugs by CS-tropic vehicles such as TRX-20 liposomes.

In summary, we have demonstrated that TRX-20 liposomes that preferentially bind to certain CSs can be successfully used for the delivery of anticancer drugs to tumors that express large amounts of CS *in vivo*. TRX-20 liposomes are particularly effective in suppressing tumor metastasis to the liver. Although much additional study of relevant tumor models is required to improve the efficacy of TRX-20 liposomes, the results in this study warrant additional development of these liposomes for potential investigation into their use in clinical tumor chemotherapy.

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This practical, clinically oriented reference provides the veterinarian with all the relevant clinical pharmacology information needed when selecting drug regimes for pets. It compiles easy-to-find data on dosages, routes of administration, formulation, metabolism, side effects, interactions, special considerations, and more, all in one convenient volume. Information on each drug class is presented in a consistent format, making it easy to locate key information. Chapters on all the main drug classes are complemented by additional chapters on key body systems, as well as introductory chapters on pharmacokinetics and pharmacological principles.

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- Cimetidine has been reported to cause a cutaneous drug eruption in a cat.
- The dose of the H_2-antagonists should be reduced by 50% in patients with impaired renal function.

Known drug interactions

- Cimetidine can decrease hepatic microsomal enzyme systems and thus theoretically can decrease hepatic metabolism of various drugs, including benzodiazepines, barbiturates, propranolol, calcium-channel blockers, metronidazole, phenytoin, quinidine, theophylline and warfarin. This has been demonstrated in the dog in a study of the pharmacokinetics of verapamil when cimetidine was administered concurrently. The clinical significance of this effect has not been established, although there are anecdotal reports of cimetidine therapy adversely affecting dogs receiving phenobarbital. Ranitidine inhibits microsomal enzyme systems to a much lesser (five- to 10-fold) degree.
- The increased intragastric pH associated with H_2-antagonist administration may reduce the absorption of drugs that require an acid medium for dissolution and absorption, such as ketoconazole.
- It is recommended that at least 2 h elapses between dosing with cimetidine and giving antacids, metoclopramide, digoxin or ketoconazole.

SUCRALFATE

Clinical applications

Sucralfate is indicated for the symptomatic treatment of gastric ulceration from various causes. In humans, sucralfate is as effective as antacids or H_2-receptor antagonists in healing ulcers. It does not appear to be successful, however, in preventing corticosteroid-induced ulceration in dogs subjected to spinal surgery. Its efficacy in preventing NSAID-induced ulcers is unproven in the dog. Sucralfate has also been used to treat oral and esophageal ulcers and esophagitis.

Mechanism of action

Sucralfate is composed of sucrose octasulfate and aluminum hydroxide, which dissociate in the acid environment of the stomach. Minimal systemic absorption of either compound occurs. Sucralfate is structurally related to heparin but does not possess any appreciable anticoagulant activity. It is also structurally related to sucrose but it not used as a sugar by the body.

When given orally, sucrose octasulfate reacts with hydrochloric acid and is polymerized to a viscous sticky substance that binds to the proteinaceous exudate usually found at ulcer sites. Because of electrostatic charges, sucralfate preferentially adheres to ulcerated tissues. It protects the ulcer against hydrogen ion back-diffusion, pepsin and bile and therefore promotes ulcer healing. The aluminum hydroxide theoretically neutralizes gastric acid but this antacid activity is not believed to be clinically important.

It was believed that the formation of a physical protective barrier was the major mechanism by which sucralfate assisted ulcer healing. However, it is now believed that the major drug actions of sucralfate are related to stimulation of mucosal defense and reparative mechanisms. Sucralfate also inactivates pepsin, adsorbs bile acids and is believed to be cytoprotective by stimulating prostaglandin synthesis. It does not significantly affect gastric acid output but may slow gastric emptying appreciably.

Formulations and dose rates

Large dogs
- 1 g PO q.8 h

Small dogs
- 0.5 g PO q.8 h

Cats
- 0.25–0.5 g PO q.8–12 h

Pharmacokinetics

Only 3–5% of an oral dose of sucralfate is absorbed and this is excreted unchanged in urine within 48 h. The remainder of the drug is excreted in feces within 48 h. Sucralfate binds to the ulcer site for up to 6 h after oral dosing.

Adverse effects

- Because very little drug is absorbed systemically, no systemic toxicities have been reported.
- The only reported side effect in humans is constipation.

Known drug interactions

- Recommendations vary concerning whether concurrent administration of H_2-antagonists

JOP. J Pancreas (Online) 2011 Mar 9; 12(2):110-113.

HIGHLIGHT ARTICLE

Treatment for Refractory Pancreatic Cancer

Highlights from the "2011 ASCO Gastrointestinal Cancers Symposium". San Francisco, CA, USA. January 20-22, 2011

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Summary

While gemcitabine-based regimens are currently accepted as the standard first-line treatment of patients with locally advanced or metastatic pancreatic adenocarcinoma, there is no consensus regarding treatment in the second-line setting. This review is an update from the 2011 American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium regarding recent developments in the treatment of refractory pancreatic cancer, as these were presented in Abstracts #237 and #272 of the meeting.

Introduction

Pancreatic cancer remains the fourth leading cause of cancer-related mortality with an estimated total of 43,100 new cases and 36,800 deaths in 2010 in the USA alone [1]. Overall survival remains poor despite advances in therapeutics. Gemcitabine-based regimens represent the standard systemic first-line treatment in patients with advanced pancreatic cancer, offering a better quality of life as well as a small survival benefit [2]. Only a small percentage of patients who exhibit disease progression after first-line treatment continue to receive second-line therapy, mainly because of poor performance status. Therefore, few randomized trials have been conducted and there is currently no consensus on the standard of care for refractory pancreatic cancer [3].

What Did We Know Prior to the 2011 ASCO GI Cancer Symposium?

Oettle *et al.* [4] evaluated folinic acid plus 5-FU plus oxaliplatin (FOLFOX) as second-line treatment in advanced pancreatic cancer and they were the first to establish that chemotherapy offers better overall survival to refractory patients as compared to best supportive care (21 *vs.* 10 weeks, P=0.007). According to the final results of the Charité Onkologie trial (CONKO-003), the addition of oxaliplatin to 5-FU and leucovorin improves overall survival and progression-free survival when compared to 5-FU and leucovorin [5]. Based on the above, it has been suggested that FOLFOX become a standard second-line regimen [6]. Some studies have demonstrated that the doublet of gemcitabine and oxaliplatin can be used as second-line treatment in patients refractory to standard gemcitabine regimen [7, 8]. Activity of oxaliplatin has also been shown in combination with capecitabine after gemcitabine failure [9]. These results were confirmed in a phase II study by Dr. Mane *et al.*, presented at the 2011 ASCO GI Cancer Symposium (Abstract #308) [10], but it should be noted that the latter trial enrolled patients with pancreatic or biliary adenocarcinoma and that results were reported on the total of patients.

Regarding taxanes, paclitaxel monotherapy has been suggested as an additional therapeutic option with considerable efficacy and low toxicity in second-line treatment [11]. A recent retrospective study evaluated docetaxel monotherapy as well as docetaxel-based doublets in the treatment of refractory pancreatic cancer and mild activity was shown with no grade 3 or 4 toxicity [12].

Irinotecan has been evaluated in combination with oxaliplatin in patients with advanced pretreated pancreatic cancer exhibiting modest activity and manageable toxicity [13] and offering median overall survival of 4.1 months [14].

Key words gemcitabine; irinotecan; Pancreatic Neoplasms; Treatment Failure

Abbreviations ASCO: American Society of Clinical Oncology; FOLFIRI: irinotecan with 5-FU and folinic acid; FOLFOX: folinic acid plus 5-FU plus oxaliplatin; PEP02: liposome irinotecan

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Irinotecan with 5-FU and folinic acid (FOLFIRI) showed notable activity and a good toxicity profile after gemcitabine failure [15, 6].
S-1, an oral fluoropyrimidin, has also been investigated in Japanese populations after failure of gemcitabine-based treatment. It seems that this agent is tolerable and marginally effective, offering median overall survival of 5.8 months [16, 17].
Targeted therapies are also being studied in refractory pancreatic cancer. The combination of bevacizumab and erlotinib was recently evaluated in this setting but despite good tolerability, the results were poor [18], as were the results of the use of everolimus [19] and sunitinib [20] as single agents. Bevacizumab monotherapy or its administration in combination with docetaxel did not show any antitumor activity after gemcitabine failure [21].

What Did We Learn at the 2011 ASCO GI Cancer Symposium?

With regard to treatment of refractory pancreatic cancer after failure of at least one line of therapy, two important abstracts were presented at the 2011 ASCO GI Cancer Symposium (Table 1). Both evaluated the use of irinotecan-based regimens in this treatment setting.
Dr. Ko *et al.* presented a phase II trial conducted in three centers in the USA and Taiwan (Abstract #237) [22]. They studied the use of single-agent PEP02, a novel nanoparticle liposome formulation of irinotecan, in refractory pancreatic cancer. It is characterized by improved pharmacokinetics and better tumor localization of irinotecan and of its active metabolite SN38, as compared to the free form of the drug. Favorable safety and efficacy of PEP02 were shown in previous phase I studies of patients with refractory solid tumors, such as pancreatic cancer [23, 24]. PEP02 has been studied as monotherapy [23] as well as in combination with 5-FU and leucovorin [24] and tumor response was reported. In the 2011 Ko *et al.* study [22], 37 patients with metastatic pancreatic cancer received triweekly PEP02 at a dose of 120 mg/m^2 as second-line treatment after gemcitabine failure. According to results based on the first 31 evaluable patients, a 52% disease control rate was achieved. CA 19-9 levels decreased more than 50% in one third of patients whose baseline levels were originally elevated. The study met its primary endpoint of 3-month overall survival as the latter reached 74%, with one patient surviving for more than one year. Toxicity was considered acceptable, with fatigue (31%) and neutropenia (25%) being the two most common grade equal to, or greater than, 3 adverse events.
Regimens combining irinotecan with 5-FU and folinic acid (FOLFIRI) have been administered to patients with advanced pancreatic cancer in the first- [25] and second-line setting [15] and data from phase II studies have shown modest efficacy with tolerable toxicity. Gebbia *et al.* [6] conducted a relevant retrospective study in 40 patients with refractory pancreatic cancer. A new larger retrospective study, conducted in two French institutions, was presented by Dr. Neuzillet *et al.* at the 2011 ASCO GI Cancer Symposium reporting on the use of FOLFIRI after one or more lines of treatment (Abstract #272) [26]. It included 70 patients with unresectable, locally advanced or metastatic, pancreatic cancer with an overall maspin score less than 3. These patients had previously received gemcitabine and platinum-based chemotherapies. Approximately one third of patients had been administered one prior regimen, 57% had received two lines of treatment and only 8.8% had received three or more lines. Sixty of 70 patients (85.7%) received FOLFIRI-1 (irinotecan 180 mg/m^2 day 1) and the rest were administered FOLFIRI-3 (irinotecan 100 mg/m^2 days 1 and 3). Disease control rate was 44.3%. One-year and two-year progression-free survival was 17% and 3%, respectively, whereas overall survival rates were 24% and 9%, respectively. Dosage adjustment was necessary in 21 patients (30%) and adverse events were considered tolerable with no toxic deaths reported.

