Featured Article

Randomized Phase I Clinical and Pharmacologic Study of Weekly *versus* Twice-Weekly Dose-intensive Cisplatin and Gemcitabine in Patients with Advanced Non-Small Cell Lung Cancer[1]

Mirjam Crul,[2] Nadja E. Schoemaker, Dick Pluim, Marc Maliepaard, René W. M. Underberg, Margaret Schot, Rolf W. Sparidans, Paul Baas, Jos H. Beijnen, Nico van Zandwijk, and Jan H. M. Schellens

Netherlands Cancer Institute, Departments of Medical Oncology [M. C., N. E. S., D. P., M. M., R. W. M. U., M. S., J. H. M. S.] and Thoracic Oncology, Amsterdam [P. B., N. v. Z.]; Slotervaart Hospital, Department of Pharmacy, 1066 EC Amsterdam [M. C., N. E. S., J. H. B.]; Faculty of Pharmacy, Department of Biomedical Analysis, Utrecht University, Utrecht [R. W. S.]; and Division of Drug Toxicology, Faculty of Pharmacy, Utrecht University, Utrecht [J. H. B., J. H. M. S.], the Netherlands

Abstract

Purpose: **To establish the maximum dose intensity of cisplatin plus gemcitabine on a weekly or two-weekly schedule in patients with advanced non-small cell lung cancer (NSCLC).**

Methods: **Patients with NSCLC stage IIIB or IV were randomized to receive weekly or two-weekly courses of gemcitabine on day 1 and cisplatin on day 2. An interpatient dose escalation scheme was used, and pharmacokinetics were determined for both agents in plasma and WBCs.**

Results: **Seventy-three patients were included, 32 on the weekly schedule and 41 on the two-weekly schedule. Fifty patients received all planned courses. Dose-limiting toxicities were leukocytopenia, neutropenia, and trombocytopenia on the weekly schedule and ototoxicity on the two-weekly schedule. Most common nonhematological toxicities consisted of nausea, vomiting, and fatigue. The highest dose intensity of cisplatin could be achieved on the two-weekly schedule, and therefore, further development of the weekly schedule was abandoned. The maximum tolerated dose was established at 1500 mg/m^2 gemcitabine in combination with cisplatin 90 mg/m^2. More than half (53%) of patients achieved an objective response on the two-weekly schedule, *versus* 23% in the weekly treatment arm. The pharmacokinetic studies revealed a significant interaction: gemcitabine reduced both GG and AG platinum-DNA intrastrand adducts in WBCs.**

Conclusion: **The combination of gemcitabine (1500 mg/m^2) with cisplatin at a dose intensity of 50 mg/m^2/week is feasible on a two-weekly administration scheme in NSCLC patients.**

Introduction

Cisplatin and gemcitabine are cytotoxic drugs with proven activity in NSCLC.[3] Monotherapy with these agents produced response rates ~20% (1, 2), which led to the initiation of studies evaluating the combination. At present, several Phase II and III studies in advanced NSCLC combining gemcitabine and cisplatin have been completed. Initially, 28-day schedules were tested administering gemcitabine at 1000–1500 mg/m^2 on days 1, 8, and 15 every 4 weeks and cisplatin at 100 mg/m^2 on days 1, 2, or 15 or at 30 mg/m^2 on days 1, 8, and 15 (3). Later, the 21-day regimen became popular with gemcitabine at 1000–1250 mg/m^2 on days 1 and 8 and cisplatin at 70–100 mg/m^2 on day 2 (4–6). Attempts were also made to ameliorate cisplatin toxicity by splitting the dose, resulting in schedules like gemcitabine weekly ×3 at 800 mg/m^2 and cisplatin weekly ×2 at 50 mg/m^2 (7). Response rates in these studies ranged from 25 to 65% in stage IIIB-IV patients, and substantial symptom relief occurred in more than half of the patients treated (8, 9). 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. This approach has been the subject of our study.

