Phase I clinical and pharmacologic study of a 2-weekly administration of cisplatin and gemcitabine in patients with advanced non-small cell lung cancer

Jeany M. Rademaker-Lakhai[a,b], Mirjam Crul[a,b], Dick Pluim[a], Rolf W. Sparidans[c], Paul Baas[a], Jos H. Beijnen[a–c], Nico van Zandwijk[a] and Jan H. M. Schellens[a–c]

Our objective was to study the feasibility of schedule- and dose-intensive cisplatin plus gemcitabine in patients with non-small cell lung cancer (NSCLC) when given on 1 day in four 2-weekly cycles. Cisplatin was administered as a 3 h i.v. infusion followed by gemcitabine as a 30-min i.v. infusion on the same day, every 2 weeks. An interval of 1 h between the two infusions was applied. Patients received four courses without any break. An interpatient dose-escalation scheme was used. The starting dose was 87.5 mg/m^2 of cisplatin and 1350 mg/m^2 of gemcitabine. The pharmacokinetics of cisplatin and gemcitabine were determined in plasma and white blood cells. In total, 23 patients were included in the study. Median age of the patients was 56 years (range 27–76) and most patients were in good clinical condition. Thirteen patients received all planned courses. Dose-limiting toxicity was Common Toxicity Criteria grade 2 ototoxicity. The maximum tolerated dose was established at cisplatin 90 mg/m^2 in combination with gemcitabine 1500 mg/m^2. This short induction schedule is practical and convenient for the patient. We conclude that the combination of cisplatin at a dose intensity of 51 mg/m^2/week followed by gemcitabine (1500 mg/m^2) on the same day is clinically feasible in NSCLC patients when given as a 2-weekly cycle.
Anti-Cancer Drugs **16:1029–1036** © **2005 Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2005, **16:**1029–1036

Keywords: adverse events, cisplatin, dose intensity, gemcitabine, maximum tolerated dose, pharmacokinetics

[a]The Netherlands Cancer Institute, Amsterdam, The Netherlands, [b]Slotervaart Hospital, Amsterdam, The Netherlands and [c]Faculty of Pharmaceutical Sciences, Utrecht, The Netherlands.

Sponsorship: This work was supported by The Netherlands Cancer Institute/Slotervaart Hospital, Amsterdam.

Correspondence to J. M. Rademaker, Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute/Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, The Netherlands.
Tel: +31 20 512 4481; e-mail: jeanyrademaker@quicknet.nl

Received 22 June 2005 **Accepted** 5 July 2005

Introduction

Approximately one-third of patients with non-small cell lung cancer (NSCLC) have advanced disease at the time of diagnosis [1]. The treatment of advanced NSCLC has continued to evolve over recent years. Doublet chemotherapy combinations have become the current standard of care for patients with advanced NSCLC [2]. Combination chemotherapy, particularly cisplatin-based regimens, results in higher response rates than single-agent chemotherapy. The combination of cisplatin and gemcitabine is one of the most active regimens currently available in the treatment of solid tumors. In advanced NSCLC, platinum-containing regimens, in particular gemcitabine and cisplatin, achieve response rates ranging from 28 to 54%, with significant survival benefit [3–6]. Monotherapy with these agents produced response rates of around 20% [7,8]. At present, several phase II and III studies in advanced NSCLC using the gemcitabine and cisplatin regimen have been completed. One of the most commonly used schedules for this combination was cisplatin 100 mg/m^2 on day 1 or 2, and gemcitabine 1000 mg/m^2 on days 1, 8 and 15 of a 4-week cycle [9]. However, the incidence of grade 3 thrombocytopenia compromises the gemcitabine dose intensity just as ototoxicity and nephrotoxicity compromises the dose intensity of cisplatin. Nowadays, cisplatin 75 mg/m^2 and gemcitabine 1250 mg/m^2 are administered in a 3-weekly schedule since thrombocytopenia was less severe on day 15. In general, the development of this combination has largely aimed at increasing the dose and dose intensity of gemcitabine. Increasing the dose intensity of cisplatin can possibly further optimize the efficacy of this combination.

Cisplatin has documented high activity as a single agent in a variety of solid tumors, including head and neck cancer, ovarian cancer, testicular cancer, and NSCLC [6,10,11]. The principal mechanism of action of cisplatin is the formation of DNA–platinum adducts. Cisplatin binds covalently to DNA, leading to the formation of inter- and intrastrand crosslinks. The major adducts formed are the intrastrand crosslinks between two guanine nucleotides (GG), which are the most abundant and potentially damaging lesions, and between adenine

0959-4973

and guanine nucleotides (AG) [12]. Gemcitabine [(22-difluoro-2-deoxycytidine (dFdC)] is a deoxycytidine analog with activity as a single agent and in combination therapy against several chemotherapy-resistant tumors, including pancreatic cancer and NSCLC [13]. Gemcitabine is a pro-drug, which after intracellular phosphorylation exerts its cytotoxic effects through its active intracellular metabolites, gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP). DFdCTP acts by incorporation into DNA, subsequently leading to inhibition of DNA replication and repair, whereas dFdCDP inhibits *de novo* deoxyribonucleotide synthesis and thereby decreases the cellular nucleotide pool [14]. Preclinical studies have shown additive and synergistic effects of the gemcitabine and cisplatin regimen. Gemcitabine may increase the formation of DNA–platinum adducts, while cisplatin may increase the incorporation of gemcitabine into DNA [13,15–17]. It has been shown that this synergistic interaction is related to a decrease in repair of DNA–platinum adducts [18]. Since the toxicity profiles of the two drugs only partly overlap, combination therapy at full dosage could be feasible.

