Cancer Chemother Pharmacol (2007) 59:549–557
DOI 10.1007/s00280-006-0297-3

ORIGINAL ARTICLE

A phase I study of oxaliplatin in combination with gemcitabine: correlation of clinical outcome with gene expression

Stephen Shibata · Warren Chow · Paul Frankel · Agnes Juhasz · Lucille Leong · Dean Lim · Kim Margolin · Robert Morgan · Edward Newman · George Somlo · Yun Yen · Timothy Synold · David Gandara · Heinz-Josef Lenz · James Doroshow

Received: 17 May 2006 / Accepted: 19 July 2006 / Published online: 19 October 2006

Abstract
Purpose Oxaliplatin has in vitro activity similar to or higher than other platinum agents. Preclinically, gemcitabine has demonstrated synergy when combined with platinum compounds. These facts formed the rationale for determining the maximum tolerated dose (MTD) of gemcitabine in combination with oxaliplatin.
Methods Eligible patients with advanced incurable solid tumors were given oxaliplatin 130 mg/m^2 as a 2-h infusion on day 1 followed by escalating doses of gemcitabine given over 30 min on day 1 and 8 of a 21-day cycle.
Results A total of 43 patients were enrolled, including 30 patients at the MTD in an expanded cohort. At a gemcitabine dose of 800 mg/m^2, 1/6 patients had a dose limiting toxicity (DLT) (grade 3 blurred vision and memory loss). At 1,000 mg/m^2, 1/6 patients had a DLT (grade 3 increase in AST). At 1,200 mg/m^2, 2/3 patients had a DLT (grade 4 thrombocytopenia and grade 3 confusion). The MTD of gemcitabine with 130 mg/m^2 of oxaliplatin was therefore 1,000 mg/m^2. The clearances of gemcitabine and ultrafilterable platinum are within the ranges previously reported for single agents. A patient with colon cancer had a partial response, and 21 patients had a best response of stable disease. In patients with tumor biopsies treated at the MTD, decreased ribonucleotide reductase M2 expression correlated with response.
Conclusion Treatment with gemcitabine and oxaliplatin was well tolerated with primarily hematologic toxicity at the MTD. Study of biochemical correlates of response remain of interest althought current results remain exploratory.

Keywords Oxaliplatin · Gemcitabine · Ribonucleotide reductase · Pharmacokinetics

This study was supported by National Cancer Institute grants U01 CA62505 and P30 CA33572.

S. Shibata (✉) · W. Chow · P. Frankel · A. Juhasz · L. Leong · D. Lim · K. Margolin · R. Morgan · E. Newman · G. Somlo · Y. Yen · T. Synold · J. Doroshow
Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010, USA
e-mail: sshibata@coh.org

D. Gandara
University of California at Davis, Cancer Center, Sacramento, CA, USA

H.-J. Lenz
University of Southern California Norris, Cancer Center, Los Angeles, CA, USA

Introduction

Oxaliplatin is a platinum analog of the diaminocyclohexane (DACH) family [29], and based on several recent phase III clinical trials, it has been approved for use in combination with 5-fluorouracil in the first [26] and second line treatment [44] of metastatic colon cancer as well as in the adjuvant setting [5].

Oxaliplatin is of interest in other tumor types due to preclinical data demonstrating a non-overlapping spectrum of activity with cisplatin [36, 42]. The DACH-platinum adducts are more effective at inhibiting DNA synthesis [31, 54] and are more cytotoxic than *cis*-diammine-platinum adducts formed from cisplatin and carboplatin [42, 46, 54]. DACH-platinum adducts also show the ability to retain activity in cases of mismatch repair

mutations associated with cisplatin resistance [2, 20, 21] [41]. Also, enhanced replicative bypass, another mechanism of cisplatin resistance, is not seen with oxaliplatin in either cisplatin resistant or sensitive cell lines [50].

Clinical studies have shown evidence of single agent activity in lung [33], breast [24], and ovarian cancer [11]. Importantly, oxaliplatin has also shown evidence of clinical activity in platinum-refractory patients including those with ovarian cancer [11, 17, 48].

When combined with other cytotoxic agents (5-FU, SN38, cisplatin, carboplatin, taxanes, or gemcitabine), oxaliplatin has additive or synergistic antitumoral effects in various in vitro and in vivo models [19, 41]. Therefore, clinical testing of combination therapy involving oxaliplatin is of significant interest.

Gemcitabine is a cytidine analogue with activity against pancreatic cancer, as well as other solid tumors such as lung, and bladder cancer. It is an inhibitor of ribonucleotide reductase and is also incorporated into DNA with subsequent chain termination and inhibition of DNA repair [37]. It has shown evidence of synergy with cisplatin [8, 51] and oxaliplatin [19] in preclinical studies, which may be due to inhibition of the repair of platinum related intra-strand cross-links. The combination of cisplatin and gemcitabine has demonstrated activity in non-small cell lung cancer [45], bladder cancer [53], breast [13], and pancreatic cancer [12].

Based on this information, we conducted a phase I trial of a combination of gemcitabine and oxaliplatin with the goal of determining a recommended phase II dose. A second important goal was to determine the expression of ribonucleotide reductase-M1, M2 (RRM1/M2), excision repair cross complementation (ERCC1), thymidylate synthase (TS), deoxycytidine kinase (DCK), and dihydropyrimidine dehydrongenase (DPD) in tumor biopsies in an expanded cohort of patients treated at the recommended phase II dose. The levels of messenger RNAs for these genes were correlated in exploratory fashion with clinical outcome.