The mechanisms of action of cisplatin and gemcitabine are substantially different; cisplatin binds covalently to DNA, leading to the formation of inter and intrastrand cross-links. The major adducts formed are the intrastrand cross-links between two guanine nucleotides (GG) and adenine and guanine nucleotides (AG). Minor but potentially damaging adducts are the interstrand GG adducts (10). In contrast, gemcitabine is incorporated into the DNA (after phosphorylation on cell entry), which blocks DNA polymerization at the next nucleotide (11). In *in vitro* and animal studies, synergistic cytotoxicity of these agents has been demonstrated (12–14). The mechanism underlying this synergism probably involves a positive effect of gemcitabine on cisplatin-DNA adduct formation, either by facilitation of the binding of cisplatin to DNA or by decreasing

Received 12/18/02; revised 4/4/03; accepted 4/12/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by an educational grant from Eli Lilly Co. (Indianapolis, IN).
[2] To whom requests for reprints should be addressed, at Department of Pharmacy and Pharmacology, Slotervaart Hospital/Antoni van Leeuwenhoek Hospital, Louwesweg 6, 1066 EC Amsterdam, the Netherlands. Phone: 31 20 5124481; Fax: 31 20 5124753; E-mail: apmcr@slz.nl.

[3] The abbreviations used are: NSCLC, non-small cell lung cancer; AUA, area under the adduct curve; MTD, maximum tolerated dose; CTC, common toxicity criteria; AUC, area under the curve; Pt, platinum; PS, performance status; dFdU, 2′,2′-difluoro-2′-deoxy-uridine.

DNA repair (12). On the other hand, a stimulatory effect of cisplatin on gemcitabine incorporation into DNA has been observed as well (15). Because the toxicity profiles of cisplatin and gemcitabine are only partly overlapping, combination therapy at full dosage could be feasible.

Here, we present the results of a Phase I study in which cisplatin delivery is investigated on a weekly basis and compared with a two-weekly schedule. Increasing the dose intensity of cisplatin is expected to result in a higher response rate, as has been demonstrated previously for ovarian cancer (16). This cannot be achieved by administering higher doses of cisplatin in the standard schedule, because 100 mg/m^2 is around the MTD of a single cisplatin administration. The other option is to give lower doses more frequently, as we have extensively explored previously in other combination regimens (17). In addition, combining cisplatin to gemcitabine in each administration would take better advantage of the synergism ascribed to these agents, as compared with the standard schedule, where gemcitabine is given as a single agent in half of its administrations. Positive results with weekly cisplatin have been obtained in several tumor types (18, 19) but has been reported as overly toxic as well (20). Hence, it is presently unknown whether weekly cisplatin in combination with gemcitabine is the optimal schedule to achieve the highest dose intensity for both drugs. This has prompted us to design a randomized Phase I study comparing weekly cisplatin plus gemcitabine (weeks 1, 2, and 3 and 5, 6, and 7) with once every 2 weeks (weeks 1, 3, 5, and 7) in patients with advanced NSCLC. For comparability, the dose intensity of cisplatin in each of these schedules was equal. Our aim was to establish the schedule that would result in the highest dose intensity of this combination and manageable toxicity. Secondary objectives were: (*a*) to determine the MTD of the combination of gemcitabine and cisplatin in the two different schedules; (*b*) to assess the safety of the combination; (*c*) to describe the pharmacokinetics of Pt and Pt-DNA-adducts in WBC; and (*d*) to describe the pharmacokinetics of gemcitabine.

Materials and Methods

Patient Eligibility Criteria. Patients were eligible if they had histologically and evaluable confirmed advanced NSCLC (stage IIIB or IV) and a performance status of 0–2 (WHO Eastern Cooperative Oncology Group). Other eligibility criteria were: age >18 years, acceptable hematological parameters (absolute neutrophil count $\geq 2 \times 10^9$/liter, platelets $\geq 150 \times 10^9$/liter) and adequate hepatic and renal function (liver: bilirubin <25 μM, asparatate aminotransferase/alanine aminotransferase <2 × upper level of normal and <5 × upper level of normal in case of liver metastases; renal: serum creatinine <125 μM or creatinine clearance >50 ml/min). Patients were excluded if they had previous chemotherapy for NSCLC, symptomatic brain metastases, carcinomatous leptomeningitis, or ototoxicity of CTC grade >1. The study was approved by the local ethics committee, and all patients gave written informed consent.