Crul *et al.* [19] performed a randomized phase I study in which cisplatin and gemcitabine delivery was investigated on a weekly basis and compared with a 2-weekly schedule in advanced NSCLC. Patients on the weekly schedule received six courses of gemcitabine on day 1 and cisplatin on day 2, with 2 weeks of rest after the first 3 cycles, and patients on the 2-weekly schedule received four courses without rest. Both schedules resulted in a treatment duration of 7 weeks. In the weekly schedule the dose intensity of cisplatin could not be increased beyond 42 mg/m^2/week and any further increase was limited by moderate leukocytopenia. The highest dose intensity of cisplatin, 50 mg/m^2/week, could be reached on the 2-weekly schedule in combination with 1500 mg/m^2 of gemcitabine [19]. Overall, both schedules were well tolerated. After determining the maximum tolerated dose (MTD) the following cohorts of patients were treated with a reversed schedule. In order to improve the feasibility and patient convenience of the phase I dose-escalation study described by Crul *et al.* [19] the two drugs were given on a single day in the present study. Cisplatin preceded gemcitabine and an interval of 1 h between the two infusions was applied to give cisplatin sufficient time to be taken up by tissues in order to prevent an interaction with gemcitabine. The motivation for this order of administration was to improve the patient convenience with this short induction schedule, instead of the administration of gemcitabine and cisplatin in a 3- or 4-weekly schedule on days 1 and 2, respectively. Another motivation to administer gemcitabine and cisplatin on a single day was that results obtained from Crul *et al.* [19] revealed a significant interaction at the level of the formation of platinum–DNA adducts when gemcitabine was administered prior to cisplatin. The platinum–DNA adduct levels in white blood cells (WBCs) decreased with increasing gemcitabine doses. The objectives of this study were (i) to determine the MTD of the combination of gemcitabine and cisplatin at this reversed schedule, (ii) to assess the safety of the combination, (iii) to describe the pharmacokinetics of platinum and DNA–platinum adducts in WBCs, and (iv) to describe the pharmacokinetics of gemcitabine. This study relates to that of Crul *et al.* [19] in that it is a confirmatory study, differing only in administering both drugs on day 1.

Patients and methods

Patient eligibility criteria

Patients were eligible if they had histologically and evaluably confirmed advanced NSCLC (stage IIIB or IV) and a performance status of 0–2 (WHO ECOG). Other eligibility criteria were: age > 18 years, acceptable hematologic parameters (ANC $\geq 2.0 \times 10^9$/l and platelets $\geq 150 \times 10^9$/l), and adequate hepatic and renal functions [liver: bilirubin $< 25\,\mu$M, AST/ALT $< 2 \times$ upper limit of normal (ULN) and $< 5 \times$ ULN in the case of liver metastases; renal: serum creatinine $< 125\,\mu$M or creatinine clearance > 50 ml/min). Patients were excluded if they had symptomatic brain metastases, carcinomatous leptomeningitis or ototoxicity Common Toxicity Criteria (CTC) above grade 1. The study was approved by the local ethics committee and all patients gave written informed consent.

Treatment plan

In each combination cycle, cisplatin was administered as a 3-h i.v. infusion followed by gemcitabine as a 30 min i.v. infusion on the same day, every 2 weeks. An interval of 1 h between the two infusions was applied. Patients received four courses without rest, which resulted in a treatment period of 7 weeks. Pre- and post-hydration consisted of 2000 ml NaCl 0.45%/glucose 2.5% over 14 h before treatment and 3000 ml NaCl 0.45%/glucose 2.5% over 18 h after cisplatin infusion, and was given as inpatient therapy. During the post-hydration, 20 mmol KCl, 500 mg magnesium sulfate and 290 mg calcium gluconate were added. The gemcitabine and cisplatin combination was given according to an interpatient dose-escalation scheme (Table 1). For safety reasons this schedule started at the dose level below the MTD of the 2-weekly schedule where gemcitabine was followed by cisplatin, which was 90 and 1500 mg/m^2 for cisplatin and gemcitabine, respectively [19]. The starting dose of cisplatin and gemcitabine at the current schedule was therefore 82.5 and 1350 mg/m^2, respectively. Six patients per dose level were treated. The MTD was defined as on e dose level below the dose level at which two out of six patients experienced a dose-limiting toxicity (DLT).