Patients and methods

Adult patients 18 years of age and older with histologically confirmed incurable malignancies were eligible. Three or fewer previous chemotherapy regimens and radiation to no more than 30% of bone marrow were allowed [16]. Karnofsky performance status ≥60%, leukocytes ≥3,000/μl, absolute neutrophil count ≥1,500/μl, platelets ≥100,000/μl, total bilirubin within institutional normal limits, and alanine and aspartate transferase levels ≤2.5× institutional upper limits of normal were required as was a normal serum creatinine or a measured creatinine clearance ≥60 ml/min. Patients with clinically significant peripheral neuropathy were excluded, as were patients with brain metastasis, platinum allergies, HIV patients on anti-retroviral treatment, and patients with pulmonary fibrosis. Prior chemotherapy or radiation must have been completed at least 4 weeks prior to protocol treatment.

Treatment consisted of a fixed dose of oxaliplatin 130 mg/m^2 over 2 h on day 1 of a 21 day cycle. Gemcitabine was given following oxaliplatin in escalating doses over 30 min on days 1 and 8. Day 8 dosing was modified based on neutrophil and platelet counts on that day (see Table 1). Patients were evaluated for tolerance using the CTC v. 2.0. In addition, a scale developed by Sanofi-Synthelabo was used to grade and dose adjust for neurotoxicity (see Table 2).

Non-hematologic dose limiting toxicities (DLT) were any treatment-related grade 3 toxicities not reversible to grade 2 or less in 96 h, or a grade 4 toxicity of any duration. Exceptions included grade 3 peripheral neuropathy, which was a DLT only if persistent between cycles, and grade 3 nausea, which was a DLT if IV hydration was required for more than 24 h despite optimal antiemetics. Additionally, grade 3 or 4 diarrhea treated with suboptimal anti-diarrheal medication was not a DLT. Dose limiting hematologic toxicities were defined as grade 4 thrombocytopenia of any duration, or grade 4 neutropenia either lasting for more than 5 days or complicated by neutropenic fever. A treatment delay of ≥4 weeks due to toxicity constituted a DLT. Course 1 toxicities were used in determining the maximally tolerated dose (MTD).

Patients were enrolled in cohorts of three. If one DLT occurred at a treatment level, further patients would be entered up to a total of six. If no more than 1/6 patients ultimately experienced a DLT, then further gemcitabine dose escalation would occur. The DLT level was defined as the level at which ≥2/6 patients experienced a DLT. The maximally tolerated dose was defined as the highest level that ≤1/6 patients experienced a DLT.

Oxaliplatin and gemcitabine pharmacokinetics were determined in a subset of patients during the first cycle. Blood samples for ultrafilterable platinum concentrations

Table 1 Day 8 gemcitabine hematologic dose adjustments

AGC (×10^6)	Platelet	% dose
>1500	>100,000	100%
1000–1499	75,000–99,000	75%
750–999	50,000–74,000	50%
<750	<50,000	Hold

Table 2 Oxaliplatin dose modifications for neurologic toxicity

Toxicity	Duration of toxicity		Persistent[a] between cycles
	1–7 days	>7 days	
Paresthesias/dysesthesias[b] of short duration that resolve and do not interfere with function (Grade 1)	No change	No change	No change
Paresthesias/dysesthesias[b] interfering with function, but not activities of daily living (ADL) (Grade 2)	No change	No change	20% decrease
Paresthesias/dysesthesias[b] with pain or with functional impairment that also interfere with ADL (Grade 3)	First time: 20% decrease second time: 20% decrease	First time: 30% decrease second time: 30% decrease	Stop
Persistent paresthesias/dysesthesias that are disabling or life-threatening(Grade 4)	Stop	Stop	Stop
Pharyngo-laryngeal dysesthesias	No change	Eight duration of infusion to 6 h	Eight duration of infusion to 6 h

[a] Not resolved by the beginning of the next cycle

[b] May be cold-induced

were obtained with the first oxaliplatin dose; prior to treatment, at the end of the 2-h infusion, and then 0.5, 1, 2, 4, 24, and 48 h after the end of the infusion. Plasma ultrafiltrate samples were prepared as previously described [49]. All oxaliplatin assays were performed in the Analytical Pharmacology Core Facility at the City of Hope utilizing a validated atomic absorption spectroscopic assay [14]. The method has a limit of quantitation of 20 ng/ml, and an intra- and inter-day precision and accuracy within ±10%. For determination of gemcitabine pharmacokinetics, serial blood samples were collected around doses 1 and 8 at the following times; prior to treatment, then 5, 10, 15, 30, and 60 min after the end of the infusion. Gemcitabine in plasma was determined by HPLC using a minor modification of the method of Freeman et al. [23]. Compartmental pharmacokinetic data analyses of ultrafilterable platinum and gemcitabine were performed using ADAPT II (USC, Biomedical Simulations Resource). Descriptive statistics (mean ± standard deviation) were calculated for the model-derived PK parameters.