Treatment Plan. The gemcitabine-cisplatin combination was given according to an interpatient dose escalation scheme (Table 1). For safety reasons, only the dose of one of each agents was increased in each escalation step. Patients were randomly assigned to a weekly or a two-weekly schedule, which was considered an induction regimen. The dose intensity of cisplatin at the starting level corresponded with the dose intensity of the 28-day schedule of this combination, 100 mg/m^2 q 4 weeks. At each dose level, the overall dose intensity of both agents was equal in the weekly and two-weekly schedule. Three patients were enrolled per schedule per level. Patients on the weekly schedule received six courses of gemcitabine on day 1 and cisplatin on day 2, with 1 week of rest after the first three cycles, and patients on the two-weekly schedule received four courses without rest. Gemcitabine was given as a 30-min and cisplatin as a 3-h infusion. If one of the three enrolled patients developed dose-limiting toxicity, 3 more patients were recruited at that dose level. The MTD was defined one dose level below the dose level at which two of 6 patients experienced a dose-limiting toxicity. Stage IV patients who had a favorable clinical response on the induction regimen received gemcitabine monotherapy during a consolidation phase, in which patients with stage IIIB continued with radiotherapy.

Table 1 Dose levels and number of patients included per level

	Level	Cisplatin (mg/m^2)	Gemcitabine (mg/m^2)	*n*
Weekly schedule	1	25	600	3
	2	30	700	3
	3	35	800	3
	4	40	900	3
	5	45	900	4
	6	50	900	4
	7	55	900	3
	8	60	900	4
	9	60	1000	5
Two-weekly schedule	1	37.5	900	3
	2	45	1050	3
	3	52.5	1200	3
	4	60	1350	3
	5	67.5	1350	3
	6	75	1350	3
	7	82.5	1350	3
	8	90	1350	3
	9	90	1500	6
	10	97.5	1500	6
	11	105	1500	6

Patient Evaluation. A complete medical history and physical examination were completed before registration. Before each course, the physical examination was repeated, and hematology and serum chemistry were checked. All toxicities were graded according to the Common Toxicity Criteria (21). Dose-limiting toxicity was defined as: any grade 4 neutropenia lasting >5 days, any grade 3–4 neutropenia accompanied by fever (≥38.5°C) and/or infection, any grade 4 thrombocytopenia and any grade 3 nonhematological toxicity, or grade 2 neuro and ototoxicity (except alopecia and untreated nausea and vomiting). At retreatment, minimal values for neutrophils had to be $>1.5 \times 10^9$/liter and for platelets $>75 \times 10^9$/liter. Otherwise, the next course was delayed by 1 week. Dose-limiting delays were defined as any cycle interrupted unplanned for >1 week and were regarded as equal to dose-limiting toxicities (resulting in inclusion of 3 extra patients at the same dose level). Tumor evaluations were performed by computed tomography scan after completion of the courses.

Table 2 Patient characteristics

	No. of patients		
	Weekly schedule (with randomization)	Two-weekly schedule (with randomization)	Two-weekly schedule[a] (without randomization)
Total	32	26	15
Gender			
Male	17	13	6
Female	15	13	9
Age			
Median	52	51	55
Range	33–75	41–76	43–68
PS			
0	12	12	5
1	14	10	10
2	6	4	0
Tumor Stage			
IIIB	13	11	1
IV	19	15	14
Previous therapy			
Radiotherapy	6	4	2
Chemotherapy	0	0	0
Surgery	4	2	2
No previous therapy	23	21	11

[a] The weekly schedule was abandoned after dose level nine, and subsequent patients were included at the two-weekly schedule without randomization.

