Docetaxel and gemcitabine combination therapy in advanced transitional cell carcinoma of the urothelium: results of a phase II and pharmacologic study

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Our objective was to determine the response to gemcitabine plus docetaxel in advanced urothelial transitional cell carcinoma in a phase II trial, and gemcitabine distribution between plasma and erythrocytes, following docetaxel administration. Patients with locally advanced or metastatic transitional cell carcinoma, following a maximum of one prior chemotherapy regimen, were given gemcitabine 800 mg/m^2 on days 1 and 8 plus docetaxel 85 mg/m^2 on day 8, every 21 days. Gemcitabine was measured in the plasma and erythrocytes of nine patients before and after docetaxel administration. Thirty-four patients (median 63 years; range 49–79 years), of whom seven had prior chemotherapy and 27 were chemotherapy-naive, received a median of six cycles (range 1–6). Complete and partial remissions were observed in two and 16 (including three pretreated) patients, respectively, for an overall response rate of 53%. Median response duration was 5 months (range 1–39+). Haematoxicity was manageable, despite grade 3 infections in 24% of patients, but other toxicities were mostly mild. An apparent shift of gemcitabine from plasma to erythrocytes occurred after docetaxel in five of six patients evaluable for this analysis. We conclude gemcitabine plus docetaxel is tolerable and highly active in treated and untreated patients with advanced transitional cell carcinoma. ***Anti-Cancer Drugs*** **18:211–218 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Transitional cell carcinoma (TCC) of the urothelium is considered a chemosensitive malignancy. For more than a decade, the combination regimen of methotrexate/vinblastine/doxorubicine/cisplatin (M-VAC) has been considered the standard for these patients. On the basis of encouraging results with the combination of gemcitabine and cisplatin (GC), a phase III trial comparing GC with M-VAC randomized 405 patients, and showed that the two regimens are associated with similar response rates, time to progression and overall survival, whereas GC is less toxic than M-VAC. On the basis of this superior risk–benefit ratio, the GC regimen should be favoured as a new standard treatment in patients with locally advanced and metastatic TCC of the urothelium [1]. The overall response rate for GC ranges from 41 to 57%, with a complete remission (CR) rate of 15–22% and a median survival of 12.5–14.3 months. This combination chemotherapy can provide palliation, but only a moderate survival advantage, and few patients achieve long-term disease control. These modest results and the unsuccessful attempt to increase efficacy with dose-intensive M-VAC schedules have prompted the identification of new active combinations in TCC, such as taxanes and gemcitabine. Triplets of these drugs with platinum are also currently being compared with GC and M-VAC [2,3]. We favoured the combination of gemcitabine and docetaxel because it is useful as a second-line treatment after M-VAC and as a first-line treatment in patients with impaired renal function.

Gemcitabine is an analogue of the nucleoside deoxycytidine and is inactive in its parent form. Intracellular phosphorylation of the parent drug yields the active diphosphate and triphosphate metabolites. The diphosphate form inhibits ribonucleotide reductase (an enzyme important for DNA biosynthesis), whereas the triphosphate form is incorporated into DNA, in competition with the normal nucleotide base deoxycytidine, as a fraudulent base [4]. Once the gemcitabine triphosphate metabolite is incorporated into DNA, one additional nucleoside is incorporated, after which DNA chain synthesis is terminated. This 'masked chain termination' leaves the fraudulent base relatively

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resistant to excision repair by DNA repair enzymes and thus may overcome a key mechanism in the development of drug resistance [5].

Docetaxel, a semisynthetic analogue of paclitaxel, functions by stabilizing tubulin, which results in the inhibition of mitotic and interphase cellular functions [6]. Docetaxel induces phosphorylation of bcl-2, promoting apoptosis. The combination of DNA synthesis termination by gemcitabine and the promotion of apoptosis by docetaxel may lead to a synergistic effect of these two agents *in vivo*.