Additional patients were enrolled at the MTD level to allow baseline tumor biopsies prior to treatment for exploratory studies correlating parameters of tumor biology with clinical outcome. Given the exploratory nature of these studies, the goal was for six or more patients with biopsies. Ultimatelyfresh frozen tumor biopsies from 14 patients treated at the MTD were examined for the expression of RRM1/M2, ERCC1, DPD, TS, DCK and the 18 S ribosome genes. The biopsy specimens were divided: half for pathologic review, and the remainder immediately frozen in liquid nitrogen and stored at −80°C. RNA was isolated using RNAzol B (Tel-Test, Inc.) per the manufacturer's directions. After Dnase I treatment, cDNA was prepared and used for relative quantification of gene expression by real-time polymerase chain reaction (PCR). Primers for target genes were designed according to Applied Biosystems guidelines. Expression of target genes was quantified as a ratio in relation to 18 S with adjustments for differences in PCR efficiency [28].

Results

A total of 43 patients with advanced solid tumors were enrolled (see Table 3). At a level of gemcitabine 1,200 mg/m^2, two of three patients experienced a DLT during the first cycle of treatment. One patient experienced a grade 3 platelet level with a nadir of 18 K/μl felt to be dose limiting. A second patient experienced grade 3 confusion without evidence of central nervous system metastasis. Subsequently, a cohort of six patients was treated with gemcitabine 1,000 mg/m^2 with one colon cancer patient with liver metastasis experiencing grade 3 liver function test elevation during the first cycle. Five other patients were treated without experiencing a DLT during the first cycle, establishing this level as the MTD and recommended phase II dose.

Other DLTs at lower levels included reversible grade 3 blurred vision and memory loss in a patient treated at 800 mg/m^2 gemcitabine without evidence of central nervous system metastasis. Further evaluation did not detect glaucoma, or change in a baseline cataract, and corrective lenses were prescribed. The only case of febrile neutropenia also occurred in a patient treated at this level.

Table 3 Patient characteristics

Characteristics	Number
Age	
Median(56 year)	
Range(22–84 year)	
Race	
Caucasian	39
Afro-American	1
Asian	3
Gender	
Male	27
Female	16
Diagnosis	
Colorecta	L23
Stomach	4
Pancreatobiliary	2
Melanoma	2
Unknown primary	2
Other	10

An additional 24 patients were enrolled at the MTD level and evaluated for response and tolerance (see Table 4). These patients were enrolled with the goal of obtaining tumor biopsies. Of these patients, only one DLT level toxicity was noted (grade 3 fatigue > 96 h). Grade 3 platelet nadirs were seen in 11/30 patients in 23 treatment courses with platelet transfusions given in seven cases. Three patients had grade 3 thrombocytopenia complicating the first course of treatment. No clinically significant episodes of bleeding were seen.

Grade 3 ANC was seen in 9/30 patients at the MTD in 22 treatment courses. No episodes of grade 4 ANC or neutropenic sepsis were noted. Four patients had grade 3 neutropenia complicating the first course.

Non-hematologic toxicity was moderate with no events greater than grade 3 at the MTD (see Table 4). Neuropathy was relatively uncommon with only two patients experiencing grade 3 acute toxicity after 1 and 3 cycles, respectively. No chronic toxicity was reported. Grade 3 dyspnea was seen in five patients, including four at the MTD. In each case, pulmonary metastatic disease was felt to be the primary factor and two patients received further treatment without incident.

Dose reductions were required in 6/30 patients treated at the MTD with two patients requiring adjustment after cycle 1. The median number of treatments given was three with a range of 1–10. Treatment was stopped after 1 course in four cases, in no case with toxicity greater than grade 2 (two for progressive disease, one for poor tolerance not qualifying as a DLT, and one at the request of the patient). Eleven patients received four or more cycles. Of the 30 patients at the MTD, 18 patients stopped treatment due to progressive disease, with other patients stopping due to cumulative side effects, and for personal reasons.

Among all patients, one patient with colorectal cancer had a partial response by RECIST criteria, and a second patient with bile duct cancer had an unconfirmed partial response. Stable disease was documented in 20 patients, with progressive disease in 17 cases. Four patients were inevaluable for response.

Gemcitabine and unbound platinum pharmacokinetics were evaluated in patients enrolled at each of the dose levels. Estimated secondary pharmacokinetic parameters for gemcitabine following the dose administered on day 1 are summarized in Table 5. Gemcitabine elimination from plasma followed a bi-exponential decay, with mean alpha and beta half-lives of 2.3 ± 0.8 and 19.5 ± 6.7 min, respectively. The mean gemcitabine clearance (CL_{sys}) was $2.2 \pm$ l/min/m^2, and was unrelated to dose over the range tested. The average apparent volume of distribution (V_d) was 21 ± 8 l/m^2. Gemcitabine pharmacokinetics were also determined following doses on days 1 and 8 in a subset of eight patients and no significant differences were observed in the pharmacokinetics on the two different days (data not shown and Fig. 1).

Unbound platinum pharmacokinetics following the first dose of oxaliplatin were determined in 15 patients and the secondary parameters are summarized in Table 6. The mean maximum plasma concentration (C_{max}) measured at the end of the infusion was 1.5 ± 0.5 μg/l. Following the drug administration, free platinum concentrations in plasma were best described by a bi-exponential pattern of elimination, with mean

Table 4 Toxicities at the MTD (Grade 3–4)

Course # (cycles)	1 (30)	2 (26)	3 (15)	4 (11)	5 (9)	6 (8)	7 (4)	8 (4)	9 (2)	10 (2)
Neutropenia	4	4	5	3	2	2	1	2	0	0
Thrombocytopenia	3	5	5	3	3	3	2	1	0	1
Fatigue	5	3	3	2	1	0	0	0	0	0
Neuropathy										
Acute	1	0	1	0	0	0	0	0	0	0
Chronic	0	0	0	0	0	0	0	0	0	0
Nausea/vomiting	3	1	0	1	1	0	0	0	1	0