Table 1 Interpatient dose-escalation scheme

Level	Cisplatin (mg/m^2)	Gemcitabine (mg/m^2)
82.5/1350	82.5	1350
90/1350	90	1350
90/1500	90	1500
97.5/1500	97.5	1500

Patient evaluation

A complete medical history and physical examination were completed prior to registration. Before each course, the physical examination was repeated, and hematology and serum chemistry were checked. All toxicities were graded according to the CTC [20]. DLT was defined as: any grade 4 neutropenia lasting longer than 5 days, any grade 3/4 neutropenia accompanied by fever ($\geq 38.5^{\circ}$C) and/or infection, any grade 4 thrombocytopenia and any grade 3 non-hematologic toxicity, or grade 2 neuro- and ototoxicity (except alopecia, and untreated nausea and vomiting). At re-treatment, minimal values for neutrophils had to be $> 1.5 \times 10^9$/l and for platelets $> 75 \times 10^9$/l; otherwise, the next course was delayed by 1 week. Dose-limiting delays were defined as any cycle interrupted unplanned for more than 1 week and were regarded as equal to DLTs (resulting in inclusion of three extra patients at the same dose level). Tumor evaluations were performed by computed tomography scan after completion of the courses. Although assessment of the antitumor activity was not a primary objective of this study, patients with measurable disease were evaluated according to the RECIST criteria [21].

Pharmacokinetic studies and analysis

Gemcitabine and cisplatin pharmacokinetics were studied during the first week of treatment. For cisplatin, 5-ml blood samples were obtained at 0, 1, 2, 3, 3.25, 3.50, 4, 4.5, 5, 6.5, 8, 11.5 and 23.5 h after the start of the 3 h infusion. Unbound platinum was obtained by a validated ethanol precipitation method [22] and bioanalysis of platinum was performed by atomic absorption spectrometry [23]. At 0, 4 and 23.5 h after the start of the cisplatin infusion, 15 ml of blood was collected from which WBCs were isolated for the measurement of platinum–DNA adducts by a sensitive and validated ^{32}P post-labeling assay, enabling the selective determination of GG- and AG-intrastrand platinum adducts [24]. For gemcitabine, 2-ml blood samples were taken at 0, 10, 20, 30, 45, 60 and 75 min after the start of the 30-min infusion, and at 1.5, 4, 18, 26 and 42 h after the end of the infusion. Of each blood sample, 1 ml was immediately added to 10 µl of tetrahydrouridine (10 mg/ml), after which it was centrifuged for 5 min at 4°C and 1500 *g*. Subsequently, the plasma layer was stored at -20°C until analysis. Both gemcitabine and its metabolite 2′2′-difluoro-2′-deoxyuridine (dFdU) were measured in plasma. All gemcitabine levels were measured using a validated high-performance liquid chromatography method, analogous to the method of Freeman *et al.* [25] and Sparidans *et al.* [26].

Table 2 Patient characteristics

	n	%
Total no.	23	
Male/female	11/12	48/52
Median age [years (range)]	56 (27–73)	
WHO performance status		
0	2	9
1	21	91
2	0	
Previous therapy		
radiotherapy	3	13
systemic therapy	3	13
Tumor type		
larynx carcinoma	1	
NSCLC stage II	1	
NSCLC stage IIIA	1	
NSCLC stage IIIB	5	
NSCLC stage IV	15	

The area under the curve (AUC), total plasma clearance (Cl), volume of distribution (V_d) and the elimination half-life ($t_{1/2}$) of gemcitabine and its metabolite dFdU as well as of non-protein bound (free) platinum were determined by non-compartmental analysis. In addition, the area under the adduct curve (AUA) of platinum–DNA adducts was calculated in WBCs [27]. The pharmacokinetics parameters were reported as mean ± SD.

Results

Patient characteristics

A total of 23 patients were included in the study. Patient characteristics are presented in Table 2. Median age of the patients was 56 years (range 27–76) and most patients were in good general condition. One patient had advanced head and neck carcinoma, and 22 patients had NSCLC (one with stage II, one with stage IIIA, five with stage IIIB and 15 with stage IV). Since this was a feasibility and pharmacokinetics study it was allowed to include the patient who had advanced head and neck carcinoma, and this patient was not excluded based on the inclusion criteria. Some patients received prior radiotherapy or systemic therapy.

In total, 75 courses of gemcitabine and cisplatin were administered. The number of patients treated at each dose level and the number of courses administered are summarized in Table 3. Most patients received at least two courses of the gemcitabine and cisplatin combination. On this schedule, 13 of 23 patients included (57%) received all planned courses. Three patients went off-study after one course because of severe hearing loss.