We performed this trial to assess the antitumour effect of the combination of gemcitabine (Gemzar) and docetaxel (Taxotere) in patients with advanced TCC of the urothelium, and to further evaluate the toxic effects of this combination [7]. Blood sampling was performed in nine patients to determine the gemcitabine concentrations in plasma and erythrocytes, and assess the alterations in gemcitabine distribution following docetaxel administration (as detected in our phase I study [8]).

Patients and methods

Eligibility

Patients with histologically confirmed TCC of the bladder or urinary tract, with measurable locally advanced disease considered unresectable for cure or with measurable metastases, and who had received a maximum of one previous chemotherapy regimen for the treatment of their TCC were eligible if they met the following criteria: adequate organ function [defined as absolute neutrophil count (ANC) $\geq 2000/\mu l$, platelet count $\geq 100\,000/\mu l$, normal bilirubin, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) $< 2 \times$ upper normal limit (UNL), alkaline phosphatase < 2.5 UNL or < 5 UNL if of bone origin; creatinin clearance > 20 ml/min]; age ≥ 18 years; a World Health Organization performance status < 2; and an estimated life expectancy > 12 weeks. Previous chemotherapy or radiotherapy had to be completed at least 4 weeks before enrolment. Patients with other severe medical illnesses were excluded: uncontrolled congestive heart failure, angina pectoris, hypertension or arrhythmia, myocardial infarction within 1 year, significant neurological or psychiatric disorders, active infection, active ulcer disease and unstable diabetes mellitus or other contraindications to corticotherapy, pre-existing ascites, pleural or pericardial effusion. Pregnant or lactating women and patients unwilling to use adequate contraception were also excluded. Other exclusion criteria were: symptomatic or known central nervous system metastases; existing peripheral neuropathy grade 2 or more; previous or concurrent second primary malignancies; concurrent treatment with experimental drugs; participation in clinical trials with experimental agents within 30 days of study entry; and prior use of taxoids, gemcitabine or other nucleoside analogues. All patients provided written informed consent and agreed to regular follow-up. The protocol was approved by the institutional medical ethics committee.

Treatment plan

Patients received gemcitabine 800 mg/m^2 in 250 ml NaCl 0.9% intravenously over 30 min on days 1 and 8. Docetaxel (provided by Aventis Pharma, Brussels, Belgium) was administered intravenously at a dose of 85 mg/m^2 in 250 ml glucose 5% over 1 h on day 8 before gemcitabine. A 1-h interval exists between the end of docetaxel infusion and the start of gemcitabine administration on day 8. Treatment cycles were repeated every 21 days in an outpatient setting. This schedule was determined by our phase I study [7].

The premedication for docetaxel was methylprednisolone 32 mg orally twice daily, starting on day 7 in the evening and continuing for the next 2 days. Prophylactic granulocyte colony-stimulating factor or other haematopoietic growth factors were not allowed during the first cycle of treatment. In cases of neutropenic fever or prolonged neutropenia grade 4 ($< 500/\mu l$ for a minimum of 5 days), granulocyte colony-stimulating factor was given prophylactically on subsequent cycles. Further supportive treatment (e.g. antiemetics, antiallergics, analgesics) was given as medically indicated and reported in the case report forms. Concomitant radiotherapy was not allowed, unless for local pain control to a small area not encompassing the solely measurable lesion.

Treatment continued for six cycles unless there was evidence of unacceptable toxicity or progressive disease (PD), patient refusal, or when it was no longer in the best interests of the patient according to the treating physician.

Evaluations during treatment

Pretreatment evaluations included: history and physical examination; complete blood count with differential and platelet count; biochemical profile (calculation of the creatinin clearance with the Cockroft and Gault formula); electrocardiogram; chest radiograph; and computed tomography (CT) scan of the chest, abdomen and pelvis with documentation of tumour measurements within 2 weeks before the start of the study.

Complete blood counts were performed weekly during treatment. A history was taken, including concurrent illness and concomitant medication, and a physical examination was performed before each treatment cycle; toxicities were scored according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC version 2.0) on days 1 and 8 of every cycle before each treatment.

The biochemical profile was also assessed on days 1 and 8 of each cycle. The weight of the patient was recorded on every cycle to document any weight gain attributable to oedema.