Table 5 Gemcitabine pharmacokinetics on day 1

Dose level (mg/m^2)	N	C_{max} (μg/l)	AUC (μg/l min)	V_d (l/m^2)	CL_{sys} ($ml/min/m^2$)	$t_{1/2}$ alpha (min)	$t_{1/2}$ beta (min)
700	3	13.3	422.4	9.8	1,685	1.7	16.5
800	2	7.0	242.6	25.3	3,487	1.9	14.9
1,000	6	17.0	506.9	22.0	2,078	2.1	29.5
1,250	1	20.0	735.3	26.1	1,663	3.5	17.2
			Average	20.8	2,228.2	2.3	19.5
			SD	7.6	860.6	0.8	6.7
			Median	23.6	1,881.4	2.0	16.8

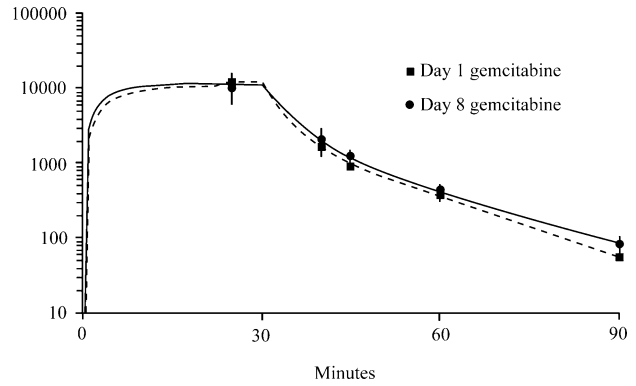

Fig. 1 Gemcitabine plasma concentration versus time curves following doses administered on days 1 and 8 of the first cycle ($n = 8$ patients). The squares represent the mean data following the dose on day 1 and the circles depict the mean data following the dose on day 8

alpha and beta half-lives of 0.23 ± 0.16 h and 17.1 ± 13.6 h. Average unbound platinum CL_{sys} and V_d were 7.4 ± 3.6 l/h/m^2 and 106 ± 34 l/m^2, respectively.

The subset 14 patients with pre-treatment biopsies studied at the MTD, included the patient with partially responding colorectal cancer (PR), as well as eight patients with stable disease (SD) and six with progressive disease (PD). In total, eight patients in this group had colorectal cancer. Using a standard two-sample *t* test, RR-M2 expression rates across groups were significantly lower in patients with SD versus PD (0.0004 vs. 0.001, $P = 0.05$) (Fig. 2). No significant differences were noted in the expression of other genes, or in the subset of patients with colorectal cancer.

Discussion

Combinations of oxaliplatin and gemcitabine have been studied in the treatment of various malignancies including ovarian [40], biliary [6], testicular [35], mesothelioma [47], renal [38], cervical [15], pancreatic [4, 30], and lung cancer [18, 22]. This study provided further information regarding the combination of oxaliplatin and gemcitabine, and provides information related to biochemical parameters that may affect the efficacy of this combination. Moreover, the pharmacokinetic

Table 6 Unbound platinum pharmacokinetics

Patient #	C_{max} (μg/l)	AUC (μg/l h)	CL_{sys} (l/h/m^2)	V_d (l/m^2)	$t_{1/2}$ alpha (h)	$t_{1/2}$ beta (h)
1	2.2	28.2	4.6	59.3	0.19	12.0
2	1.5	79.8	1.6	144.3	0.24	65.1
3	2.3	35.4	3.7	69.5	0.21	16.9
4	1.0	36.0	3.6	32.9	0.16	12.5
5	2.2	19.3	6.7	76.8	0.19	13.0
6	2.0	19.4	6.7	88.1	0.17	14.2
7	1.3	12.8	10.1	116.0	0.18	12.0
8	1.0	13.8	9.4	156.1	0.16	15.0
9	0.7	7.8	16.8	115.7	0.80	6.2
10	1.6	16.1	8.1	104.3	0.17	13.4
11	1.5	18.7	6.9	124.0	0.20	17.3
12	1.4	20.7	6.3	124.8	0.19	17.5
13	1.3	13.9	9.4	134.3	0.28	14.7
14	1.4	15.6	8.3	120.3	0.21	14.4
15	1.1	13.9	9.4	124.9	0.18	12.0
Average	1.5	23.4	7.4	106.1	0.23	17.1
SD	0.5	17.5	3.6	34.1	0.16	13.6
Median	1.4	18.7	6.9	116.0	0.19	14.2
Low	0.7	7.8	1.6	32.9	0.17	6.2
High	4.8	79.8	16.8	144.3	0.80	65.1