Dose escalations and adverse events

All patients were evaluable for toxicity. Six patients were treated with the starting dose of 82.5 mg/m^2 of cisplatin

Table 3 Dose escalation

	Dose level gemcitabine/cisplatin			
	82.5/1350	90/1350	90/1500	97.5/1500
No. patients	6	6	6	5
No. cycles	21	21	23	10

and 1350 mg/m^2 of gemcitabine. The main treatment-related non-hematological adverse events at this dose level were CTC grade 2 nausea and vomiting, and CTC grade 2 fatigue, which were not considered to be dose limiting. The third patient at this dose level experienced CTC grade 2 ototoxicity, which was considered to be dose limiting. Since no other patients developed serious drug-related toxicities it was considered safe to treat the next cohort of six patients with 90 mg/m^2 of cisplatin and 1350 mg/m^2 of gemcitabine. One patient experienced CTC grade 3 vomiting and other patients experienced CTC grade 2 fatigue. These adverse events were not considered dose limiting. One patient experienced CTC grade 2 ototoxicity, which was dose limiting. This DLT occurred in only one patient at this dose level. Therefore, the dose was escalated to 90 mg/m^2 of cisplatin and 1500 mg/m^2 of gemcitabine. Initially, three patients were included at this dose level. One patient experienced CTC grade 3 anemia, but the other patients did not develop serious drug-related toxicities, and therefore the dose was escalated to 97.5 mg/m^2 of cisplatin and 1500 mg/m^2 of gemcitabine. Five patients were included at this dose level. One patient suffered from CTC grade 3 fever and one patient experienced CTC grade 3 dehydration. Three patients suffered from CTC grade 2 ototoxicity and this was considered to be dose limiting. Ototoxicity, generally manifesting as hearing loss of the higher tones, was not reversible after cessation of treatment. Moreover, three out of five patients experienced a DLT at this dose level (97.5 mg/m^2 of cisplatin and 1500 mg/m^2 of gemcitabine), and the advised dose for further testing was therefore 90 mg/m^2 of cisplatin and 1500 mg/m^2 of gemcitabine on this schedule. The dose intensity of cisplatin was 51 mg/m^2/week. Three other patients were treated at this lower dose level. One patient experienced CTC grade 3 nausea and vomiting.

The main treatment-related non-hematological adverse events per patient as a function of dose are presented in Table 4. The main treatment-related hematological adverse events per patient as a function of dose are presented in Table 5. Overall, hematological toxicity was negligible, rarely exceeding CTC grade 2.

Other clinical abnormalities per patient as a function of dose are presented in Table 6. The CTC grade never exceeded grade 2.

Responses

All patients were evaluable for response. One patient (4%) with NSCLC stage IV had a complete response after the four 2-weekly cycles. Five patients achieved a partial response (22%), six patients had stable disease (26%) and 11 patients (48%) showed disease progression. One patient with stable disease had surgery after the induction treatment was completed. Two patients received two courses and three patients received one course with gemcitabine and cisplatin. Some of these patients were responding to the combination. However, these patients experienced ototoxicity, and therefore they were further treated with gemcitabine and carboplatin. Table 7 outlines response rates for subsets of patients.

Gemcitabine pharmacokinetics

Pharmacokinetic sampling was performed in 18 patients. Gemcitabine was detectable (>0.2 μg/ml) until 1.4 h after the end of infusion. The pharmacokinetic parameters C_{max} and AUC did not increase significantly with the dose. However, only two gemcitabine dose levels were applied, differing by only 150 mg/m^2 (1350 versus 1500 mg/m^2). Furthermore, gemcitabine had a mean terminal half-life of 12 ± 2.4 min, a mean Cl of 236 ± 58 l/h and a mean V_d of 40 ± 15 l. The pharmacokinetics parameters per dose level are depicted in Table 8.

For the analysis of the metabolite dFdU, samples were obtained until 42 h after the end of infusion. Also for this metabolite, the C_{max} and AUC did not increase significantly with the dose. Peak plasma levels of dFdU were reached approximately 30 min after the end of infusion, and dFdU had a mean terminal half-life of 15.3 ± 9.7 h, a mean Cl of 9 ± 3.2 l/h and a mean V_d of 150 ± 78 l. The pharmacokinetics parameters per dose level are depicted in Table 8.

Cisplatin pharmacokinetics

Pharmacokinetic sampling was performed in 23 patients. For ultrafilterable platinum, the following pharmacokinetics parameters were calculated: mean terminal half-life of 4.3 ± 4.8 h, mean Cl of 69.5 ± 36.5 l/h and mean V_d of 446 ± 435 l. Both C_{max} and AUC showed no positive correlation with the dose. However, only three dose levels were tested differing maximally by 7.5 mg/m^2 between the dose levels. The pharmacokinetics parameters per dose level are depicted in Table 8.