CT scans and chest radiographs were repeated every two cycles to assess response. It was intended that all patients with stable disease (SD), CR or partial response (PR) had CT scans approximately 3-monthly following the end of the study, until there was evidence of disease progression.

Dose adjustments

If the ANC was less than 1500/μl or the platelet count less than 100 000/μl on day 1 of a treatment cycle, treatment was delayed 1 week. Patients in whom the ANC or platelet counts had not recovered after a 2-week delay were withdrawn from the study. If the ANC was 1000–1499/μl or the platelets 75 000–99 000/μl on day 8, docetaxel and gemcitabine were given at 75% of the day 1 doses. If the ANC was less than 1000/μl or the platelet count less than 75 000/μl on day 8, docetaxel and gemcitabine at the day 8 dose were postponed until recovery (with a maximum of 2 weeks postponement).

Hypersensitivity reactions occurring during docetaxel infusions despite premedication were appropriately treated according to their severity by slowing the infusion rate, interrupting the infusion, giving dexamethasone and promethazine, and resuming the infusion, or discontinuation in the most severe cases (bronchospasm, generalized urticaria, hypotension and angio-oedema). The dose was not reduced in cases of fluid retentions. The clinical tolerance of the patient, the overall tumour response and the medical judgement of the investigator determined if it was in the best interest of the patient to continue in association with diuretics or to discontinue the study drug. In cases of grade 3/4 neurotoxicity, the patient was withdrawn from the protocol. Nephrotoxicity, with a reduction in creatinine clearance to < 20 ml/min, delayed gemcitabine administration until recovery (maximum 2 weeks). The directives concerning hepatic dysfunction were as follows: ASAT and ALAT > 2.5 and ≤ 5 UNL, respectively, with alkaline phosphatase levels ≤ 2.5 UNL warranted a dose reduction of 25% of both drugs; ASAT and ALAT > 1.5 and ≤ 5 UNL, respectively with alkaline phosphatase levels > 2.5 and ≤ 5 UNL also warranted a dose reduction of 25% of both drugs; ASAT and ALAT > 5 UNL or alkaline phosphatase levels > 5 UNL (unless bone metastases were present in the absence of any liver disorder) warranted a dose delay of a maximum of 2 weeks. In cases of an abnormal bilirubin, drug administration was delayed by a maximum of 2 weeks. Patients who did not recover to the minimum requirements after 2 weeks delay were withdrawn from the study. If patients experienced other grade 3 or 4 nonhaematological toxicities (except alopecia and nail changes), treatment was postponed for 2 weeks, and resumed if the toxicity resolved to grade 2 or less.

Bioanalytical method for gemcitabine determination in plasma and red blood cells

In nine patients, after obtaining appropriate informed consent, blood samples were collected immediately before gemcitabine infusion on day 1, at the end of the infusion (30 min) and 90 min thereafter to determine gemcitabine concentrations in plasma and erythrocytes. On day 8, blood samples were drawn at the following time points: before the start of the docetaxel infusion, 30 min thereafter, 1 h after the end of the docetaxel infusion (i.e. just before the start of gemcitabine infusion), 90 min after the end of the gemcitabine infusion, and once between 13:30 and 21:30 h after the end of the gemcitabine infusion.

Erythrocytes were separated from plasma with the MESED device in three centrifugation steps [8] and both specimens were immediately frozen at -20°C until further analysis.

To extract gemcitabine from plasma, 100 μl of the internal standard 2′-deoxycytidine (1.0 μg/ml) was added to 200 μl of plasma. After vortexing, the sample was treated with 6 ml of isopropanol (15%) in ethyl acetate and mixed thoroughly. After centrifugation, the organic phase was transferred to another polypropylene tube and evaporated until dry. The residue was redissolved in 1 ml of the mobile phase (see below, a $5 \times$ dilution) and filtered over a 0.45-μm polyvinylidine diflouride high-performance liquid chromatographic (HPLC) filter (Acrodisc; Waters Milford, USA) for HPLC injection (20 μl).