Pharmacokinetic Studies. Gemcitabine pharmacokinetics were studied during the 1st week and cisplatin pharmacokinetics during the 1st and 3rd week of treatment. Twenty-four h urine was collected on days 1 and 2 of the first week and day 2 of the 3rd week of treatment to measure gemcitabine and Pt renal excretion. For gemcitabine, 2-ml blood samples were taken at 0–10-20–30-45–60-75 min and 2.5–5-7.5–23.5–30-47.5 h after the start of the 30-min infusion on day 1. Of each blood sample, 1 ml was 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 dFdU were measured in plasma. Additionally, 15-ml blood samples were taken at 0–1.5–4-24 h after start of infusion for the determination of the triphosphate metabolite of gemcitabine (dFdCTP) in WBCs. Isolation of WBCs was performed using a Ficoll density gradient (Pharmacia, Stockholm, Sweden), after which nucleotides were extracted as described previously (22). All gemcitabine levels were measured using a validated high-performance liquid chromatography method, analogous to the method of Freeman *et al.* (23). For cisplatin, 5-ml blood samples were obtained at 0–1-2–3-3.25–3.50–3.75–4-4.5–5-6–6.5–22 h after the start of the 3-h infusion. Unbound Pt was obtained by a validated ethanol precipitation method (24), and bio-analysis of Pt was performed by atomic absorption spectrometry (25). At 0, 4, and 22 h after start of the infusion, 15 ml of blood were collected from which WBCs were isolated for the measurement of Pt DNA adducts by a sensitive and validated ^{32}P postlabelling assay, enabling the selective determination of GG- and AG-intrastrand Pt adducts (26).

The AUC, total plasma clearance (Cl), volume of distribution (V), and the elimination half-life ($t_{1/2}$) of gemcitabine and its metabolite dFdU as well as of nonprotein bound (free) Pt were determined by noncompartmental analysis. In addition, the AUC of dFdCTP and the AUA of Pt-DNA adducts were calculated in WBC (17). From the urine samples, the total amount of excreted gemcitabine, dFdU, and Pt was determined per 24-h time interval. To investigate a possible influence of gemcitabine on cisplatin pharmacokinetics, the dose of gemcitabine was plotted against pharmacokinetic parameters of free Pt and Pt-DNA adducts, corrected for the dose of cisplatin by division. A linear correlation between cisplatin dose and pharmacokinetics of both the unbound fraction and the adducts in WBCs has been established previously (17), enabling us to use this method for dose correction.

Statistical Analysis. To quantitate the relationship between the different schedules, toxicity, and response of patients, logistic regression was used, correcting for the variables gender, age, tumor stage, and performance status. The interaction effects between gemcitabine and cisplatin pharmacokinetics were studied by linear regression analysis.

Results

Patients. Seventy-three patients (25 with stage IIIB and 48 with stage IV) were included in the study (Table 2), first at nine different dose levels by randomization on each schedule ($n = 58$). Fifteen more patients were included at higher dose levels in the two-weekly schedule (6 at levels 10 and 11 each and 3 additional patients at the MTD, level 9; Table 1). Patient characteristics matched well in both randomization groups. On the weekly schedule, 23 of 32 patients (72%) finished all six courses. At the highest dose level (1000 mg/m^2 gemcitabine and 60 mg/m^2 cisplatin), all 5 patients included went off study, one because of ototoxicity, three to clinical deterioration, and one patient refused to continue treatment. In view of the increasing hematological toxicity, further dose escalation on this schedule was not considered feasible. On the two-weekly schedule, 31 of

Table 3 Occurrence of hematologic toxicity at all dose levels
Number of patients per schedule.

		Weekly schedule (n = 27)	Two-weekly schedule (n = 41)
Leukocytopenia	CTC 1	6 (22%)	9 (22%)
	CTC 2	4 (15%)	3 (7%)
	CTC 3[a]	3 (11%)	
Neutropenia	CTC 1	4 (15%)	5 (12%)
	CTC 2	3 (11%)	8 (20%)
	CTC 3	6 (22%)	1 (2%)
Trombocytopenia	CTC 1	7 (26%)	11 (27%)
	CTC 2	2 (7%)	3 (7%)
	CTC 3	5 (19%)	
Anemia	CTC 1	5 (19%)	8 (20%)
	CTC 2	7 (26%)	14 (34%)
	CTC 3	3 (11%)	3 (7%)

[a] Hematologic toxicity never exceeded CTC grade 3.