The intracellular platinum–DNA intrastrand adducts Pt–GG and Pt–AG were measured in WBCs in 17 patients. Adducts were determined in three patients receiving cisplatin at a dose of 82.5 mg/m^2, 11 patients receiving 90 mg/m^2 and in three patients at a cisplatin dose of 97.5 mg/m^2. Peak levels of up to 2 fmol/μg DNA for Pt–GG and 0.5 fmol/μg DNA for Pt–AG were reached at 90

Table 4 Occurrence of possibly, probably or definitely drug-related non-hematological toxicities at all dose levels [*n* (%)]

Toxicity	Total dose level gemcitabine/cisplatin	Grade				No. patients
		1	2	3	4	
Gastrointestinal toxicity						
nausea	82.5/1350	2 (33)	2 (33)	0	0	4 (66)
	90/1350	3 (50)	3 (50)	0	0	6 (100)
	90/1500	4 (66)	1 (17)	1 (17)	0	6 (100)
	97.5/1500	2 (40)	1 (20)	0	0	3 (60)
vomiting	82.5/1350	0	2 (33)	0	0	2 (33)
	90/1350	2 (33)	2 (33)	1 (17)	0	5 (83)
	90/1500	0	1 (17)	1 (17)	0	2 (33)
	97.5/1500	1 (20)	1 (20)	0	0	2 (40)
diarrhea	82.5/1350	1 (17)	0	0	0	1 (17)
	90/1350	1 (17)	0	0	0	1 (17)
	90/1500	0	0	0	0	0
	97.5/1500	2 (40)	0	0	0	2 (40)
constipation	82.5/1350	0	0	0	0	0
	90/1350	0	1 (17)	0	0	1 (17)
	90/1500	0	1 (17)	0	0	1 (17)
	97.5/1500	0	0	0	0	0
Ototoxicity						
tinnitus	82.5/1350	0	1 (17)	0	0	1 (17)
	90/1350	0	1 (17)	0	0	1 (17)
	90/1500	1 (17)	0	0	0	1 (17)
	97.5/1500	0	3 (60)	0	0	3 (60)
Main other toxicities						
fatigue	82.5/1350	0	3 (50)	0	0	3 (50)
	90/1350	0	4 (66)	0	0	4 (66)
	90/1500	0	0	0	0	0
	97.5/1500	0	0	0	0	0
dehydration	82.5/1350	0	0	0	0	0
	90/1350	0	0	0	0	0
	90/1500	0	1 (17)	0	0	1 (17)
	97.5/1500	0	0	1 (20)	0	1 (20)
fever	82.5/1350	1 (17)	0	0	0	1 (17)
	90/1350	1 (17)	0	0	0	1 (17)
	90/1500	0	0	0	0	0
	97.5/1500	0	0	1 (20)	0	1 (20)
reflux	82.5/1350	1 (17)	0	0	0	1 (17)
	90/1350	0	0	0	0	0
	90/1500	1 (17)	0	0	0	1 (17)
	97.5/1500	0	0	0	0	0

Table 5 Occurrence of possibly, probably or definitely drug-related hematological toxicities at all dose levels per patient [*n* (%)]

Item	Total dose level gemcitabine/cisplatin	CTC grade [n (%)]				No. of patients
		1	2	3	4	
Hemoglobin (mM)	82.5/1350	0	0	0	0	0
	90/1350	1 (17)	0	0	0	1 (17)
	90/1500	0	0	1 (17)	0	1 (17)
	97.5/1500	0	0	0	0	0
Platelets (10^9/l)	82.5/1350	0	0	0	0	0
	90/1350	1 (17)	0	0	0	1 (17)
	90/1500	0	0	0	0	0
	97.5/1500	0	0	0	0	0
Leucocytes (10^9/l)	82.5/1350	0	0	0	0	0
	90/1350	0	0	0	0	0
	90/1500	0	0	0	0	0
	97.5/1500	0	0	0	0	0
Neutrophils (10^9/l)	82.5/1350	0	0	0	0	0
	90/1350	2 (33)	0	0	0	2 (33)
	90/1500	0	1 (17)	0	0	1 (17)
	97.5/1500	0	0	0	0	0

and 97.5 mg/m^2 cisplatin, respectively. AUAs for both types of adducts correlated to cisplatin dose. Median C_{max} of Pt–GG adducts was 1.4 fmol/μg DNA (range 0.7–2.0). Median C_{max} of Pt–AG adducts was 0.3 fmol/μg DNA (range 0.1–0.5). Crul *et al.* [19] showed a median C_{max} of Pt–GG adducts of 1.1 fmol/μg DNA (range 0.4–3.3). Median C_{max} of Pt–AG adducts was 0.2 fmol/μg DNA (range 0.1–0.4).