Discussion

Very few options are available for patients with advanced pancreatic cancer after failure of

Table 1. Summary of the 2011 ASCO GI Cancer Symposium abstracts reporting clinical study results on treatment of refractory pancreatic cancer.

	Ko *et al.* (Abstract #237) [22]	Nauzillet *et al.* (Abstract #272) [26]
Study design	Phase II (Simon's 2-stage design) Metastatic disease	Retrospective Locally advanced or metastatic disease
Countries	USA, Taiwan	France
No. of patients	37 (31 evaluable)	70
Drugs	Liposome irinotecan (PEP02)	FOLFIRI-1 or FOLFIRI-3
Dose	120 mg/m^2 3-week cycle	Irinotecan 180 mg/m^2 day 1 Irinotecan 100 mg/m^2 days 1 and 3
Line of treatment	Second	Second or further
Previous treatment	Gemcitabine-based	Gemcitabine- and platinum-based
Disease control rate	52%	44.3%
Survival	3-month: 74% (ongoing)	Progression-free survival: 23 weeks Overall survival: 24 weeks
Grade 3/4 toxicity	Fatigue, neutropenia, nausea/vomiting, diarrhea	Hematological, digestive

Disease control rate: partial response plus stable disease

gemcitabine-based regimens. Irinotecan monotherapy has already been evaluated in patients treated with first-line gemcitabine-based chemotherapy: in 2009, Yi *et al.* [27] reported the results of a phase II trial evaluating biweekly doses of irinotecan monotherapy (150 mg/m^2) as salvage treatment in this setting. However, Ko *et al.* [22] presented the first phase II study of a novel liposomal irinotecan formulation in the second-line treatment of these patients. In both trials, disease control rates were comparable (48 *vs.* 52% in the Yi and Ko studies, respectively) as were the percentages of patients that exhibited more than 50% decrease in their CA 19-9 levels (33% in both studies). In terms of survival, three-month overall survival seems considerably higher in the liposomal irinotecan study according to the preliminary data presented at the 2011 ASCO GI Cancer Symposium (74% *vs.* approximately 40% in the Yi *et al.* trial). However, it should be noted that with regard to toxicity, the liposomal formulation of irinotecan seems to be associated with a significant greater percentage of grade 3/4 adverse events. In this study, fatigue grade equal to, or greater than, 3 is reported in 31% of patients whereas in the Yi *et al.* study this adverse event was not reported. This difference in toxicity needs to be taken into account as treatment in the second-line setting is often palliative and one of its main objectives is maintaining quality of life.

FOLFIRI regimens have been studied in the past in the treatment of gemcitabine refractory pancreatic cancer. The Yoo *et al.* [15] phase II study was the first to show favorable efficacy and toxicity profile in gemcitabine pretreated patients. Gebbia *et al.* [6] retrospectively examined 40 patients who received standard biweekly FOLFIRI after gemcitabine failure and suggested this regimen be used selectively in patients with good performance status or good response to first-line treatment. The 2011 Neuzillet *et al.* [26] study was also retrospective and showed comparable efficacy results (50% *vs.* 44.3% disease control rates in the Gebbia *et al.* and Neuzillet *et al.* studies, respectively). Estimated median overall survival was 6 months in both studies and toxicity was mainly hematological and gastrointestinal. The Neuzillet *et al.* trial is the first study to report considerable efficacy and manageable toxicity in patients receiving third- and further-line of chemotherapy. However, what needs to be noted is that patients included were of significantly better performance status (42.9% had performance status equal to 0 *vs.* 15% and 0.5% in the Yoo *et al.* and Gebbia *et al.* studies, respectively), despite the fact that more than 65% of patients had already received 2 or more lines of treatment. In the Neuzillet *et al.* trial there is also great heterogeneity in the results despite the similar median overall survival of 6 months: the range of overall survival is 0.5-36.8 months *vs.* 2-8.2 months in the Gebbia *et al.* study, respectively. Finally, the Neuzillet *et al.* study does not state whether results or toxicity differed between patients receiving FOLFIRI-1 or FOLFIRI-3 regimens.

In conclusion, little progress has been made in the field of second-line treatment of gemcitabine-refractory pancreatic cancer; therefore, there is no evidence-based treatment recommendation for these patients. There is need for larger randomized trials that will study novel agents as well as new treatment combinations in an effort to improve survival while maintaining quality of life.

Conflict of interest None

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March 24, 2016

Merrimack Announces Inclusion of ONIVYDE® (irinotecan liposome injection) as a Category 1 Treatment Option in the 2016 NCCN Guidelines for Pancreatic Adenocarcinoma

Updated guidelines recognize the ONIVYDE regimen as a standard-of-care for treatment of patients with post-gemcitabine metastatic pancreatic cancer

CAMBRIDGE, Mass., March 24, 2016 /PRNewswire/ -- Merrimack Pharmaceuticals, Inc. (Nasdaq: MACK) today announced the National Comprehensive Cancer Network (NCCN) has included ONIVYDE® (irinotecan liposome injection) in combination with fluorouracil (5-FU) and leucovorin in its 2016 Clinical Practice Guidelines in Oncology for pancreatic adenocarcinoma. The new guidelines recognize the ONIVYDE regimen as a category 1 second-line therapy for patients with metastatic adenocarcinoma of the pancreas who have previously been treated with gemcitabine-based therapy. A category 1 classification represents the highest level of evidence and uniform NCCN consensus that the intervention is appropriate. The new guidelines are published on www.nccn.org .

"The addition of the ONIVYDE regimen to the 2016 NCCN guidelines further validates the importance of this treatment option for patients battling metastatic pancreatic cancer," said Edward J. Stewart, Head of Commercial at Merrimack. "ONIVYDE is the only FDA approved therapy available to patients whose disease has progressed after gemcitabine-based therapy and, more importantly, it addresses a critical unmet need in a patient population with very limited options. We believe these guidelines will further support the adoption of the ONIVYDE regimen as a standard-of-care in metastatic pancreatic cancer."

Pancreatic cancer is a rare and deadly disease. Each year approximately 53,000 patients are diagnosed with pancreatic cancer in the United States with only 7% surviving five years or longer[1]. The NCCN's recommendation was based on a review by a multidisciplinary panel of experts from NCCN member institutions and supported by data from the NAPOLI-1 study, published in *The Lancet* in 2015, and the U.S. Food and Drug Administration (FDA) approval of the ONIVYDE regimen. NAPOLI-1 was a randomized, open label Phase 3 study in patients with metastatic adenocarcinoma of the pancreas who received prior gemcitabine-based therapy, and was the largest Phase 3 study in this setting to date. Patients were enrolled at 76 sites in North America, South America, Europe, Asia and Oceania.

NCCN is an alliance of 26 world-class cancer centers dedicated to the development of treatment guidelines for most cancers and to research that will ultimately improve the quality of patient care and outcomes. The NCCN guidelines are widely recognized as the standard of clinical practice in oncology and provide evidence-based treatment recommendations to assist key stakeholders, including physicians, patients and payers, in directing cancer patient care.

About ONIVYDE® [pronounced \ 'on - ĭh - vide **]**

ONIVYDE® (irinotecan liposome injection), also known as MM-398 or "nal-IRI," is a novel encapsulation of irinotecan in a liposomal formulation. The activated form of irinotecan is SN-38, which functions by inhibiting topoisomerase I (an essential enzyme involved in DNA transcription and replication) and promoting cell death. ONIVYDE was recently approved by the U.S. Food and Drug Administration in combination with fluorouracil and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy. For full prescribing information, including Boxed WARNING, please visit www.ONIVYDE.com.

About Merrimack

Merrimack is a fully integrated biopharmaceutical company that views cancer as a complex engineering challenge. Through

systems biology, which brings together the fields of biology, computing and engineering, Merrimack aims to decrease uncertainty in drug development and clinical validation, and move discovery efforts beyond trial and error. Such an approach has the potential to make individualized treatment of patients a reality. Merrimack's first commercial product, ONIVYDE® (irinotecan liposome injection), was approved by the U.S. FDA on October 22, 2015. With four additional candidates in clinical studies, several in preclinical development and multiple biomarkers designed to support patient selection, Merrimack is building one of the most robust oncology pipelines in the industry. For more information, please visit Merrimack's website at www.merrimack.com or connect on Twitter at @MerrimackPharma.

Forward-Looking Statements

To the extent that statements contained in this press release are not descriptions of historical facts, they are forward-looking statements reflecting the current beliefs and expectations of management made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995, as amended. Forward-looking statements include any statements about Merrimack's strategy, future operations, future financial position, future revenues and future expectations and plans and prospects for Merrimack, and any other statements containing the words "anticipate," "believe," "estimate," "expect," "intend," "may," "plan," "predict," "project," "target," "potential," "will," "would," "could," "should," "continue," and similar expressions. In this press release, Merrimack's forward-looking statements include, among others, statements about the potential adoption of the ONIVYDE regimen as a standard-of-care. Such forward-looking statements involve substantial risks and uncertainties that could cause Merrimack's clinical development programs, future results, performance or achievements to differ significantly from those expressed or implied by the forward-looking statements. Such risks and uncertainties include, among others, the uncertainties inherent in the initiation of future clinical trials, availability of data from ongoing clinical trials, expectations for regulatory approvals, development progress of Merrimack's companion diagnostics, availability of funding sufficient for Merrimack's foreseeable and unforeseeable operating expenses and capital expenditure requirements, and other matters that could affect the availability or commercial potential of Merrimack's products, product candidates or companion diagnostics. Merrimack undertakes no obligation to update or revise any forward-looking statements. Forward-looking statements should not be relied upon as representing Merrimack's views as of any date subsequent to the date hereof. For a further description of the risks and uncertainties that could cause actual results to differ from those expressed in these forward-looking statements, as well as risks relating to Merrimack's business in general, see the "Risk Factors" section of Merrimack's Annual Report on Form 10-K filed with the Securities and Exchange Commission (SEC) on February 26, 2016 and other reports Merrimack files with the SEC.