In the sample pretreatment of the erythrocytes, 400 μl of demineralized water was added to 100 μl of red blood cells (RBCs). After vortexing and lysis of the RBCs, 100 μl of the internal standard was added and the sample was then treated in the same way as described for the plasma extraction.

A HPLC method was used and validated for the determination of gemcitabine and its main circulating metabolite 2′-difluoro-2′,2′-deoxyuridine in human plasma and erythrocytes, with 2′-deoxycytidine as internal standard. Separation was achieved on a Chrompack Spherisorb ODS-2 (Varian Chrompack International, Middelburg, The Netherlands) reversed-phase column (25 m $\times$ 4.6 mm, 5 μm). The mobile phase was Pic B7 reagent (Waters Milford, USA) in 15% methanol (pH 3.1) with a flow rate of 1.0 ml/min. Gemcitabine and 2′-deoxycytidine are detected by ultraviolet detection at 270 nm. The limit of quantitation was about 100 ng/ml for gemcitabine. Within-run and between-run precisions were less than 10%, and average accuracies were between 90 and 110%.

The erythrocyte sample pretreatment procedure for gemcitabine was not essentially different from that reported previously. The internal standard work solution (10 μg/ml; 300 μl) and 50 μl of the tetrahydrouridine solution (10 mg/ml) extracted with isopropanol (15%) in ethyl acetate (2 × 5 ml) was transferred to another polypropylene tube and evaporated (as described above). The residue was resuspended in 1 ml of mobile phase (a 5 × dilution) and filtered over a 0.45-μm polyvinylidine diflouride filter for HPLC injection (20 μl).

Separation was achieved on a Chrompack Spherisorb ODS-2 reversed-phase column (250 × 4.6 mm ID, 5 μm). The mobile phase was Pic B7 reagent (Waters Milford, USA) in 15% methanol (pH 3.1) with a flow rate of 1.0 ml/min. Gemicitabine and 2′-deoxycytidine were detected by ultraviolet detection at 270 nm. The order of elution was 2′-deoxycytidine followed by gemcitabine.

Response criteria

Patients who had received at least one cycle of treatment were evaluable for response, on the basis of a set of target lesions selected before the first treatment. A maximum of five lesions were chosen from the same organ site, with a maximum of 10 representative lesions. A target lesion was defined as a lesion of at least 1 cm diameter on spiral CT scan, or at least 2 cm diameter on chest radiograph or clinical measurements with callipers. Lesions in an irradiated area could be used as targets if the radiotherapy had ended 3 months before study entry and provided that they had since either progressed or appeared. Response Evaluation Criteria in Solid Tumours were used to determine best overall response [9]. A CR was defined as the disappearance of all lesions; a PR defined as at least a 30% decrease in the sum of the longest diameter of the target lesions, taking as a reference the baseline sum; and PD defined as at least a 20% increase in the sum of the longest diameter of the target lesions, taking as a reference the smallest sum recorded since the start of the treatment or the appearance of new lesions. SD was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, at least 6 weeks after the start of the treatment. Best overall response (OR) was the best response designation recorded from the start of treatment until disease progression or recurrence. CR and PR had to be confirmed by two evaluations at least 4 weeks apart, but the date of best response was the date that the best response was first detected. The duration of response was measured from the date CR or PR criteria were first met to the date of recurrent disease, or objective progression in the absence of other treatment.

All participants underwent CT scans to assess response approximately every 6 weeks (two cycles) during treatment and approximately every 3 months after completion of treatment.

Statistical analysis

A Simon two-stage phase II study design was used where the unacceptable and promising response rates were designated as 10 and 30%, respectively. A test was initially performed when the first seven patients were evaluable for response. The trial would have been stopped if one response or less was observed in these first seven patients, with the conclusion that the combination was ineffective and should not be investigated further. As two or more responses were seen, accrual of additional patients was planned until a minimum of 25 patients were evaluable for response. The regimen was to be declared promising if five or more responses were observed among these 25 patients. The high response rates observed prompted the inclusion of additional patients, who were also included in this analysis.