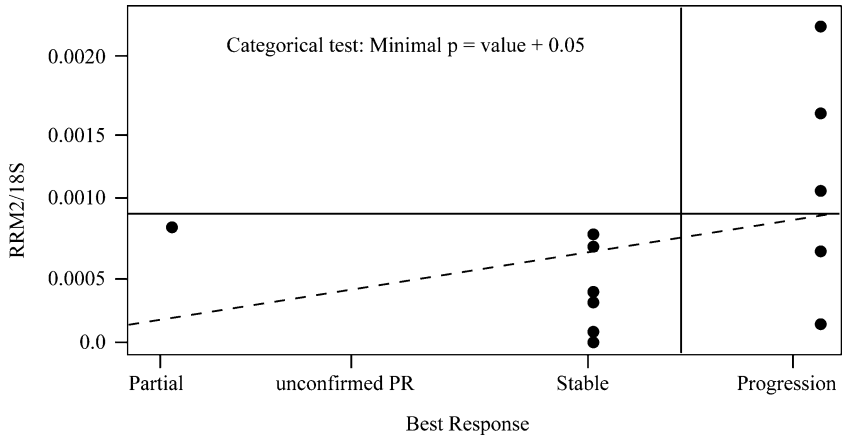

Fig. 2 Response in patients treated with oxaliplatin and gemcitabine at the maximally treated dose was correlated with RR-M2 expression. Lower expression was found to correlate with stable disease and response in comparison to progressive disease

data presented here are consistent with single agent data [1, 27, 52], as well as with previous reports of the combination [18, 32], suggesting that drug–drug interactions between oxaliplatin and gemcitabine are minimal. Hematologic changes were the primary toxicity at the MTD. No episodes of grade 4 toxicity were seen, and only one episode of neutropenic fever was noted. Grade 3 fatigue was seen in approximately 1/3 of patients at the MTD, but repeated treatment was possible despite this, indicating that symptoms were not progressive.

Dyspnea was seen in several patients. Pulmonary toxicity, which can be severe, has been reported with gemcitabine [34] and cases of interstitial fibrosis have rarely been reported with oxaliplatin. However, none of the cases appeared due to this and instead appeared disease related. No clear instances of oxaliplatin related laryngopharygo dysesthesias, which clinically may present as dyspnea, were documented.

Neuropathy is a common complication with oxaliplatin treatment [10]. Both acute and chronic dose-related neuropathy are seen. Grade 3 acute neuropathy was noted in two cases, which were managed by dose reduction. Lack of significant chronic neuropathy is related to the relatively short median duration of treatment. Previous reports suggest that chronic neuropathy becomes more significant with cumulative doses greater than 800 mg/m^2.

Several previous phase I trials have studied the combination of oxaliplatin and gemcitabine. In one report, gemcitabine at 1,800 mg/m^2 on days 1 and 8 with oxaliplatin on day 8 was reported as the DLT level defined by hematologic toxicity and asthenia. The recommended phase II dose was gemcitabine 1,200–1,400 mg/m^2 on days 1 and 8 and oxaliplatin 130 mg/m^2 on day 8. Cumulative hematologic toxicity was not reported and grades 2 and 3 neurotoxicity was the primary cumulative non-hematologic toxicity. Ten partial responses were reported among 56 patients evaluable for response [32].

Another study recommended a phase II dose of gemcitabine 1,500 mg/m^2 with oxaliplatin 85 mg/m^2 every 2 weeks [18]. No specific DLTs were noted at any level, and the recommended dose was based on achievement of therapeutic levels of each drug. Hematologic toxicity was mild with no episodes of neutropenic sepsis reported with two treatment delays were needed for grades 3–4 thrombocytopenia. Asthenia and neurologic toxicity were the primary cumulative non-hematologic toxicities. Eleven of 33 patients with non-small cell lung cancer treated across a range of doses had an objective response.

A phase I trial focusing on non-small cell lung cancer used a fixed dose of gemcitabine 1,250 mg/m^2 on day 1 and 8 of a 21 day cycle with oxaliplatin 130 mg/m^2 given on day 1 at the MTD. The primary toxicities were hematologic with no significant neurotoxicity reported. All patients were pre-treated and 2/15 had a partial response [9].

A report focusing on pancreatic cancer recommended a phase II dose of gemcitabine 1,000 mg/m^2 on day 1 and 8 with oxaliplatin 100 mg/m^2 on day 1 of a 21-day schedule [3]. The DLT level occurred at a gemcitabine dose of 1,250 mg/m^2 and consisted of grade 4 neutropenia and neutropenic fever complicated by a fatal myocardial infarction. Grades 3–4 neutropenia occurred in 2/6 patients at the MTD but did not prevent repeated cycles. Only sporadic grade 3 non-hematologic toxicities were noted. Three of 18 patients showed evidence of a response including one with a complete response.

Gemcitabine 1,000 mg/m^2 at a rate of 10 mg/m^2 on day 1 with oxaliplatin 100 mg/m^2 on day 2 over 2 h repeated every 2 weeks (GEMOX) was compared to bolus gemcitabine alone in patients with advanced pancreatic cancer [30]. An improved response rate (26.8 vs. 17.3%, $P = 0.04$), progression free survival (5.8 vs. 3.7 m, $P = 0.04$), and clinical benefit (38.2 vs. 26.9%, $P = 0.03$) were reported. The ECOG-6201 trial was

recently presented and although there was a trend toward improvement in overall survival with infusional gemcitabine and GEMOX versus bolus gemcitabine, this was not statistically significant [39]. The routine use of GEMOX or infusional gemcitabine is therefore not supported in advanced pancreatic cancer.

The recommended phase II dose of gemcitabine is combined with 130 mg/m^2 is somewhat lower than that recommended in other studies (1,000 mg/m^{2}vs. 1,200–1,400 mg/m^2). Given the infrequency of grade 4 and DLT level toxicities at the MTD, it is possible that a modest increase in the gemcitabine dose would be possible. The number of grade 3 and 4 hematologic toxicities suggests that marked further dose escalation would not be possible.