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SOURCE Merrimack Pharmaceuticals, Inc.

Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and *28

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Objectives SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.

Methods Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.

Results Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.

Conclusions The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.

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Keywords: diplotypes, genetic polymorphism, haplotype, irinotecan, SN-38, UGT1A1

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Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

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studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT 1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)], *1A9*22* (-126_-118T_9 > T_{10}), *1A9*5* [766G > A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

Methods

Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin $\leq$ 2 mg/dl, aspartate aminotransferase (GOT) $\leq$ 105 IU/l, alanine aminotransferase (GPT) $\leq$ 120 IU/l, creatinine $\leq$ 1.5 mg/dl, white blood cell count $\geq$ 3000/μl, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

Genetic polymorphisms of UGT1As and pharmacokinetics

Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Fig. 1

UGT1A1

Region		Enhancer	Promoter	Exon 1		Frequency
Nucleotide change		-3279 T>G	-40_-39 insTA	211 G>A	686 C>A	
Amino acid change				G71R	P229Q	
Marker allele		*60	*28	*6	*27	
Haplotype	*1					0.548
	*6					0.167
	*60					0.147
	*28 *28b					0.138
	*28 *28c					
	*28 *28d					

UGT1A10

Region		Exon 1				Frequency
Nucleotide change		4 G>A	177 G>A	200 A>G	605 C>T	
Amino acid change		A2T	M59I	E67G	T202I	
Marker allele		*2T	*2	*67G	*3	
Haplotype	*1					0.981
	*2					0.006
	*2T					0.003
	*3					0.010
	*67G					0.000

UGT1A7

Region		Exon 1				Frequency
Nucleotide change		387 T>G	391C>A	392 G>A	622 T>C	
Amino acid change		N129K	R131K		W208R	
Marker allele		*2,*3	*2,*3	*2,*3	*3,*4	
Haplotype	*1					0.630
	*2					0.147
	*3					0.223

Block C

Region		Exon.4	Exon.5		3'-UTR			Frequency
Nucleotide change		1091 C>T	1456 T>G	1598 A>C	*211(1813) C>T	*339 (1941) C>G	*440(2042) C>G	
Amino acid change		P364L	Y486D	H533P				
Marker allele		*364L	*7	*533P	*1B	*1B	*1B	
Haplotypes	*1A							0.864
	*1B *1b-*1j							0.127
	*1B *533P							
	*7							0.003
	*364L							0.006

UGT1A9

Region		Promoter		Exon1			Frequency
Nucleotide change		-126_-118 T9>T10	-126_-118 T9>T11	422 C>G	726 T>G	766 G>A	
Amino acid change				S141C	Y242X	D256N	
Marker allele		*22	*T11	*141C	*4	*5	
Haplotype	*1						0.347
	*22						0.644
	*141C						0.000
	*4						0.000
	*5						0.006
	*T11						0.003

Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the χ^2 test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or *28'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

Results

Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6)* haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

Table 1 Characteristics of Japanese cancer patients in this study

		No. of participants	
Age			
Mean/range	60.5/26–78	177	
Sex			
Male/female		135/42	
Performance status	0/1/2	84/89/4	
Combination therapy and tumor type (initial dose of irinotecan; mg/m^2)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing drug[a]	Lung (60)	58[b]	48 [60][c]
	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil (including tegafur)	Colon (100 or 150)	34	
	Others (90 or 100)	2	
With mitomycin-C	Stomach (150)	10	
	Colon (150)	1	
With amrubicin	Lung (60)	2	
Previous treatment			
Surgery	Yes/no	85/92	
Chemotherapy	Yes/no	97/80	
Radiotherapy	Yes/no	26/151	
Smoking history	Yes/no	29/148	

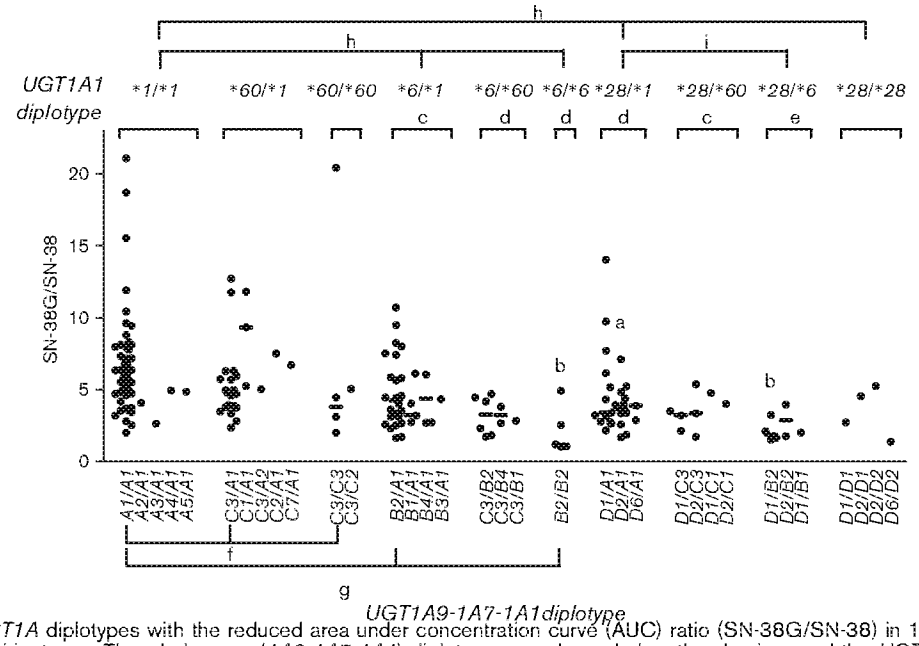
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1*

	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
Haplotype	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	*N*[b]	Frequency
A1[c]	**I*	**1*	**I*	**22–*1–*1*	189	0.534
	**I*	**3*	**I*			
A3	**III*	**1*	**I*	**1–*2–*1*	2	0.006
A2	**II*	**1*	**I*	**1–*3–*1*	1	0.003
A4	**IV*	**1*	**I*	**22–*3–*1*	1	0.003
A5				**T11–*1–*1*	1	0.003
B2[c]	**II*	**1*	**III*			
	**II*	**1*	**VI*	**1–*3–*6*	47	0.133
	**II*	**4*	**VI*			
B4	**IV*	**1*	**III*	**22–*3–*6*	6	0.017
B1	**I*	**1*	**III*	**22–*1–*6*	5	0.014
	**I*	**1*	**VI*			
B3	**III*	**1*	**III*	**1–*2- *6*	1	0.003
C3[c]	**III*	**3*	**IV*			
	**III*	**1*	**IV*			
	**III*	**3*	**V*	**1–*2–*60*	44	0.124
	**III*	**1*	**V*			
C1	**I*	**3*	**IV*	**22–*1–*60*	5	0.014
	**I*	**1*	**IV*			
C2	**II*	**3*	**IV*	**1–*3–*60*	2	0.006
C7	**VII*	**3*	**V*	**22–*2–*60*	1	0.003
D1	**I*	**1*	**IIa*	**22–*1–*28*	23	0.065
	**I*	**1*	**IIc*			
D2	**II*	**1*	**IIa*			
	**II*	**3*	**IIa*	**1–*3–*28*	22	0.062
	**II*	**1*	**IIc*			
D6	**VI*	**1*	**IIb*	**1–*2–*28*	4	0.011
				Total	354	1.000

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.

Fig. 2



The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part of the figure. Each point represents a patient value, and the median is indicated by a bar. Significant reductions in the AUC ratio were detected in the *B2/B2*, *D2/A1*, and *D1/B2* compared with *A1/A1* for the whole gene diplotypes [Kruskal–Wallis test ($P=0.0009$) followed by Dunnett's multiple comparison test]. As for the *1A1* diplotypes, significant reductions were detected in the **6/*1*, **6/*60*, **6/*6*, **28/*1*, **28/*60*, and **28/*6* compared with the **1/*1* group [Kruskal–Wallis test ($P<0.0001$) followed by Dunnett's multiple comparison test]. Gene–dose effects on the reduced AUC ratio were significant for **6* and **28* (Jonckheere–Terpestra test). A significant additive effect of **6* on the reduced AUC ratio by **28* was detected by comparing **28/*1* and **28/*6*. [a]$P<0.05$ and [b]$P<0.01$ against *A1/A1* group (Dunnett's multiple comparison test); [c]$P<0.05$, [d]$P<0.01$, and [e]$P\&<0.001$ against the **1/*1* group (Dunnett's multiple comparison test); [f]$P<0.05$, [g]$P<0.001$, and [h]$P<0.0001$ (Jonckheere–Terpestra test for gene–dose effect); [i]$P<0.01$ (Wilcoxon test).

($P = 0.1134$). No significant effects on the AUC ratio were observed for Block C (exon 2–5) haplotypes or rare variations including *1A10* (**2T*, **2*, or **3*) and *1A9* (**5*, **T11*).

Multiple regression analysis of the area under concentration curve ratio

We further assessed the impact of *UGT1A* genetic factors on the AUC ratio by multiple regression analysis. First, we used the *1A9-1A7-1A1* and Block C haplotypes as genetic factors. The AUC ratio was significantly associated with the haplotypes *B2*, *D1*, and *D2* and serum biochemistry parameters indicating hepatic or renal function before treatment. The Groups B and D haplotypes harbor *1A1*6* and **28*, respectively. The dependency on specific *1A7* or *1A9* polymorphisms, however, was not obtained, considering the contributions of both *D1* and *D2*. As *1A1*6* and **28* are mutually exclusive and their effects are comparable, we grouped *1A1*6* and **28* into the same category in the final multiple regression model (Table 4). The final model confirmed the significant contribution of this genetic marker (**6* or **28*) to the AUC ratio.