The duration of response was calculated by Kaplan–Meier methodology for all patients who had an objective response. In this analysis, the duration of response was measured from the date of start of treatment to the date of documented progression. If another treatment was started before documented progression, the duration of response was 'censored' on the day of the start of the new treatment. Overall survival was also depicted by Kaplan–Meier methodology.

Results

Patient characteristics

Thirty-four patients with advanced TCC of the urothelium, nine women and 25 men, were enrolled into the study between March 1999 and April 2001 (Table 1). The median age was 63 years (range 49–79 years) with a median WHO performance status of 1 (range 0–2; only two patients had a WHO performance status of 2). The primary tumour site was the bladder in majority of the patients (26), the kidney (pyelon, calyx) in five and the ureter in three. Seven had progressed after first-line systemic chemotherapy (M-VAC, cisplatin, methotrexate and vinblastine or carboplatin, vinblastine and adriamycin) and 27 (79%) had not received prior chemotherapy. Twenty-eight patients (82%) underwent surgery for their

Table 1 Patient characteristics (*N* = 34)

Age (years) median (range)	63 (49–79)
Female/male	9/25
WHO performance status median (range)	1 (0–2)
Primary tumour site	
bladder	26
kidney	5
ureter	3
Prior systemic chemotherapy	
M-VAC	3
CMV	3
CVA	1

M-VAC, methotrexate, vinblastine, adriamycin and cisplatin; CMV, cisplatin, methotrexate and vinblastine; CVA, carboplatin, vinblastine and adriamycin; WHO, World Health Organization.

primary tumour, but only one had been irradiated. Sixteen had loco-regional advanced disease and/or pathological lymph node invasion at the start of the study. Thirteen patients presented with visceral metastases (liver and/or lung), six had bone metastases and two had muscle involvement. The median number of cycles delivered per patient was six (range 1–6), with half the patients receiving six cycles.

Adverse events

Thirty-four patients received a total of 152 cycles of gemcitabine plus docetaxel chemotherapy during this phase II trial.

The percentage of patients experiencing grade 3/4 adverse events is listed in Table 2.

Haematologic toxicity was common, including neutropenia grade 3 (13% of cycles), and grade 4 (9% of cycles), thrombocytopenia grade 3 (3% of cycles), and anaemia grade 3 (5% of cycles) plus one episode of anaemia grade 4. Five episodes of neutropenic fever (3% of cycles) occurred, but there were no treatment-related deaths.

Important nonhaematologic toxicities included grade 3 cardiovascular toxicity (decompensation) in two patients, hypotension grade 4 in one patient, diarrhoea grade 3 in one patient, infection grade 3/4 in eight patients (26%), pulmonary toxicity grade 3 in two patients and severe peripheral oedema in two patients (moderate oedema was present in another two). Alopecia developed in all patients and most experienced mild fatigue or asthenia. Almost half reported grade 1 or 2 nausea and/or vomiting, and one-third developed mild anorexia. Nine patients experienced flu-like symptoms and a cumulative sensory neuropathy grade 1–2 occurred in five patients. One was withdrawn from the protocol after his fifth cycle because of pulmonary toxicity (deterioration in diffusion capacity). All these toxicities were reported by the investigator as possibly or probably study drug related.

Table 2 Frequency of grade 3 and 4 toxicities (152 cycles)

Toxicity	Grade 3		Grade 4	
	No. of patients	Percentage	No. of patients	Percentage
Infection	8	24	1	3
Dyspnoea	2	6		
Peripheral oedema	2	6		
Diarrhoea	1	3		
Hypotension			1	3
Heart failure	2	6		
Anaemia	8		1	0.65
Thrombocytopenia	4	5		
Neutropenia	19	3	14	9
Neutropenic fever (±infection)		13	5	3

Treatment delays were required in 21 patients [in 43 (28%) cycles] and 12 patients required dose reductions or omissions [in 20 (13%) cycles].