As a group, it appears that patients, who had evidence of benefit, albeit primarily with stable disease, had a lower expression of RR-M2. This conclusion is limited by the small number of patient samples, and the heterogeneous nature of the samples. Although preliminary, this result is of interest because preclinical studies have suggested that RR-M2 levels correlate with resistance to gemcitabine [25]. Clinical studies have suggested a role for ribonucleotide reductase and specifically that RR-M1 levels may correlate with response to gemcitabine in patients with non-small cell lung cancer [7, 43]. In addition, dNTP pool size changes associated with inhibition of RR-M2 may affect repair to oxaliplatin related DNA adducts [55].

In conclusion, the regimen reported in this study is well tolerated with primarily hematologic toxicity. Further study of biochemical correlates including RR-M2 may assist with determining patients most likely to respond to benefit from this treatment.

References

1. Abbruzzese JL, Grunewald R, Weeks EA, Gravel D, Adams T, Nowak B, Mineishi S, Tarassoff P, Satterlee W, Raber MN et al (1991) A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J Clin Oncol 9:491–498
2. Aebi S, Kurdi-Haidar B, Gordon R, Cenni B, Zheng H, Fink D, Christen RD, Boland CR, Koi M, Fishel R, Howell SB (1996) Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res 56:3087–3090
3. Alberts SR, Townley PM, Goldberg RM, Cha SS, Moore DF Jr, Krook JE, Pitot HC, Fitch TR, Wiesenfeld M, Mailliard JA, Sargent DJ (2002) Gemcitabine and oxaliplatin for patients with advanced or metastatic pancreatic cancer: a North Central Cancer Treatment Group (NCCTG) phase I study. Ann Oncol 13:553–557
4. Alberts SR, Townley PM, Goldberg RM, Cha SS, Sargent DJ, Moore DF, Krook JE, Pitot HC, Fitch TR, Wiesenfeld M, Mailliard JA (2003) Gemcitabine and oxaliplatin for metastatic pancreatic adenocarcinoma: a North Central Cancer Treatment Group phase II study. Ann Oncol 14:580–585
5. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zaninelli M, Clingan P, Bridgewater J, Tabah-Fisch I, de Gramont A (2004) Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 350:2343–2351
6. Andre T, Tournigand C, Rosmorduc O, Provent S, Maindrault-Goebel F, Avenin D, Selle F, Paye F, Hannoun L, Houry S, Gayet B, Lotz JP, de Gramont A, Louvet C (2004) Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma: a GERCOR study. Ann Oncol 15:1339–1343
7. Bepler G, Sharma S, Cantor A, Gautam A, Haura E, Simon G, Sharma A, Sommers E, Robinson L (2004) RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol 22:1878–1885
8. Bergman AM, Ruiz v HV, Veerman G, Kuiper CM, Peters GJ (1996) Synergistic interaction between cisplatin and gemcitabine in vitro. Clin Cancer Res 2:521–530
9. Bidoli P, Stani SC, Mariani L, De Candis D, Cortinovis D, Aglione S, Zilembo N, Toffolatti L, Formisano B, Bajetta E (2004) Phase I study of escalating doses of oxaliplatin in combination with fixed dose gemcitabine in patients with non-small cell lung cancer. Lung Cancer 43:203–208
10. Cassidy J, Misset JL (2002) Oxaliplatin-related side effects: characteristics and management. Semin Oncol (Suppl 15) 29:11–20
11. Chollet P, Bensmaine MA, Brienza S, Deloche C, Cure H, Caillet H, Cvitkovic E (1996) Single agent activity of oxaliplatin in heavily pretreated advanced epithelial ovarian cancer. Ann Oncol 7:1065–1070
12. Colucci G, Giuliani F, Gebbia V, Biglietto M, Rabitti P, Uomo G, Cigolari S, Testa A, Maiello E, Lopez M (2002) Gemcitabine alone or with cisplatin for the treatment of patients with locally advanced and/or metastatic pancreatic carcinoma: a prospective, randomized phase III study of the Gruppo Oncologia dell'Italia Meridionale. Cancer 94:902–910
13. Doroshow J, Tetef M, Margolin K, Somlo G, Frankel P, Longmate J, Synold T, Gandara D, Lenz HJ, Albain K (2000) Significant activity of gemcitabine and cisplatin in both heavily and minimally-pretreated metastatic breast cancer patients: a California Cancer Consortium/Loyola University Chicago Trial Proc ASCO 19:155a
14. Doroshow JH, Synold TW, Gandara D, Mani S, Remick SC, Mulkerin D, Hamilton A, Sharma S, Ramanathan RK, Lenz HJ, Graham M, Longmate J, Takimoto CH, Ivy P (2003) Pharmacology of oxaliplatin in solid tumor patients with hepatic dysfunction: a preliminary report of the National Cancer Institute Organ Dysfunction Working Group. Semin Oncol Aug (Suppl 15) 30:14–19
15. Duenas-Gonzalez A, Lopez-Graniel C, Gonzalez A, Gomez E, Rivera L, Mohar A, Chanona G, Trejo-Becerril C, de la GJ (2003) Induction chemotherapy with gemcitabine and oxaliplatin for locally advanced cervical carcinoma. Am J Clin Oncol 26:22–25
16. Ellis RE (1961) The distribution of active bone marrow in the adult. Phys Med Biol 5:255–258
17. Faivre S, Kalla S, Cvitkovic E, Bourdon O, Hauteville D, Dourte LM, Bensmaine M A, Itzhaki M, Marty M, Extra JM (1999) Oxaliplatin and paclitaxel combination in patients with platinum-pretreated ovarian carcinoma: an investigator-originated compassionate-use experience. Ann Oncol 10:1125–1128
18. Faivre S, Le Chevalier T, Monnerat C, Lokiec F, Novello S, Taieb J, Pautier P, Lhomme C, Ruffie P, Kayitalire L,