Effects of the genetic marker '*6 or *28' on pharmaco kinetic parameters

Then, a dose effect of the genetic marker '**6* or **28*' on pharmacokinetic parameters was further analyzed

Table 3 AUC ratio of SN-38 glucuronide to SN-38 for *UGTIAI* diplotypes

Diplotype	Number of patients	AUC ratio		*P*-value[a] (vs. **1/*1*)
		Median	Interquartile range	
**1/*1*	55	6.13	4.72–7.79	
**1/*60*	25	5.04	3.85–6.52	0.9803
**60/*60*	5	4.48	2.57–12.74	0.8141
**6/*1*	32	4.03	2.74–5.97	0.0126
**6/*60*	9	2.84	2.09–4.33	0.0021
**6/*6*	5	1.19	1.06–3.74	0.0012
**28/*1*	26	3.65	2.76–5.21	0.0040
**28/*60*	8	3.44	2.68–4.40	0.0261
**28/*6*	7	2.03	1.65–3.26	<0.0001
**28/*28*	4	3.65	2.05–4.92	0.2322

AUC, area under concentration curve.
[a]Dunnett's multiple comparison test.

(Fig. 3). Patients with one haplotype harboring either **6* or **28* (**6/*1*, **6/*60*, **28/*1*, and **28/*60*) had lower SN-38G/SN-38 AUC ratios (median, 3.62; interquartile range, 2.74–5.18) than patients without **6* or **28* (**1/*1*, **60/*1*, and **60/*60*) (5.55, 4.13–7.26), and patients with two haplotypes harboring **6* or **28* (**6/*6*, **28/*28*, and **28/*6*) had the lowest AUC ratio (2.07, 1.45–3.62) ($P < 0.0001$, Fig. 3a). Similarly, the number of the **6* or **28*-containing haplotypes affected the AUC ratios of SN-38 to irinotecan (Fig. 3b). When the correlations

between irinotecan dosage and the AUC of SN-38 were tested, different correlations were obtained according to the number of the haplotypes (Fig. 3c). The slope of regression line for one and two haplotypes harboring **6* or **28* was 1.4-fold and 2.4-fold greater, respectively, than that for the diplotype without **6* or **28*.

Associations of UGT1A1 genetic polymorphisms with toxicities

Association between genetic polymorphisms and toxicities was investigated in patients receiving irinotecan as a single agent. One patient was referred to another hospital 3 days after the first administration of irinotecan without evaluating toxicities and was lost in terms of follow-up. Therefore, association between genetic polymorphisms and toxicities was investigated in 55 patients. Six (11%) and 14 (25%) patients experienced grade 3 or greater diarrhea and neutropenia, respectively. As for the *1A9-1A7-1A1* diplotypes, a higher incidence of grade 3 or greater neutropenia was observed in *D1/B2* (*1A1*28/*6*) (100%, $n = 3$) than in *A1/A1* (11.8%, $n = 17$) ($P = 0.0088$, Fisher's exact test), indicating clinical impact of the genetic marker *1A1*6* or **28*. As for the dose effect of '**6 or *28*', incidences of grade 3 or 4 neutropenia were 14, 24, and 80% for 0, 1, and 2 haplotypes harboring these markers, respectively (Table 5). A significant association between '**6* or **28*' and neutropenia was also observed for 62 patients who received irinotecan in combination with cisplatin (Table 5). No association, however, was observed between diarrhea and the marker '**6* or **28*'.

Multivariate analysis for irinotecan toxicities

We further evaluated the effect of the genetic marker '**6* or **28*' on neutropenia in multivariate analysis, and confirmed a significant correlation of '**6* or **28*' with the nadir of absolute neutrophil counts (Table 6). Elevated alkaline phosphatase levels and the absolute neutrophil count at baseline were also significant.

Discussion

The association study with the *1A9-1A7-1A1* diplotypes revealed that the reduction in inactivation of SN-38, as well as neutropenia, was dependent on the Groups B and D haplotypes which corresponded to the *1A1*6* and **28* segmental haplotypes. Also, multivariate analyses clearly showed clinical significance of the genetic marker '**6* or **28*' for both pharmacokinetics and toxicity of irinotecan in Japanese patients (Tables 3 and 6). *UGT1A1*6* and **28* were mutually exclusive [14] and contributed to the reduction in glucuronidation of SN-38 to the same extent. Therefore, the activity of SN-38 glucuronidation in individuals depended on the number of the haplotypes harboring **6* or **28*. Although the role of *1A1*28* for irinotecan toxicity has been focused on [8–12], this study strongly suggests that **6* should be tested in addition to **28* before starting chemotherapy with irinotecan in Japanese patients.

The clinical importance of **6* for neutropenia by irinotecan was also supported by a recent report in Korean patients who received irinotecan and cisplatin [31]. Although no patients with irinotecan as a single agent were homozygous for **6* in our study, clinical significance of the double heterozygote, **6/*28*, was clearly demonstrated. Among patients treated with irinotecan in combination chemotherapy, the majority of patients received platinum agents in our study. A significant association of '**6* or **28*' with a higher incidence of grade 3 or 4 neutropenia was also observed in patients who received irinotecan and cisplatin (Table 5). These findings further support the necessity of testing '**6* or **28*' before irinotecan is given to patients.

As possible enhancement of toxicities by the **27* allele was suggested [8], we evaluated the effect of the **28c* haplotype, which had an additional single-nucleotide polymorphism [**27*; 686C > A(P229Q)] to the **28* allele (-40_-39insTA). In our cohort of patients, there were three **28c* heterozygotes (**28c/*1*) and one double heterozygote (**28b/*28c*). The values of the AUC ratio were within the range of variations of the **28* group, and no additional impact of **28c* was observed in relation to toxicities.

Although the decreasing trend of the AUC ratio for *1A1*60* (and combinatorial haplotype *C3*) was observed (Fig. 2), the contribution of *1A1*60* to toxicities was not clearly demonstrated in this study as reported in the Japanese retrospective study [32].

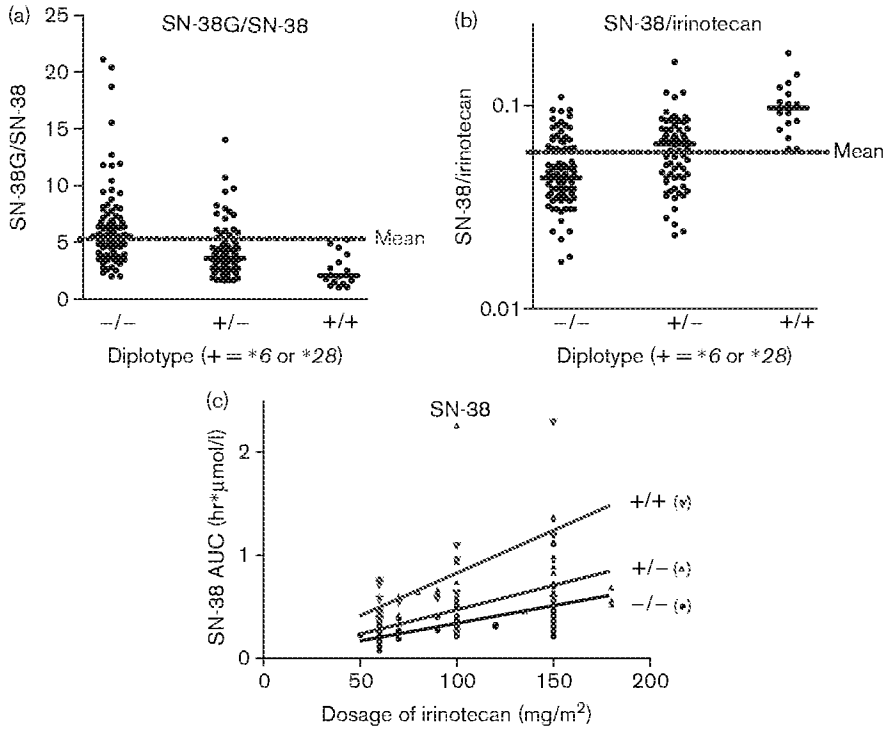
In addition to UGT1A1, recent studies have suggested possible contributions of UGT1A7, 1A9, and 1A10 to SN-38G formation [15–17]. An in-vitro study demonstrated that *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T > C(W208R)] had reduced activity in terms of SN-38G formation [16]. Results of clinical studies, however, on the association between *1A7* polymorphisms and irinotecan toxicity/efficacy are inconsistent, whereas different populations with different combination therapies were used [19,20]. Furthermore, it was reported that the *UGT1A7* polymorphisms (**2* and **3*), which were linked to *1A9*1*, were associated with a lowered incidence

Table 4 Multiple regression analysis toward the AUC ratio (SN-38G/SN-38)[a]

Variable	Coefficient	F-value	*P*-value	R^2	Intercept	*N*
				0.410	0.8869	176
**6* or **28*	−0.189	70.2	<0.0001			
Age	0.005	8.88	0.0033			
Serum albumin level[b]	−0.136	9.92	0.0019			
Serum GOT and ALP[c]	0.070	8.88	0.0033			
Serum creatinine[d]	0.210	7.23	0.0079			

ALP, alkaline phosphatase; AUC, area under concentration curve.
[a]The values after logarithmic conversion were used as an objective variable.
[b]The absolute value (g/dl) before irinotecan treatment.
[c]Grade 1 or greater scores in both serum GOT and ALP before irinotecan treatment.
[d]Grade 1 or greater scores in serum creatinine before irinotecan treatment.

Fig. 3



Effects of the genetic marker of *UGT1A1* '*6 or *28' on the area under concentration curve (AUC) ratios of SN-38G/SN-38 (a) and SN-38/irinotecan (b), and SN-38 by irinotecan dosage (c) in 176 Japanese cancer patients after irinotecan treatment.

Table 5 Association of *UGT1A1*6* and *28 with irinotecan toxicities

Diplotype (+ = *6 or *28)	Number of patients	Diarrhea (grade 3)	Neutropenia (grade 3 or 4)
Irinotecan monotherapy			
−/−	21	3 (14.3%)[a]	3 (14.3%)
+/−	29	2 (6.90%)	7 (24.1%)
+/+	5	1 (20.0%)	4 (80.0%)
P-value[b]		0.8500	**0.0117**
P-value[c]		0.3889	**0.0124**
With cisplatin			
−/−	35	1 (2.9%)	20 (57.1%)
+/−	20	2 (10.0%)	14 (70.0%)
+/+	7	1 (14.3%)	7 (100%)
P-value[b]		0.1747	**0.0315**
P-value[c]		0.3886	0.0863

[a]Percentage of the patient number in each diplotype is indicated in parentheses.
[b]Chi-squared test for trend.
[c]Fisher's exact test, (−/− and +/−) vs. +/+.