Table 6 Biochemistry at all dose levels, worst per patient [*n* (%)]

Item	Total dose level gemcitabine/cisplatin	CTC grade [n (%)]				No. of patients
		1	2	3	4	
Creatinine (μM)	82.5/1350	0	0	0	0	0
	90/1350	0	0	0	0	0
	90/1500	0	1 (17)	0	0	1 (17)
	97.5/1500	0	0	0	0	0
AST (U/l)	82.5/1350	0	0	0	0	0
	90/1350	0	0	0	0	0
	90/1500	1 (17)	0	0	0	1 (17)
	97.5/1500	0	0	0	0	0
ALT (U/l)	82.5/1350	0	0	0	0	0
	90/1350	0	1 (17)	0	0	1 (17)
	90/1500	1 (17)	0	0	0	1 (17)
	97.5/1500	0	0	0	0	0

Table 7 Responses, number of patients per response group and percentage [*n* (%)]

	Complete response	Partial response	Stable disease	Progressive disease
Total ($n=23$)	1 (4)	5 (22)	6 (26)	11 (48)
Tumor type				
larynx ($n=1$)	0	0	0	1 (100)
NSCLC stage II ($n=1$)	0	0	0	1 (100)
NSCLC stage IIIA ($n=1$)	0	0	0	1 (100)
NSCLC stage IIIB ($n=5$)	0	1 (20)	1 (20)	3 (60)
NSCLC stage IV ($n=15$)	1 (7)	4 (27)	5 (33)	5 (33)
PS 0 ($n=2$)	0	0	1 (50)	1 (50)
PS 1 ($n=21$)	1 (4)	5 (24)	5 (24)	10 (48)

Table 8 Summary of non-compartmental pharmacokinetic parameters

Dose level (mg/m^2)	C_{max} (μg/ml)	$t_{1/2}$ (h)	AUC (h · μg/ml)	Cl (l/h)	V_d (l)
Gemcitabine					
1350	20.0 ± 2.8	0.2 ± 0.03	9.9 ± 3.7	239.6 ± 54.3	39.6 ± 18.4
1500	20.3 ± 4.8	0.2 ± 0.05	11.9 ± 2.2	233.8 ± 64.4	40.1 ± 12.5
dFdU					
1350	37.5 ± 4.5	15.6 ± 3.5	264.4 ± 67.8	8.6 ± 2.4	169.9 ± 57.1
1500	45.0 ± 8.4	15.1 ± 13	264.2 ± 117.2	9.9 ± 3.9	133.3 ± 91.8
Ultrafilterable platinum					
82.5	0.5 ± 0.1	2.8 ± 3.9	2.2 ± 1.5	83.0 ± 38.9	226.9 ± 246.7
90.0	0.4 ± 0.1	5.0 ± 5.2	2.3 ± 0.9	69.4 ± 36.9	632.6 ± 514.0
97.5	0.6 ± 0.1	4.8 ± 5.8	3.0 ± 1.4	53.5 ± 33.2	264.4 ± 151.9

Discussion

A phase I dose-escalation and pharmacologic study of the combination of gemcitabine and cisplatin in patients with advanced NSCLC was performed. Both compounds are active in NSCLC and this combination is of great interest because of its synergy *in vitro*. Furthermore, the toxicities of the two drugs only partially overlap. High response rates of the gemcitabine and cisplatin combination have been reported in NSCLC stage III [27]. However, there is still no consensus about the optimal schedule. Initially, most studies administered cisplatin once in 28-day cycles, at a dose of 75–100 mg/m^2, with gemcitabine weekly $\times 3$, at doses of 1000–1500 mg/m^2. Next, the 21-day schedule was developed [28], which doses gemcitabine at 1250 mg/m^2 on day 1 and 8, and cisplatin at 70–100 mg/m^2 at day 2. However, it was previously shown that giving lower doses more frequently with the same dose intensity was feasible [19,29]. Furthermore, combining cisplatin and gemcitabine in each administration might allow full exploitation of the synergism ascribed to these agents and shorter hospital admission.

The cisplatin and gemcitabine combination at this order of administration was in general well tolerated. Main non-hematologic toxicities, possibly or probably related to gemcitabine and cisplatin, were ototoxicity, nausea and vomiting, fatigue, diarrhea, constipation, tinnitus, fever, and reflux (Table 4). Overall, hematologic toxicity was negligible, rarely exceeding CTC grade 2 (Table 5). The MTD was established at 90 mg/m^2 of cisplatin and 1500 mg/m^2 of gemcitabine on this schedule. The DLT was CTC grade 2 ototoxicity, generally manifesting as hearing loss of the higher tones. When gemcitabine was administered followed by cisplatin administration on the next day, the MTD was also established at 90 mg/m^2 cisplatin and 1500 mg/m^2 gemcitabine [19]. Moreover, from our studies it appears to make no difference whether gemcitabine and cisplatin are administered on

a single day compared with the administration of gemcitabine followed by cisplatin on the next day. However, our study reveals that the highest dose intensity of cisplatin, 51 mg/m^2/week, could be reached on this 2-weekly schedule. Only six patients were treated at the 90/1500 mg/m^2 dose level and this is rather too few to feel comfortable that a regimen is appropriate to take into general use.