Armand JP, Raymond E (2002) Phase I-II and pharmacokinetic study of gemcitabine combined with oxaliplatin in patients with advanced non-small-cell lung cancer and ovarian carcinoma. Ann Oncol 13:1479–1489
19. Faivre S, Raymond E, Woynarowski JM, Cvitkovic E (1999) Supraadditive effect of 2′, 2′-difluorodeoxycytidine (gemcitabine) in combination with oxaliplatin in human cancer cell lines. Cancer Chemother Pharmacol 44:117–123
20. Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, Christen RD, Howell SB (1996) The role of DNA mismatch repair in platinum drug resistance. Cancer Res 56:4881–4886
21. Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, Nehme A, Christen RD, Haas M, MacLeod CL, Howell SB (1997) In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 57:1841–1845
22. Franciosi V, Barbieri R, Aitini E, Vasini G, Cacciani GC, Capra R, Camisa R, Cascinu S (2003) Gemcitabine and oxaliplatin: a safe and active regimen in poor prognosis advanced non-small cell lung cancer patients. Lung Cancer 41:101–106
23. Freeman KB, Anliker S, Hamilton M, Osborne D, Dhahir PH, Nelson R, Allerheiligen SR (1995) Validated assays for the determination of gemcitabine in human plasma and urine using high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Appl 665:171–181
24. Garufi C, Nistico C, Brienza S, Vaccaro A, D'Ottavio A, Zappala AR, Aschelter AM, Terzoli E (2001) Single-agent oxaliplatin in pretreated advanced breast cancer patients: a phase II study. Ann Oncol 12:179–182
25. Goan YG, Zhou B, Hu E, Mi S, Yen Y (1999) Overexpression of ribonucleotide reductase as a mechanism of resistance to 2, 2-difluorodeoxycytidine in the human KB cancer cell line. Cancer Res 59:4204–4207
26. Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC, Alberts SR (2004) A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 22:23–30
27. Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E, (2000) Clinical pharmacokinetics of oxaliplatin: a critical review. Clin Cancer Res 6:1205–1218
28. Juhasz A, Frankel P, Cheng C, Rivera H, Vishwanath R, Chiu A, Margolin K, Yen Y, Newman EM, Synold T, Wilczynski S, Lenz HJ, Gandara D, Albain KS, Longmate J, Doroshow JH (2003) Quantification of chemotherapeutic target gene mRNA expression in human breast cancer biopsies: comparison of real-time reverse transcription-PCR vs. relative quantification reverse transcription-PCR utilizing DNA sequencer analysis of PCR products. J Clin Lab Anal 17:184–194
29. Kidani Y, Noji M, Tashiro T (1980) Antitumor activity of platinum(II) complexes of 1,2-diamino-cyclohexane isomers. Gann 71:637–643
30. Louvet C, Labianca R, Hammel P, Lledo G, Zampino MG, Andre T, Zaniboni A, Ducreux M, Aitini E, Taieb J, Faroux R, Lepere C, de Gramont A (2005) Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. J Clin Oncol 23:3509–3516
31. Mamenta EL, Poma EE, Kaufmann WK, Delmastro DA, Grady HL, Chaney SG (1994) Enhanced replicative bypass of platinum-DNA adducts in cisplatin-resistant human ovarian carcinoma cell lines. Cancer Res 54:3500–3505
32. Mavroudis D, Pappas P, Kouroussis C, Kakolyris S, Agelaki S, Kalbakis K, Androulakis N, Souglakos J, Vardakis N, Nikolaidou M, Samonis G, Marselos M, Georgoulias V (2003) A dose-escalation and pharmacokinetic study of gemcitabine and oxaliplatin in patients with advanced solid tumors. Ann Oncol 14:304–312
33. Monnet I, Brienza S, Hugret F, Voisin S, Gastiaburu J, Saltiel JC, Soulie P, Armand JP, Cvitkovic E, de Cremoux H (1998) Phase II study of oxaliplatin in poor-prognosis non-small cell lung cancer (NSCLC) ATTIT Association pour le Traitement des Tumeurs Intra Thoraciques. Eur J Cancer 34:1124–1127
34. Pavlakis N, Bell DR, Millward MJ, Levi JA (1997) Fatal pulmonary toxicity resulting from treatment with gemcitabine. Cancer 80:286–291
35. Pectasides D, Pectasides M, Farmakis D, Aravantinos G, Nikolaou M, Koumpou M, Gaglia A, Kostopoulou V, Mylonakis N, Skarlos D (2004) Gemcitabine and oxaliplatin (GEMOX) in patients with cisplatin-refractory germ cell tumors: a phase II study. Ann Oncol 15:493–497
36. Pendyala L, Kidani Y, Perez R, Wilkes J, Bernacki RJ, Creaven PJ (1995) Cytotoxicity, cellular accumulation and DNA binding of oxaliplatin isomers. Cancer Lett. 97:177–184
37. Plunkett W, Huang P, Searcy CE, Gandhi V (1996) Gemcitabine: preclinical pharmacology and mechanisms of action. Semin.Oncol (Suppl 10) 23:3–15
38. Porta C, Zimatore M, Imarisio I, Natalizi A, Sartore-Bianchi A, Danova M, Riccardi A (2004) Gemcitabine and oxaliplatin in the treatment of patients with immunotherapy-resistant advanced renal cell carcinoma: final results of a single-institution Phase II study. Cancer 100:2132–2138
39. Poplin E, Levy E, Berlin M, Rothenberg M, Cella D, Mitchell E, Alberts S, and Benson III A. A phase III trial of gemcitabine (30-min infusion) versus gemcitabine (fixed-dose-rate infusion [FDR]) versus gemcitabine + oxaliplatin (GEMOX) in patients with advanced pancreatic cancer. Proc ASCO 24, No 18s aLBA4004
40. Raspagliesi F, Zanaboni F, Vecchione F, Hanozet F, Scollo P, Ditto A, Grijuela B, Fontanelli R, Solima E, Spatti G, Scibilia G, Kusamura S (2004) Gemcitabine combined with oxaliplatin (GEMOX) as second-line chemotherapy in patients with advanced ovarian cancer refractory or resistant to platinum and taxane. Oncology 67:376–381
41. Raymond E, Faivre S, Woynarowski JM, Chaney SG (1998) Oxaliplatin: mechanism of action and antineoplastic activity. Semin Oncol (Suppl 5) 25:4–12
42. Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, Fojo T (1996) Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. Biochem.Pharmacol 52:1855–1865
43. Rosell R, Danenberg KD, Alberola V, Bepler G, Sanchez JJ, Camps C, Provencio M, Isla D, Taron M, Diz P, Artal A (2004) Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 10:1318–1325
44. Rothenberg ML, Oza AM, Bigelow RH, Berlin JD, Marshall JL, Ramanathan RK, Hart LL, Gupta S, Garay CA, Burger BG, Le Bail N, Haller DG (2003) Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol 21:2059–2069
45. Sandler AB, Nemunaitis J, Denham C, von Pawel J, Cormier Y, Gatzemeier U, Mattson K, Manegold C, Palmer MC, Gregor A, Nguyen B, Niyikiza C, Einhorn LH (2000) Phase III trial of gemcitabine plus cisplatin versus cisplatin alone in patients with locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 18:122–130