Table 6 Multiple regression analysis of the nadir of absolute neutrophil counts in the patients with irinotecan monotherapy

Variable	Coefficient	F-value	*P*-value	R^2	Intercept	*N*
				0.3942	643	53
Serum ALP[a]	−349.9	12.2	0.0010			
Neutrophil count before irinotecan treatment	0.2466	13.5	0.0006			
*6 or *28	−369.1	6.40	0.0146			

[a]Grade 1 or greater scores of serum ALP before irinotecan treatment.

of diarrhea in the irinotecan/capecitabine regimen, in which diarrhea was a major toxicity [20]. A highly frequent allele *1A9*22* with an insertion of T into the nine T repeats in the promoter region (-126_-118T_9 > T_{10}) was shown to have an enhanced promoter activity in an in-vitro reporter assay [21], whereas 1A9 protein expression levels did not change in the clinical samples [22]. Rare variations, *1A9*5* [766G > A(D256N)] and *UGT1A10*3* [605C > T(T202I)], were shown to cause reduced activity *in vitro*, but their clinical importance is still unknown [23,24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–28].

Our study also revealed close linkages between *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6* or *28 [28]. This fact makes it difficult to draw firm conclusions about the effects of *1A7*3* and *1A9*22* themselves. It is, however, reasonable to conclude that the degree of neutropenia depends on the activity of UGT1A1, because UGT1A1 is a major UGT1A enzyme in the liver and plays a primary role for regulating plasma concentrations of SN-38.

Taken together, for practical application to individualized irinotecan therapy, genotyping of *UGT1A1*6* and *28 would be beneficial and necessary in Japanese cancer patients to avoid severe adverse reactions. The frequency

of homozygotes for '**6* or **28*' (namely, **6/*6*, **6/*28*, and **28/*28*) is approximately 10%, which is comparable to the frequency of **28* homozygotes in Caucasian populations. In our study, it may be difficult to establish definite guidelines for dose reductions of irinotecan for patients homozygous for '**6* or **28*'. Considering, however, 2.4-fold steep relationship between the dose of irinotecan and the AUC of SN-38 for patients homozygous for '**6* or **28*' compared with patients without '**6* or **28*' (Fig. 3c), the dose for patients homozygous for '**6* or **28*' should be reduced to a half of the dosage recommended for other patients. Prospective studies are necessary to confirm the validity of the recommendation for dose reduction in Japanese cancer patients homozygous for '**6 or *28*'.

Acknowledgements

We thank Ms Chie Sudo for her secretarial assistance. This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences and by the Program for the Promotion of Studies in Health Sciences of the Ministry of Health, Labor and Welfare of Japan. Analytical standards of irinotecan and its metabolites were kindly supplied by Yakult Honsha Co. Ltd. (Tokyo, Japan).

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HUMAN CELL LINE (COLO 357) OF METASTATIC PANCREATIC ADENOCARCINOMA

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A continuous human cell line, COLO 357, with exceptional characteristics was derived from a metastasis of a pancreatic adenocarcinoma. COLO 357 grew as an adhering monolayer with a cell doubling time of 21 h and grew with 10% clonal efficiency in soft agar. COLO 357 cells had numerous lamellar inclusions. The cells elaborated the pancreatic enzymes trypsin, elastase and chymotrypsin. COLO 357 also secreted appreciable amounts of carcinoembryonic antigen and human chorionic gonadotropin. COLO 357 had a chromosome mode of 53 with 20 identifiable Giemsa-banded marker chromosomes. Nine nucleolar organizing regions were found by silver-stained metaphase preparations. COLO 357 has been "fingerprinted" for seven allelic isozymes. This cell line has been maintained in active culture for over 2 years, is preserved in a cell bank, and is available to other investigators.

Carcinoma of the pancreas has steadily increased in incidence during the past 20 years and accounts for 3% of all cancers and 5% of all cancer deaths in the United States (Hermann and Cooperman, 1979). It is the fourth commonest lethal cancer in men; only cancers of the lung, colorectum, and prostate are more frequent. In women, it is the fifth most common lethal cancer. This malignancy is more frequent in heavy smokers.

Pancreatic carcinoma is a rapidly progressive and fatal disease with on overall 5-year survival rate of less than 1% (Aoki and Ogawa, 1978), and is usually diagnosed late in the natural course of the disease. Therapeutic modalities are generally ineffective. Knowledge of the biological properties of this neoplasm has been hampered by the lack of experimental models and the identification of unique antigens or other characteristics that might aid in diagnosis and therapy.

Continuous cell lines of common human adenocarcinomas provide opportunities for characterization of biochemical properties, cytogenetic aberrations, chemotherapeutic responses, tumor-associated antigens, hormone receptors, enzyme patterns, and immune reactions.

This report concerns the establishment and characterization of an epithelial cell line derived from a human metastatic pancreatic adenocarcinoma.

MATERIAL AND METHODS

Clinical data

A 77-year-old Negro female presented in September 1977 with a 2-month history of weight loss, anemia, anorexia and progressive jaundice. Laboratory findings were unremarkable except for an elevated alkaline phosphatase level (830 mu/ml) and serum glutamine-oxaloacetic transaminase (176 mu/ml). Ultrasound showed dilation of the hepatic radicals and common bile duct and an enlarged pancreas. On September 6, 1977, an exploratory laparotomy was performed. A large mass was felt in the head of the pancreas and biopsies were taken from adjacent lymph nodes. A celiac axis lymph node was partially replaced by neoplastic foci of well-differentiated mucin-containing ducts (Fig. 1). Pathological diagnosis was metastatic adenocarcinoma of pancreatic origin. Tissue from the celiac axis lymph node was submitted for immediate tissue culture.

A cholecystojejunostomy was done to decompress the biliary tract as a curative resection was not possible. Postoperatively, the patient did well and lived at home, but succumbed to the metastatic carcinoma on December 10, 1977. The patient's ABO blood group was A+.

Culture methods

The metastatic pancreatic adenocarcinoma tissue was minced in 2-ml volumes of RPMI medium 1640 and GEM 1717 (Moore and Woods, 1977). Both media were supplemented with 20% fetal bovine serum (FBS) (heat-inactivated at 56°C/30 min) (Reheis Chemical Company, Kankakee, I11, USA), penicillin (100 IU/ml), and streptomycin (50 μg/ml). The minced tumor tissue formed a thin layer of cells in 4-oz flint-glass culture bottles that were loosely capped and incubated at 37°C in a humidified atmosphere of 10% CO_2/90% air for 24 h. Two to three ml of fresh medium were then added twice a week. Cells were selectively subcultured by mechanical removal as soon as colonies of tumor cells developed.

Criteria for the establishment of a cell line in continuous culture were the growth of the tumor cells to semiconfluency, absence of contaminating cell types such as fibroblasts or lymphocytes, and repeated successful subcultures.

Morphology

Serial morphologic observations by phase optics were made of monolayer cultures grown on Leighton-tube coverslips. Replicate coverslip preparations were stained with May-Grünwald-Giemsa, Papanicolaou, and Mucicarmine stains. For transmission electron microscopy, cell pellets were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. Embedding and staining of the cultured cells were performed as previously described (Moore *et al.*, 1975).

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Growth characteristics

The methodologies for determining plating efficiency and colony formation in soft agar have been previously described (Moore *et al.*, 1978). To determine cell population doubling time, each of 20 wells in a multi-well Test Plate II was inoculated with 10^5 viable cultured tumor cells. At various time intervals, duplicate wells were harvested by trypsinization and cell concentrations per well determined by hemocytometric counts. The $\log_{10}$ for each time stop was plotted against the time interval. The cell population doubling time was estimated from the logarithmic growth phase.

Biochemical markers

A carcinoembryonic antigen (CEA) test kit (Roche Diagnostics, Nutley, N.J., USA) was used to assay: (1) RPMI medium 1640 with 10% FBS without exposure to cells as negative control, and (2) 15 ml spent RPMI 1640 with 10% FBS 7 days post total medium replacement from a culture of 3.2×10^6 viable tumor cells.

The production of both α-fetoprotein and the β-subunit of human chorionic gonadotropin (HCG) was measured in spent media from cultures and control medium by Consolidated Biomedical Laboratories (Wichita, KA, USA).

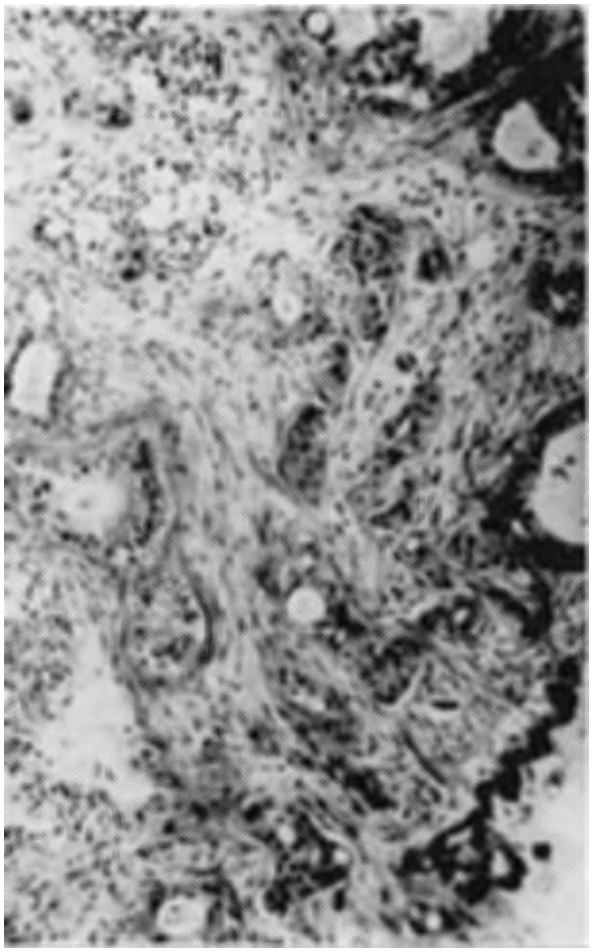

FIGURE 1 — Histological section of pancreatic adenocarcinoma metastatic to celiac lymph node. H. and E., x 84.