The interaction between gemcitabine and cisplatin has been studied extensively in preclinical experiments. Synergistic cytotoxicity was observed in a number of cell lines, including ovarian, lung, head and neck, and colon carcinoma [16,17,30]. However, this synergism was influenced by both cell type and schedule, since additivity and even antagonistic cytotoxicity have been described as well [16,17,30,31]. An effect of cisplatin on gemcitabine pharmacokinetics could not be detected [16]. Conversely, gemcitabine can influence cisplatin pharmacokinetics and pharmacodynamics. Pre-treatment with gemcitabine increased cellular platinum accumulation and platinum–DNA adduct formation. Subsequent mice studies have demonstrated the opposite effect. A decrease of intrastrand platinum–DNA adducts of almost 3-fold occurred when gemcitabine was given as the first drug [32]. In contrast, simultaneous administration of both drugs caused an increase in platinum–DNA adduct levels [32]. Interestingly, this possible negative effect of gemcitabine on platinum–DNA adduct formation was confirmed in humans. Crul *et al.* [19] also confirmed the possibility of this negative effect. There was a significant reduction in both types of platinum–DNA adducts with increasing doses of gemcitabine in WBCs and if this agent was administered first. This effect was highly significant. However, this data should be interpreted with caution, because WBCs are a surrogate tissue and might not reflect the situation in tumors, as they are non-proliferative and gemcitabine requires DNA synthesis to be active. In the present study we did not perform an interaction study. However, it was observed that the median C_{max} of Pt–GG adducts increased significantly from 1.1 [19] to 1.4 fmol/μg DNA when cisplatin preceded gemcitabine on the same day.

In conclusion, the recommended dose for cisplatin and gemcitabine when both given on 1 day every 2 weeks is 90 mg/m^2 of cisplatin (given as a 3-h infusion) and 1500 mg/m^2 of gemcitabine (given as a 30-min infusion), and was very well tolerated. The gemcitabine infusion was administered 1 h after the end of the cisplatin infusion. The dose intensity of cisplatin was 51 mg/m^2/week. CTC grade 2 ototoxicity was considered the DLT. Other adverse events were negligible at the MTD. It is clear that interactions between gemcitabine and cisplatin can occur on the DNA level. However, more insight must be gained to explain the synergism, additive or antagonistic cytotoxicity between gemcitabine and cisplatin. This short induction schedule is practical and convenient for the patient. At the advised dose it is of interest to explore the activity of gemcitabine and cisplatin as (neo-)adjuvant therapy in NSCLC patients.

References

1 Bulzebruck H, Bopp R, Drings P, *et al.* New aspects in the staging of lung cancer: prospective validation of the International Union Against Cancer TNM Classification. *Cancer* 1992; **70**:1102–1110.

2 Belani CP. Chemotherapy regimens in advanced non-small cell lung cancer: recent randomized trials. *Clin Lung Cancer* 2000; **2**:S7–S10.

3 Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in NSCLC: a meta-analysis using updated data on individual patients from 52 randomized clinical trials. *Br Med J* 1995; **311**:899–909.

4 Schiller JH, Harrington D, Sandler A. Randomized phase III trial of four chemotherapy regimens in advanced non-small cell lung cancer. *Proc Am Soc Clin Oncol* 2000; **19**:1a (abstr 2).

5 Crino L, Scagliotti V, Ricci S, De Marinis F, Rinaldi M, Gridelli C, *et al.* Gemcitabine and cisplatin versus mitomycin, ifosfamide, and cisplatin in advanced non-small cell lung cancer: a randomized phase III study of the Italian lung cancer project. *J Clin Oncol* 1999; **17**:3522–3530.

6 Sandler AB, Nemunaitis J, Denham C, von Pawel J, Cormier Y, Gatzemeier U, *et al.* 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* 2000; **18**:122–130.

7 Sweeney CJ, Sandler AB. Chemotherapy in non-small cell lung cancer. *Invest New Drugs* 2000; **18**:157–186.

8 Manegold C, Zatloukal P, Krejcy K, Blatter J. Gemcitabine in non-small cell lung cancer (NSCLC). *Invest New Drugs* 2000; **18**:29–42.

9 Soto Parra H, Cavina R, Latteri F, *et al.* Three-week versus four-week schedule of cisplatin and gemcitabine: results of a randomized phase II study. *Ann Oncol* 2002; **13**:1080–1086.

10 Neijt JP, ten Bokkel Huinink WW, Van der Burg ME, van Oosterom AT, Willemse PH, Vermorken JB, *et al.* Long-term survival in ovarian cancer. Mature data from The Netherlands Joint Study Group for Ovarian Cancer. *Eur J Cancer* 1991; **27**:1367–1372.

11 Al-Sarraf M. Head and neck cancer: chemotherapy concepts. *Semin Oncol* 1988; **15**:70–85.

12 Fichtinger-Schepman AM, Van der Veer JL, den Hartog JH, Lohman PH, Reedijk J. Adducts of the antitumor drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation. *Biochemistry* 1985; **24**:707–713.

13 Van Moorsel CJA, Peters GJ, Pinedo HM. Gemcitabine: fututre prospects of single-agent and combination studies. *Oncologist* 1997; **2**:127–134.