46. Schmidt W, Chaney SG (1993) Role of carrier ligand in platinum resistance of human carcinoma cell lines. Cancer Res 53:799–805
47. Schutte W, Blankenburg T, Lauerwald K, Schreiber J, Bork I, Wollscgkaeger B, Treutler D, Schneider CP, Bonner R (2003) A multicenter phase II study of gemcitabine and oxaliplatin for malignant pleural mesothelioma. Clin Lung Cancer 4:294–297
48. Soulie P, Bensmaine A, Garrino C, Chollet P, Brain E, Fereres M, Jasmin C, Musset M, Misset JL, Cvitkovic E (1997) Oxaliplatin/cisplatin (L-OHP/CDDP) combination in heavily pretreated ovarian cancer. Eur J Cancer 33:1400–1406
49. Takimoto CH, Remick SC, Sharma S, Mani S, Ramanathan RK, Doroshow J, Hamilton A, Mulkerin D, Graham M, Lockwood GF, Ivy P, Egorin M, Schuler B, Greenslade D, Goetz A, Knight R, Thomas R, Monahan BP, Dahut W, Grem JL (2003) Dose-escalating and pharmacological study of oxaliplatin in adult cancer patients with impaired renal function: a National Cancer Institute Organ Dysfunction Working Group Study. J Clin Oncol 21:2664–2672
50. Vaisman A, Varchenko M, Umar A, Kunkel TA, Risinger JI, Barrett JC, Hamilton TC, Chaney SG (1998) The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: correlation with replicative bypass of platinum-DNA adducts. Cancer Res 58:3579–3585
51. van Moorsel CJ, Pinedo HM, Veerman G, Bergman AM, Kuiper CM, Vermorken JB, van der Vijgh WJ, Peters GJ (1999) Mechanisms of synergism between cisplatin and gemcitabine in ovarian and non-small-cell lung cancer cell lines. Br J Cancer 80:981–990
52. Venook AP, Egorin MJ, Rosner GL, Hollis D, Mani S, Hawkins M, Byrd J, Hohl R, Budman D, Meropol NJ, Ratain MJ (2000) Phase I and pharmacokinetic trial of gemcitabine in patients with hepatic or renal dysfunction: Cancer and Leukemia Group B 9565. J Clin Oncol 18:2780–2787
53. von der Maase H, Hansen SW, Roberts JT, Dogliotti L, Oliver T, Moore MJ, Bodrogi I, Albers P, Knuth A, Lippert CM, Kerbrat P, Sanchez RP, Wersall P, Cleall SP, Roychowdhury DF, Tomlin I, Visseren-Grul CM, Conte PF (2000) Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 18:3068–3077
54. Woynarowski JM, Chapman WG, Napier C, Herzig MC, Juniewicz P (1998) Sequence- and region- specificity of oxaliplatin adducts in naked and cellular DNA. Mol Pharmacol 54:770–777
55. Zhou BS, Ker R, Ho R, Yu J, Zhao YR, Shih J, Yen Y (1998) Determination of deoxyribonucleoside triphosphate pool sizes in ribonucleotide reductase cDNA transfected human KB cells. Biochem.Pharmacol 55:1657–1665