To test for steroid hormone production, the tumor cell line was grown to near confluency (10^7 viable cells/4-oz culture flask). The culture medium was replaced for a 7-day period with 10 ml of RPMI 1640 supplemented with 5% FBS stripped of endogenous steroids by a 30-min incubation at 45°C with a dextran-coated charcoal pellet (0.25% activated charcoal and 0.0025% dextran in 0.01 M Tris-HCl, pH 8.0 at 4°C, 1 ml/ml FBS). RPMI 1640 with 5% hormone-stripped FBS was used as negative control.

Estrogen production was quantified with an Estrogen (^{3}H) (E_1/E_2) Radioimmunoassay Reagent Pak (New England Nuclear, Boston, MA). Progesterone and cortisol production were quantified with a Progesterone (^{3}H) Radioimmunoassay Pak (New England Nuclear) and Cortisol (^{3}H) Radioimmunoassay Reagent Pak (New England Nuclear).

Adrenocorticotropic hormone (ACTH) assays were performed on 3-day-old spent RPMI medium 1640 with 10% FBS from actively growing cultures and on fresh complete medium without exposure to cultured cells. The ACTH was assayed by the method of Eipper and Mains (1975).

Steroid hormone receptor proteins were measured as previously described (Woods *et al.*, 1979; Stedman *et al.*, 1979). Specific receptor proteins for estrogen, progesterone, androgen and glucocorticoids were quantified using ^{3}H-ligands of 17- β-estradiol, promegestone (R5020), 5- α-dihydrotestosterone, and dexamethasone respectively.

Allelic isozyme (allozyme) phenotype of the cultured tumor cells was determined by the methods of Harris and Hopkinson (1976). The allozymes selected for phenotypic "fingerprinting" were glucose-6-phosphate dehydrogenase (G6PD), first and third locus of phosphoglucomutase ($PGM_{1,3}$), esterase D (ESD), "mitochondrial" glutamate-oxaloacetate transaminase (GOT_m), "red cell" acid phosphatase (ACP), and adenosine deaminase (ADA). Tumor cells (10^7) were harvested by scraping, washed with phosphate-buffered saline and lysed with an equal volume of distilled water, then the cell-free supernatant was assayed directly.

The cultured tumor cells were tested for production of protease enzymes. Tissue culture medium was removed from two 75-cm^2 flasks of confluent cultured tumor cells and washed three times with phosphate-buffered saline. The washed cells were incubated in RPMI medium 1640 without serum for 20h. The supernatant was concentrated five-fold with an Amicon concentrator using a membrane with a molecular exclusion limit of 10,000 daltons. Aliquots of the concentrated medium were assayed for trypsin (Erlanger *et al.*, 1961), elastase (Saklatvala, 1977), and chymotrypsin (Walsh and Wilcox, 1970) using purified elastase, chymotrypsin and tosylphenyl chloromethyl ketone treated trypsin (Worthington Biochemicals, Freehold, NJ) as protease standards. The synthetic substrates for these assays were α-benzoyl-DL-arginine-*p*-nitroanalide (Aldrich Chemical Co., Milwaukee, WI), α-benzoyl-tyrosine ethyl ester (Schwarz Mann, Orangeburg, NY) and

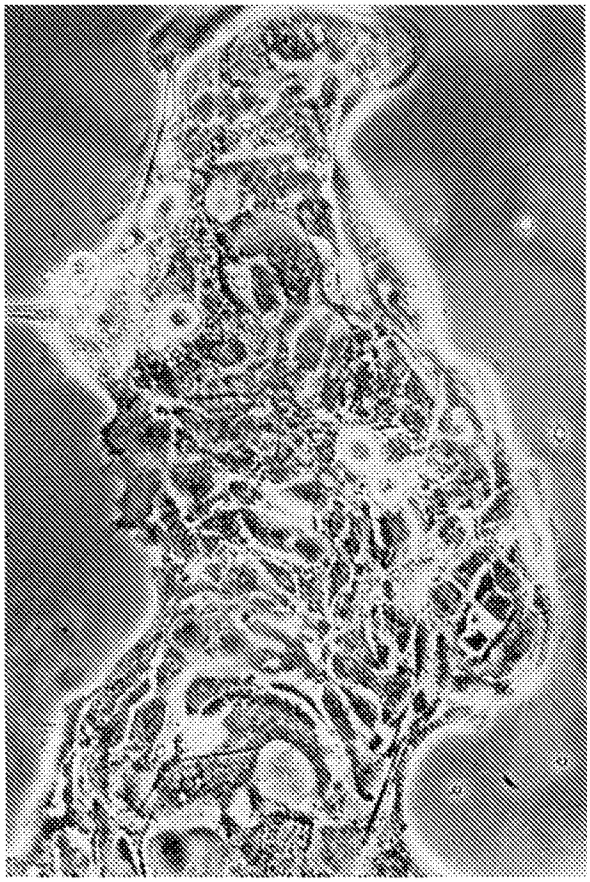

FIGURE 2 — COLO 357 cells after 19 months in continuous culture. Phase microscopy, x 210.

succinyl-L-alanyl-L-alanine-*p*-nitroanalide (Bachem Fine Chemicals, Inc., Torrence, CA). Unincubated serum-free medium was used as a blank. Since some fetal bovine serum adheres to the cells and is recovered in the serum-free medium, 1% fetal bovine serum in serum-free medium was used as an additional blank. Malignant cells produce increased amounts of plasminogen activator (Lang *et al.*, 1975) which may result in increased plasmin in culture medium. Because plasmin hydrolyzes both arginine and lysine esters, the synthetic substrate used to measure tryptic activity could be hydrolyzed by plasmin. Therefore, the trypsin assay was made 0.1 M with ε-amino caproic acid (Sigma Chemical Co., St. Louis, MO), a specific inhibitor of plasmin (Robbins and Summaria, 1976; Iwamoto *et al.*, 1968) to demonstrate that the hydrolysis was not due to plasmin activity.

Cytogenetic analysis

Subcultures of the tumor cell line were harvested (2 to 6 months post-establishment) for chromosome preparations with standard cytogenetic procedures. Giemsa- and C-banding of the chromosomes were performed as previously described (Semple *et al.*, 1978). Selected metaphase preparations were silver-stained for nucleolar organizer regions (NORs) according to the methods of Pathak and Hsu (1979) and Bloom and Goodpasture (1976). In addition, metaphase preparations were stained with Giemsa (pH 6.8) for elucidation of double minutes (DMs).

Mycoplasma tests

Tests for mycoplasma were made from smears of cultured cells stained with Hoechst 33258 fluorescent DNA stain according to the method of Chen (1975). Fluorescent observations were made using a Zeiss Photomicroscope III equipped with epi-illumination.

Cell freezing

Cultured cells were preserved for the cell bank by slow freezing. Cell suspensions of 0.5 to 1 $\times$ 10^6 cultured cells in cold RPMI medium 1640 supplemented with 20% FBS, 12.5% dimethyl sulfoxide, penicillin and streptomycin were frozen at -1°C per minute. Frozen ampules were stored at -85°C.

RESULTS

During the first 3 weeks of cell culture, fibroblast cells were the dominant cell type. However, in the culture with GEM 1717 medium supplemented with 20% FBS, one radiating cluster of tumor cells and large pleomorphic cells floating in the medium were observed. Every 3-5 days, spent culture medium was centrifuged to recover the suspended cells. The cell pellet was resuspended in 10 ml of fresh medium and returned to the original flask. Gradually, the adhering tumor cell population increased and displaced the fibroblasts. The cell culture was subcultured after the tumor cells became the dominant cell type. After several subcultures, no fibroblasts were apparent and the culture was designated COLO 357 (May 3, 1978). COLO 357 has been maintained on either RPMI 1640 or GEM 1717 medium supplemented with 5%, 10% or 20% FBS.

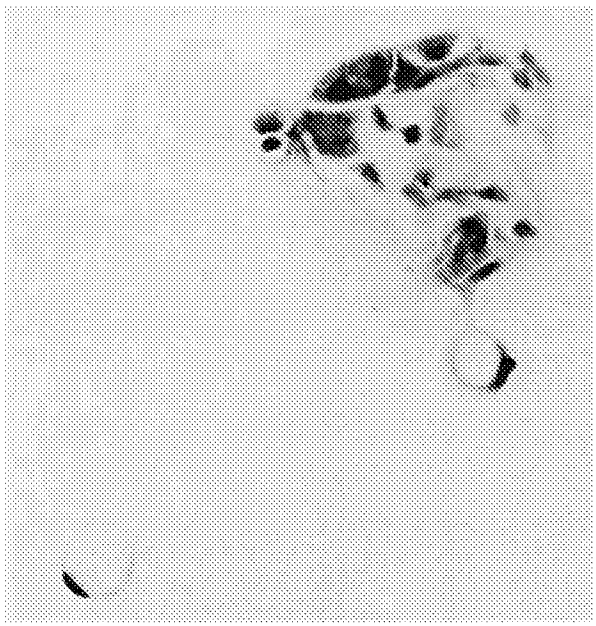

FIGURE 3 — COLO 357 cells after 19 months in continuous culture, stained for mucin. Mucicarmine, x 210.