Treatment responses and survival

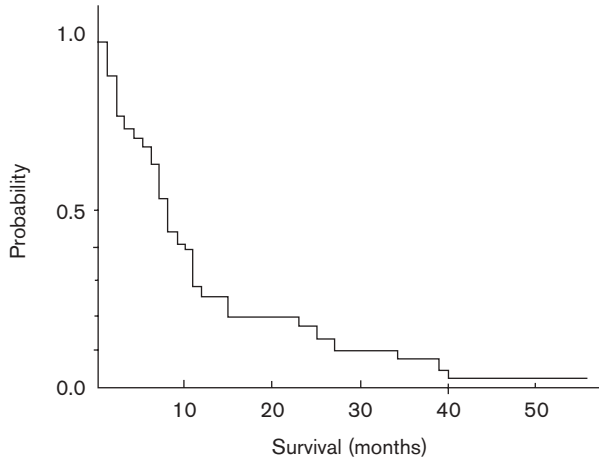
Response was not assessed in four patients: one patient died from a severe pneumonia, judged by the investigator as not drug related, before his first assessment; the second did not return after the first administration of gemcitabine on day 1 and was lost to follow-up; the third was not assessed because the target lesion was not measurable; and the fourth withdrew from the trial after two cycles without assessment because of a rapidly progressive deterioration, probably disease related. The objective response rate was calculated from all 34 eligible patients.

A CR was observed in two patients and a PR in 16, for an OR rate of 53%. One of the patients with a CR had been pretreated with mitomycin C bladder instillations after transurethral resection (TURB) of the primary bladder tumour and had presented with lymph node metastases. She was still in CR when last seen in March 2004. The second, a renal transplant patient, had a loco-regional recurrence of TCC with multiple retroperitoneal and cervical lymph nodes after nephro-ureterectomy and partial cystectomy. Four months after achieving a CR, she had a local recurrence in the bladder, removed by TURB, and remained in CR at her last evaluation in March 2004. One patient with a liver metastasis and a pathological retroperitoneal lymph node 5 months after cystoprostatectomy achieved a partial remission after six cycles of gemcitabine–docetaxel and shows no signs of progression as of March 2004 (3 years and 3 months later). No major difference was seen in response rates with respect to the primary tumour site, the site of metastases (visceral or not) or pretreatment with systemic chemotherapy. The median duration of response, measured from the date of best response to the date of disease progression, was 5 months (range 1–39 + months, ongoing). After 6 months, 29% of all patients were progression free. Overall survival and duration of response (calculated from the start of treatment to progression) are shown in Figs 1 and 2 (Kaplan–Meier).

influence of docetaxel on the distribution of gemcitabine

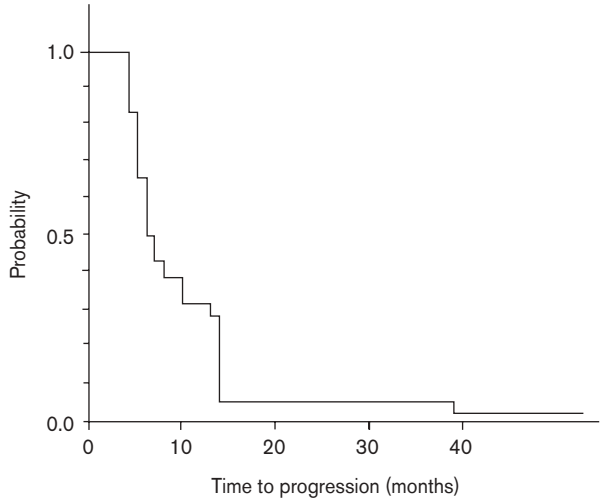
In nine patients, we had the opportunity to take blood samples to determine gemcitabine concentrations in plasma and RBCs. Partition ratios of gemcitabine (concentration in RBC/concentration in plasma) were calculated for each sample point on days 1 and 8. We noted a highly significant ($P < 0.001$, linear fit, Fig. 1) concentration-dependent rise in partition ratios. The mean partition ratio of gemcitabine on day 8 was 271%, compared with 87% on day 1, indicating a shift of

Fig. 1



Probability versus survival in months for the patients treated with docetaxel and gemcitabine.