14 Huang P, Chubb S, Hertel LW, Grindey GB, Plunkett W. Action of 2′2′-difluorodeoxycytidine on DNA synthesis. *Cancer Res* 1991; **51**:6110–6117.

15 Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman AM, Ackland SP. Basis for effective combination cancer chemotherapy with antimetabolites. *Pharmacol Ther* 2000; **87**:227–253.

16 Bergman AM, Ruiz van Haperen VWT, Veerman G, Kuiper CM, Peters GJ. Synergistic interaction between gemcitabine and cisplatin *in vitro*. *Clin Cancer Res* 1996; **2**:521–530.

17 Van Moorsel CJA, Pinedo HM, Veerman G, Bergman AM, Kuiper CM, Vermorken JB, *et al.* Mechanism of synergism between cisplatin and gemcitabine in ovarian and non-small cell lung cancer cell lines. *Br J Cancer* 1999; **80**:981–990.

18 Yang LY, Jiang H, Shen Y, Plunkett W. Expression of ERCC1 antisense RNA abrogates gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells defective in mismatch repair but proficient in nucleotide excision repair. *Clin Cancer Res* 2000; **6**:773–781.

19 Crul M, Schoemaker NE, Pluim D, Maliepaard M, Underberg RW, Schot M, *et al.* Randomized phase I clinical and pharmacologic study of weekly versus two-weekly dose-intensive cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2003; **9**:3526–3533.

20 National Cancer Institute. *Guidelines for reporting of adverse drug reactions*. Bethesda, Maryland: Division of Cancer Treatment, National Cancer Institute; 1988.

21 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, *et al.* New guidelines to evaluate the response to treatment of cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**:205–216.

22 Ma J, Stoter G, Verweij J, Schellens JHM. Comparison of ethanol plasma-protein precipitation with plasma ultrafiltration and trichloroacetic acid

protein precipitation for the measurement of unbound platinum concentrations. *Cancer Chemother Pharmacol* 1996; **38**:391–394.
23 Van Warmerdam LJC, van Tellingen O, Maes RAA, Beijnen JH. Validated method for the determination of carboplatin in biological fluids by Zeeman atomic absorption spectrometry. *Fres J Anal Chem* 1995; **351**:1820–1824.
24 Pluim D, Maliepaard M, van Waardenburg RC, Beijnen JH, Schellens JHM. ^{32}P-postlabelling assay for the quantification of the major platinum–DNA adducts. *Anal Biochem* 1999; **275**:30–38.
25 Freeman KB, Anliker S, Hamilton M, Osborne D, Dhahir PH, Nelson R, *et al.* 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* 1995; **665**:171–181.
26 Sparidans RW, Crul M, Schellens JHM, Beijnen JH. Isocratic ion-exchange chromatographic assay for the nucleotide gemcitabine triphosphate in human white blood cells. *J Chromatogr B Anal Technol Biomed Life Sci* 2002; **780**:423–430.
27 Van Zandwijk N, Smit EF, Kramer GW, Schramel F, Gans S, Festern J, *et al.* Gemcitabine and cisplatin as induction regimen for patients with biopsy-proven stage IIIA N2 non-small cell lung cancer: a phase II study of the European Organization for Research and Treatment Cancer Lung Cancer Cooperative Group (EORTC 08955). *J Clin Oncol* 2000; **18**:2658–2664.
28 Cardenal F, Lopez-Cabrerizo MP, Anton A, Alberola V, Massuti B, Carrato A, *et al.* Randomized phase III study of gemcitabine–cisplatin versus etoposide–cisplatin in the treatment of locally advanced or metastatic non-small cell lung cancer. *J Clin Oncol* 1999; **17**:12–18.
29 Schellens JHM, Ma J, Planting AS, van der Burg ME, van Meerten E, de Boer-Dennert M, *et al.* Relationship between the exposure to cisplatin, DNA–adduct formation in leucocytes and tumor response in patients with solid tumors. *Br J Cancer* 1996; **73**:1569–1575.
30 Theodossiou C, Cook JA, Fisher J, Teague D, Liebmann JE, Russo A, Mitchell JB, *et al.* Interaction of gemcitabine and paclitaxel and cisplatin in human tumor cell lines. *Int J Oncol* 1998; **12**:825–832.
31 Voigt W, Bulankin A, Muller T, Schoeber C, Grothey A, Hoang-Vu C, *et al.* Schedule-dependent antagonism of gemcitabine and cisplatin in human anaplastic thyroid cancer cells. *Clin Cancer Res* 2000; **6**:2087–2093.
32 Van Moorsel CJA, Pinedo HM, Smid K, Comijn EM, Voorn DA, Veerman G, *et al.* Schedule-dependent pharmacodynamic effects of gemcitabine and cisplatin in mice bearing Lewis lung murine non-small cell lung tumors. *Eur J Cancer* 2000; **36**:2420–2429.