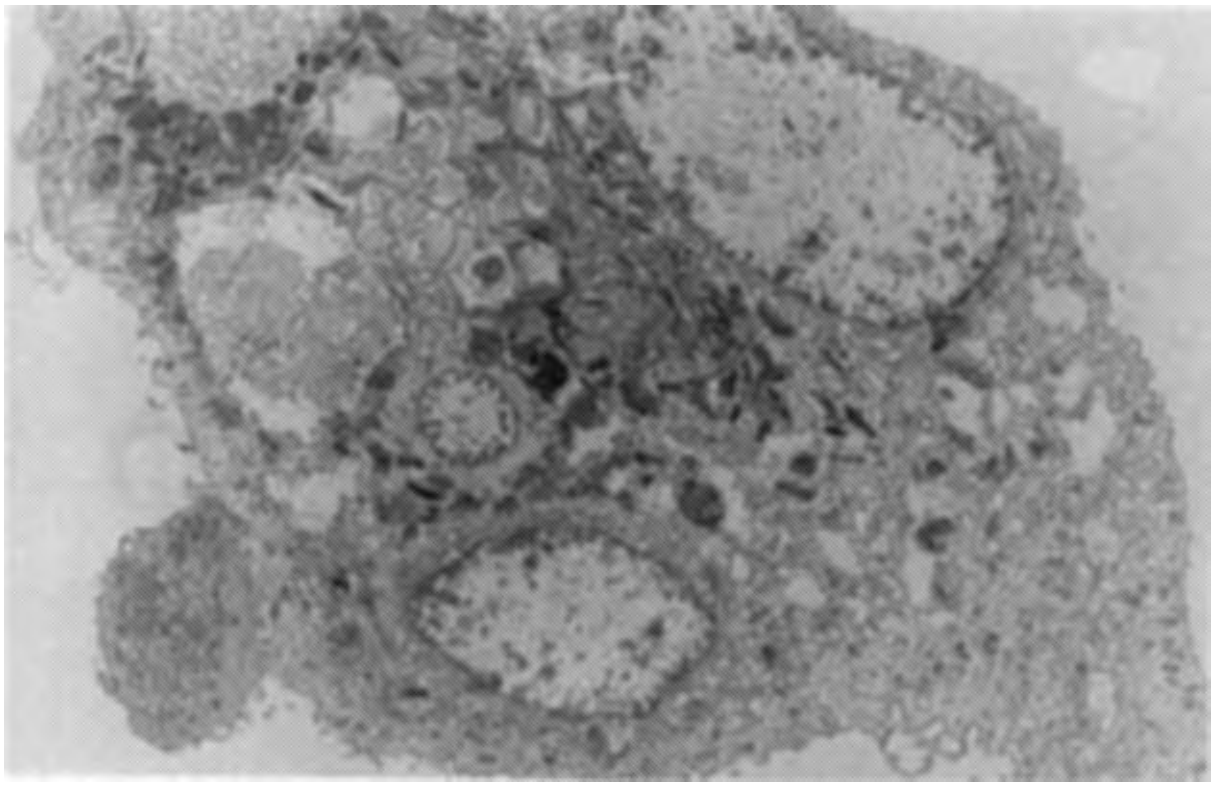

FIGURE 4 — Transmission electron micrograph of COLO 357 harvested after 8 months in culture, x 5,320.

Morphology

COLO 357 grew as highly compact colonies appressed to plastic and glass surfaces (Fig. 2). Colonies maintained well-defined contiguous boundaries with «protoplasmic connections» between colonies until semi-confluency was obtained. Compressed cells were polygonal in shape and their centric nuclei had prominent multiple nucleoli. Numerous opaque intracellular inclusions were dispersed in the cellular endoplasm. Multinuclear giant cells were occasionally observed.

Signet-ring cells were found in COLO 357 following staining with mucicarmine (Fig. 3).

At the ultrastructural level, COLO 357 cells exhibited secretory granules, possible zymogen granules, few mitochondria but abundant free ribosomes, rough endoplasmic reticulum, and Golgi apparatus (Fig. 4). Perinuclear chromatin was sparse and nucleoli were dense. Occasionally, the cultured tumor cells exhibited peculiar crystalloid structures which appeared lamellar (Fig. 5). Well-formed desmosomes were observed. No viral particles were observed.

Growth studies showed a plating efficiency of 60% for COLO 357, with a cell doubling time of 21 h and growth in soft agar with 10% efficiency.

Biochemical markers

Quantitative analyses for CEA in 7-day-old spent medium from an actively growing culture of COLO 357 showed a level of 250 ng CEA/10^6 viable cells. This compares with 105 ng CEA/10^6 cells in one of our colon cancer cell lines, COLO 205 (Semple *et al.*, 1978). No CEA was detected in complete RPMI medium 1640 with 10% FBS. In addition, 26.9 MIU of β-subunit of HCG/ml of spent medium was detected.

The following assays were carried out on conditioned medium of COLO 357 and were found to be either negative or equal to background fresh medium: ACTH, α-fetoprotein, esterone, estradiol, progesterone, and cortisol.

No steroid receptor proteins for estrogen, progesterone, androgen or glucocorticoids were detectable in cytosol extracts of COLO 357.

The allozyme phenotype of COLO 357 revealed G6PD type B, PGM_1 type 1, PGM_3 type 2-1, ESD type 1, GOT_m type 1, ACP type B, and ADA type 1. Based on the isozymes studied, the combined chance of two individuals or cell lines from two individuals being alike for all seven isozyme loci is 0.07 within a random Negro population (Povey *et al.*, 1976).

The pancreatic enzyme profile of conditioned serum-free RPMI medium 1640 was positive for trypsin, elastase and chymotrypsin. The serum-free conditioned medium sample contained 0.5 μg of trypsin. Addition of 0.1 M ε-amino caproic acid to the assay reaction did not decrease the hydrolysis of the tryptic substrate, suggesting that there was no plasmin activity in the medium. The conditioned serum-free medium contained 3.1 ng of elastase and 11.7 μg of chymotrypsin. There was no evidence of protease activity in unincubated medium containing 1% fetal bovine serum compared to the unincubated serum-free medium blank.

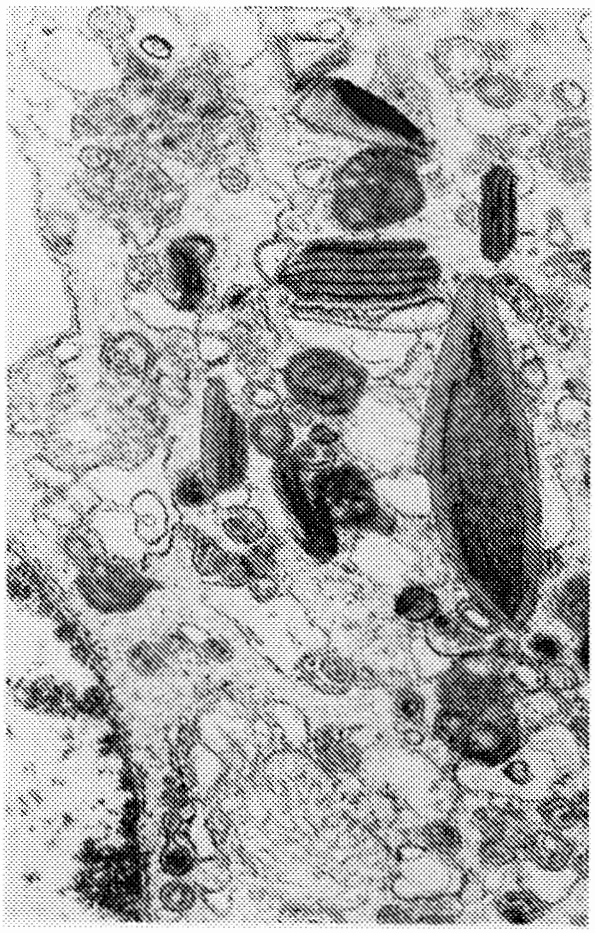

FIGURE 5 — Cytoplasmic crystalloid structures of COLO 357 showing well-defined lamellae. x 24,480.

Cytogenetics

The modal number of chromosomes for COLO 357 was 53. Eighty-five per cent of the cells had from 48 to 65 chromosomes. Chromosomes No. 2, 14, 15, 19, 20 and 22 most often had additional copies; chromosomes No. 19 and 20 most frequently had three, four or six copies. Chromosomes No. 1, 7, 10 and 16 were monosomic in the majority of cells karyotyped (Fig. 6).

The chromosome markers are described by Paris Conference (1971), Supplement (1975) nomenclature in Table I. Figures 6*A* and *B* present a karyotype of metaphase chromosomes with 12 of the marker chromosomes from one cell of COLO 357. Markers from three other cells are also represented in Figure 6*C*, *D*, and *E*. The markers in Figure 6*E* were noted in a cell from an early (2 months post-establishment) culture of COLO 357.

These markers also appeared in subsequent harvests. These harvests (6 months post-establishment) from two subcultures contained several common markers, but a few markers appeared only in one subculture. Several cells contained one or more chromosomes which, because of the poor banding and morphology, could not be described adequately. No distinctive polymorphisms were noted in the normal chromosomes by either G- or C-banding techniques.

Two markers, M_6 and M_{19}, were of interest because each may express duplication of genetic material. The translocation in M_6 had the appearance of a duplicated region of 6p21→25:. Current limitations in banding and other corroborative techniques prohibited a conclusive statement regarding this possibility. M_{19} had distinctive light and dark Giemsa

TABLE I

CHROMOSOME MARKERS IN COLO 357

Marker	Paris conference description	Percentage karyotyped[1] cells containing marker
M_1	i(1q)(qter→cen→qter)	100
M_2	del(1)(pter→q11:)	34
M_3	t(1;19)(1pter→1p13?::19p13l→19qter)	67
M_4	del(1)(qter→p13:)	17
M_5	t(2;4)(2qter→2q23::4p14→4qter)	25
M_6	t(6;?)(6qter→6p23::?)	34
M_7	t(1;6)(1pter→1p32::6p11→6q13:)	8[2]
M_8	t(7;10)(7qter→7p15::10q11→10qter)	34
M_9	i(7p)(pter→cen→pter)	67
M_{10}	t(8;13)(8qter→8p11::13q12→13qter)	67
M_{11}	i(5p)(pter→cen→pter)	17
M_{12}	t(11;5?)(11pter→11q23::5?q31→5?qter)	75
M_{13}	del(11)(pter→q13:)	8[2]
M_{14}	i(16p)(pter→cen→pter)	8[2]
M_{15}	t(12;16)(12qter→12q11::16p112?3?→16qter)	25
M_{16}	t(3;16)(3pter→3p23?::16p13→3qter)	34
M_{17}	del(17)(qter→p13?:)	34
M_{18}	del(19)(pter→q131:)	75
M_{19}	Metacentric marker possibly derived from 7q	34
M_{20}	t(5;?)(5qter→5p15?::?)	17
$M_?$	Unidentifiable markers	

[1] Number of cells karyotyped by photograph = 12. — [2] Chromosome found in one cell only (listed as marker for the sake of completeness).