Fig. 2



Probability versus time to progression in months for the patients treated with docetaxel and gemcitabine.

gemcitabine into the erythrocytes after docetaxel administration ($P = 0.0548$ unpaired Student's *t*-test, trend to significance).

Comparing the maximal concentration of gemcitabine (C_{max}) in plasma and RBCs on day 1 versus day 8, the differentials were calculated for six evaluable patients (Table 3).

Table 3 Differential (d) concentrations of gemcitabine on day 1 versus day 8

Patient no.	dC_{max} plasma (%)	(Massashift in mg)	dC_{max} red blood cells (%)	(Massashift in mg)
1	+21.4	(+1.27)	+973	(+37.3)
2	+1.29	(+0.07)	+26.3	(+0.98)
3	−4.74	(−0.19)	+1.06	(+0.04)
4	−60.0	(−1.18)	not evaluable	
5	−25.2	(−1.04)	+355	(+9.15)
6	−27.5	(−1.97)	−54.6	(−2.01)
7	+6.81	(+0.19)	+45.4	(+0.81)

Discussion

The combination of gemcitabine plus docetaxel is highly active in patients with advanced TCC of the urothelium, with an OR rate of 53%. Three PRs were observed among the seven patients who had received previous platinum-based chemotherapy. The responders were equally divided concerning primary tumour localization and site of the metastasis (visceral or pathological lymph nodes). Of the five long-term survivors (two to more than 4 years), two progressed locally in the bladder shortly after the end of the study, and needed a TURB and salvage cystectomy, respectively, although their lymph node metastases have remained in CR. The third patient, who also had lymph node metastases, is still in CR and the fourth has maintained a good PR of a liver metastasis. One patient progressed on docetaxel and gemcitabine, but responded on M-VAC and then had salvage surgery. The 29% 6-month progression-free rate observed in this study supports the conclusion that gemcitabine and docetaxel is an effective regimen in this tumour type. The regimen was generally well tolerated, with mostly mild to moderate haematologic and nonhaematologic toxicities. Only the infection rate was somewhat higher in our trial population.

The results obtained with the combination of gemcitabine plus docetaxel are surprising in view of previous studies assessing the activity of these drugs given as single agent for TCC. The OR rate for single-agent gemcitabine on the basis of five studies was 26%, which was apparently independent of whether the patients had received prior chemotherapy. Paclitaxel and docetaxel as single agents have yielded OR rates of 7–56%, depending on whether the patients had received prior chemotherapy for metastatic disease. In the light of these data, and considering the different mechanisms of action of both drugs, they may exhibit true in-vivo synergy. Recently, two phase II trials of the same drugs using different administration schedules have been published, showing OR rates of 17% (as second-line) and 33% [10,11]. The higher response rate in our study (53%) can possibly be explained by the greater fraction of chemotherapy-naive patients together with a more favourable drug administration schedule.

The other commercially available taxane, paclitaxel, has also been studied in combination with gemcitabine in advanced TCC. Two phase II studies were published in 2001, with OR rates of 54 and 60%. The response rates in second-line treatment of metastatic disease were 47 and

27%, respectively. The haematological toxicity seemed somewhat higher than in the docetaxel combinations, although this needs to be compared formerly to draw definite conclusions [12,13]. Adding cisplatin to the taxane–gemcitabine combinations results in OR rates ranging from 58 to 80%, although these triplets can only be given to chemotherapy-naive patients with adequate renal function and cause considerable toxicity [3,14].

The combination docetaxel–gemcitabine has also shown interesting activity in other tumour types. In studies of extensively pretreated anthracycline-resistant breast cancer patients, response rates ranged from 36 to 54%, with median times to disease progression of 7–8 months. In less pretreated patients, the combination produced an OR of 79% [15,16]. It is noteworthy that objective responses were also achieved with this regimen in some patients who progressed while receiving taxane-based, first-line therapy [17]. Alexopoulos *et al.* [18] performed a phase II study with gemcitabine 900 mg/m^2 on days 1 and 8 plus docetaxel 100 mg/m^2 on day 8 every 3 weeks in 50 women with metastatic breast cancer refractory or resistant to docetaxel monotherapy as first-line or second-line treatment. Forty-six per cent of patients responded, whereas 28% had SD with a median duration of response of 6.1 ± 2.4 months [18]. In randomized clinical trials, gemcitabine and taxane combinations have produced response rates and survival outcomes equal to older platinum-based regimens in non-small-cell lung cancer patients. The improvement of disease-related symptoms has outweighted toxicity in all these studies. When combined with a platinum compound, they produced the best results achieved to date in this disease [19,20]. The combination of gemcitabine and docetaxel is also a well-tolerated regimen in advanced pancreatic cancer, with OR rates ranging from 18 to 27%. The ultimate role of this combination versus single-agent gemcitabine can only be determined by a randomized phase III trial [21,22]. Sherman and Fine [23] showed *in vitro* that these two agents were minimally effective alone, but when combined they display additional biochemical antiproliferative effects [23]. A 41% OR rate in patients with advanced nonnasopharyngeal head and neck cancer with first-line paclitaxel plus gemcitabine should also be highlighted, although the median time to progression was only 4 months [24]. Of 28 patients with refractory germ cell tumours, six patients responded to the combination of paclitaxel and gemcitabine, including three complete responders [25].

As with most phase II single-institution studies, the favourable results observed in this trial may not be reproducible in the multi-institution setting or with patients who would not have met the fairly rigorous eligibility criteria of our study. Nevertheless, we have demonstrated that the combination of gemcitabine plus docetaxel is highly active in the first-line and second-line setting for patients with advanced TCC. The promising activity and limited toxicity observed in this study should be confirmed in the multi-institution setting and the regimen should be compared with the actual standard therapy for advanced TCC (gemcitabine/cisplatin and M-VAC) in a randomized phase III trial. Regardless, the docetaxel–gemcitabine combination allows patients with an impaired renal function, who cannot receive platinum-based schedules, to be treated for their liver-threatening disease.

During our phase I study with docetaxel and gemcitabine, administered in the same schedule as in this phase II study, gemcitabine pharmacokinetics were found to be changed significantly by coadministration of docetaxel. The plasma concentrations of gemcitabine were significantly lower during the distribution phase following administration of docetaxel, compared with those on day 1. In that study, no RBC sampling had been performed. We attempted to explore this finding further, with in-vitro incubation experiments using volunteer blood [26]. In the whole sample pool, no influence of docetaxel on the partitioning of gemcitabine was detected, but on subanalysis we found significantly more gemcitabine in male erythrocytes after docetaxel impregnation versus the inversed sequence of incubation. This phase II study gave us the opportunity to check these findings *in vivo*. Of the nine patients sampled, only data from six were evaluable because of various sample and technical problems. The concentration-dependent rise in partition ratios of gemcitabine between RBCs and plasma, seen in our in-vitro experiments, was confirmed by the measurements in the patients of this phase II study: at higher whole blood concentrations (RBC + plasma concentrations), a greater fraction of gemcitabine is present in the erythrocytes relative to plasma. Following treatment with docetaxel on day 8, there was a trend to higher partition ratios of gemcitabine (mean 271%) than day 1 (mean 87%), ($P = 0.0548$). All but one of our sampled patients was male, consistent with the *in vitro* data showing relatively more gemcitabine in the male erythrocytes following docetaxel incubation compared with females. The sample size of our plasma data was not sufficiently large to detect a significant decrease in plasma concentrations of gemcitabine after docetaxel infusion, as seen in our phase I study. Conversely, comparing the maximal concentrations of gemcitabine in plasma and in erythrocytes on day 1 with day 8, a shift of gemcitabine is apparent from plasma to erythrocytes after the administration of docetaxel in five of the six evaluable patients (46.3 mg in total). A similar phenomenon could explain the decrease in plasma concentrations of gemcitabine following docetaxel pretreatment in our phase I study, at least in association with male erythrocytes. The role of sex differences in the behaviour of RBCs when exposed to gemcitabine remains unclear, but docetaxel, or other substances in the formulation of this taxane, certainly influences the RBC handling of gemcitabine.

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