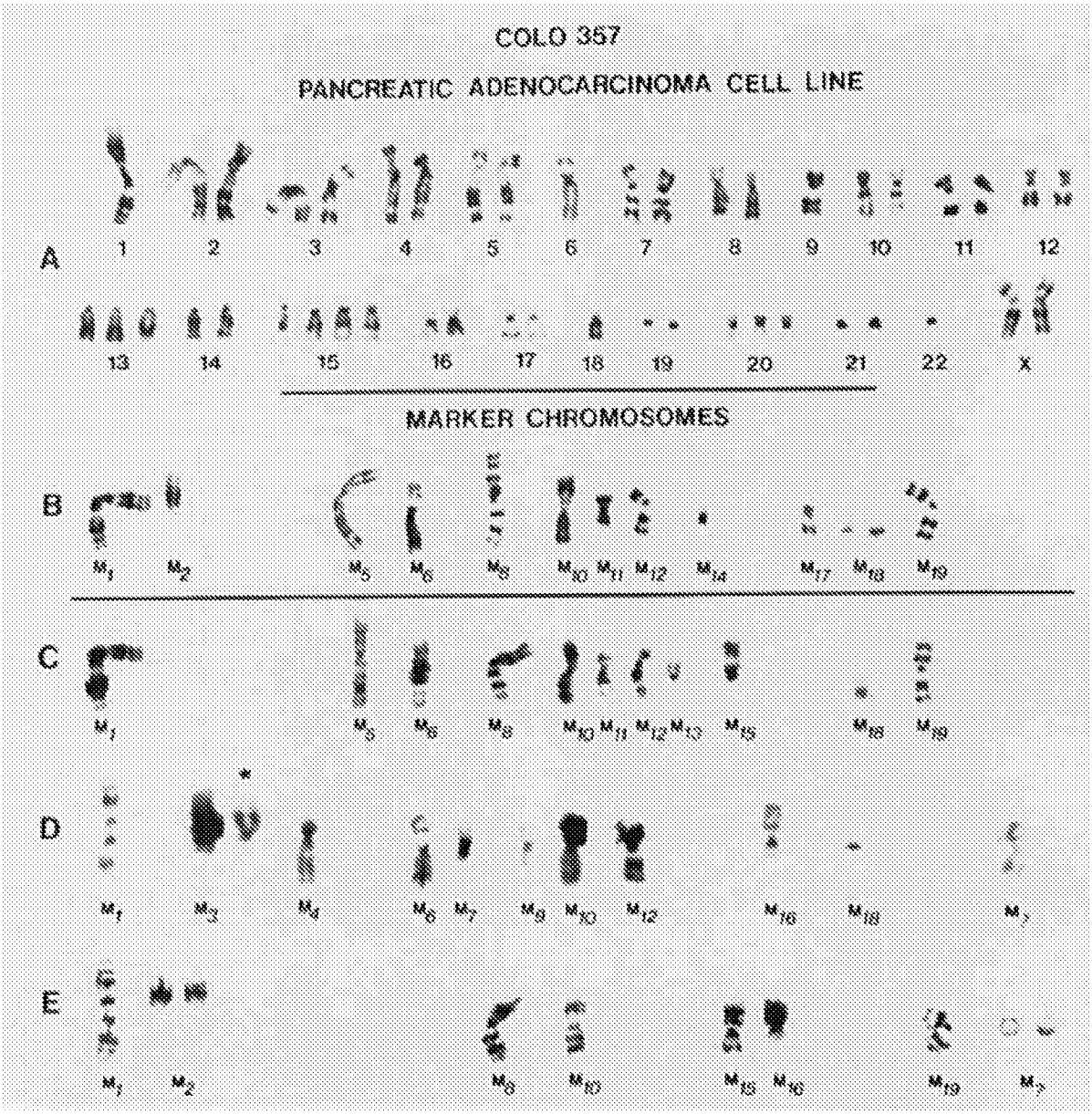

FIGURE 6 — Karyotype and marker chromosomes of COLO 357. A and B represent the normal chromosomes and marker chromosomes from a single metaphase. C, D and E are marker chromosomes from selected metaphases. *M_3 was taken from a fifth metaphase to clarify the banding morphology of M_3. M_{20} described in Table I was not present in the five metaphases depicted.

bands on both p and q arms that were similar to the 7cen→7q33: region of the No. 7 chromosome. However, the length of the 7q11-like band differed in p and q arms of the marker and the proximal dark G-band on both the p and the q arm was longer than that of the 7q21 region. Thus a definitive description was not possible in this case.

Numbers of NORs were tabulated from cells treated with $AgNO_3$ and the mean number of NORs per cell was 9. Approximately 43 metaphases were stained with Giemsa (pH 6.8) and analyzed for the presence of DMs. Two metaphases clearly had 1 DM. A microchromosome was observed in five metaphases. On the basis of morphology, it was not possible to decide whether a centromere was present in the microchromosomes because C-banding was inadequate.

Mycoplasma tests

The tumor cell line was monitored regularly for the presence of mycoplasma, and no mycoplasma contamination was detected.

DISCUSSION

Biochemical studies of human pancreatic carcinomas *in vitro* have been limited by a lack of experimental models. Studies of surgically excised tissues, body fluids and pancreatic and duodenal juices from tumor-bearing patients (Grant *et al.*, 1978)

have increased general knowledge concerning the pancreas, but added little information that would permit earlier diagnosis and successful treatment. Presently, the direct vision of the pancreatic ducts and the collection of fluid and biopsy specimens with the flexible endoscope have been very useful.

Recent reports on the establishment of pancreatic carcinoma tissues in either long-term tissue culture or in immunodeficient animals have been limited. The pancreatic cell lines, PANC-1 (Lieber *et al.*, 1975), Hs766T (Owens *et al.*, 1976) MIA PaCa-2 (Yunis *et al.*, 1977), and HDG-25 (Akagi and Kimoto, 1977) have been reported. Pancreatic carcinoma explants have been maintained in immunodeficient animals by Schmidt *et al.*, (1977) and Courtenay and Mills (1978). Grant *et al.*, (1979) established and characterized a primary human pancreatic carcinoma in both continuous cell culture and in immunodeficient *nu/nu* mice. We report the establishment in continuous culture of an adenocarcinoma cell line, COLO 357, derived from the exocrine pancreas. The unique properties of COLO 357 are production of tumor-associated proteins and protease enzymes, and a distinctive karyology and allozyme profile.

The *in vivo* production of tumor-associated proteins in patients with pancreatic adenocarcinoma has been investigated by Bender *et al.*, (1979). Serum levels of oncofetal proteins (CEA) and ectopic placental polypeptides (β-subunit of HCG) were present and elevated in only 57% and 17.5% respectively of patients with biopsy-proven, surgically staged pancreatic adenocarcinomas. *In vitro*, COLO 357 elaborates appreciable amounts of CEA and β-subunit of HCG. Yunis *et al.*, (1977) reported that neither PANC-1 nor MIA PaCa-2 produced measurable amounts of CEA.

The α-fetoprotein was not detectable in conditioned medium from COLO 357 cells. Fitzgerald *et al.*, (1978) found that only 6% of patients with cancer of the endocrine pancreas had a detectable level of α-fetoprotein in the serum.

Measurable amounts of the pancreatic enzymes, trypsin, chymotrypsin and elastase, were elaborated by COLO 357. The presence of these protease enzymes in serum-free medium from COLO 357 supports the concept that the cells are of pancreatic origin. Our findings are at variance with the work of Grant *et al.*, (1978) who suggested that most human pancreatic exocrine cancers probably do not elevate serum levels of pancreatic secretory enzymes *in vivo*. In a later study, Grant *et al.*, (1979) found no elaboration of pancreas-specific enzymes by a pancreatic cell line, though the protease enzymes were detected in pancreatic carcinoma tissues of acinar cell origin. Yunis *et al.*, (1977) did not detect trypsin or chymotrypsin activity exported by MIA PaCa-2 or PANC-1.

Eutopic production of pancreatic enzymes, especially lipase, has been associated with acinar-cell carcinomas of the pancreas (Burns *et al.*, 1974). Normal and to a lesser extent neoplastic acinar cells typically contain zymogen granules. COLO 357 cells contain secretory granules which resemble zymogen granules.

The distinctiveness of COLO 357 is further corroborated by its allozyme phenotype and karyology. Allozyme phenotypes were included in the characterization of the pancreatic carcinoma cell line of Grant *et al.*, (1979), and the G6PD type-B phenotype was included in the characterization of PANC-1 (Lieber *et al.*, 1975).

COLO 357 has an abnormal karyotype with 20 identifiable marker chromosomes, which is evidence of its malignant nature. Lieber *et al.*, (1975) reported the presence of four marker chromosomes from PANC-1 cells. One of these, a 1p-, may be similar to the del(1)(qter→p13:) marker (M) in COLO 357. This marker was observed at a low frequency. Two of the four markers of PANC-1 contained No. 1 chromosome involvement. In COLO 357, three of 20 marker chromosomes involved the No. 1 chromosome in various rearrangements. A marker which includes a No. 5 chromosome with break point in the 5p arm is observed in both COLO 357 and PANC-1.

The mean number of silver-stained NORs per cell of COLO 357 was nine. Hubbell *et al.*, (1977) suggested that NORs may be suppressed in tumor cells. Interpretation of the significance of NOR number in neoplasms awaits further analyses of other malignant cells. Low numbers of DMs in Giemsa-(pH 6.8)-stained metaphases of COLO 357 were observed.

To date, no cytogenetic studies of fresh preparations of pancreatic tumors have been reported and only one pancreatic cell line (PANC-1) has been previously analyzed with modern chromosome banding techniques. Thus, it is not yet possible to evaluate whether non-random patterns of chromosomes involved in markers of human pancreatic carcinomas exist. Utilization of Paris Conference (1971), Supplement (1975) nomenclature for tumor markers by all investigators would facilitate comparisons among cytogenetic studies.

The malignant nature of COLO 357 has been confirmed by its karyology, growth in soft agar and heterotransplantability in immunodeficient mice (personal communications, unpublished data, Dr. Michael D. Turner, Chief of Medical Services, Veterans Administration Hospital, Providence, RI).

This unique cell line may aid in the search for tumor-associated antigens (Schultz and Yunis, 1979) or the further identification of pancreatic oncofetal antigen (Gelder *et al.*, 1978). COLO 357 may be used in studies on proteolytic activity of cultured tumor cells and *in vitro* assays of chemotherapeutic agents.

COLO 357 is available to qualified investigators.

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