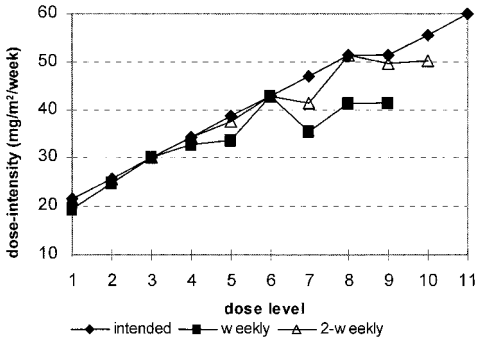

Fig. 1 Dose intensity of cisplatin in mg/m^2 per week, depicted per dose level

41 patients included (76%) received all planned courses. In total, 5 patients stopped protocol treatment before or during cycle 1, either because of clinical deterioration (4) or patient refusal (1), and these were replaced by the next eligible patient.

Hematological Toxicity. Sixty-eight patients were evaluable for toxicity. Hematological toxicity was generally mild, rarely exceeding CTC grade 2 (Table 3). As expected, bone marrow suppression was more frequent on the weekly schedule and on the higher dose levels. In the weekly schedule, at the two highest levels reached (60 mg/m^2 cisplatin with 900 or 1000 mg/m^2 gemcitabine, respectively), all patients had hematological toxicity. At level 8, 2 of 3 patients had leukocytopenia CTC grade 3, and in one of these, this was accompanied by thrombocytopenia grade 3. On level 9, 1 patient developed grade 3 neutropenia, and another patient developed grade 3 thrombocytopenia and anemia. Hematological toxicity was less frequent on the two-weekly schedule, only three episodes of grade 3 toxicity occurred. However, at the higher levels, where cisplatin was administered at doses >97.5 mg/m^2, all patients experienced leukocytopenia and neutropenia CTC grade 1–2. The development of hematological toxicity caused dose delays in an increasing number of patients at the higher dose levels, which reduced the achieved dose intensity of cisplatin. Any interruption of >1 week was considered dose limiting. The achieved *versus* intended dose intensity of cisplatin per dose level is given in Fig. 1. On the weekly schedule, the dose intensity of cisplatin did not appear to increase anymore after dose level 8. Therefore, the weekly schedule was abandoned. For the two-weekly schedule, the MTD was based on nonhematological toxicity at dose level 9 (cisplatin at 90 mg/m^2 and gemcitabine at 1500 mg/m^2). Six patients were included at this level, and the achieved dose intensity was 50 mg/m^2/week, 97% of the intended value.

Nonhematological Toxicity. Most frequent encountered toxicities (Table 4) were nausea and vomiting (occurring overall in 90 and 66% of patients, respectively). There was no significant difference between schedules for this toxicity, but frequency and severity increased at the higher dose levels. Fatigue was reported by 27 patients (40%), being severe (CTC grade 3) in 3 cases, and mild neuropathy occurred in 16 patients (24%). Other nonhematological toxicities included diarrhea, skin rash,

Table 4 Occurrence of nonhematologic toxicity at all dose levels
Number of patients per schedule.

		Weekly schedule (n = 27)	Two-weekly schedule (n = 41)
Nausea	CTC 1	16 (59%)	21 (51%)
	CTC 2	7 (26%)	16 (39%)
	CTC 3	1 (4%)	
Vomiting	CTC 1	13 (48%)	16 (39%)
	CTC 2	3 (11%)	12 (29%)
	CTC 3	1 (4%)	
Constipation	CTC 1	15 (56%)	11 (41%)
	CTC 2	4 (15%)	4 (10%)
	CTC 3		
Fatigue	CTC 1	4 (15%)	7 (17%)
	CTC 2	7 (26%)	6 (15%)
	CTC 3	2 (7%)	1 (2%)
Neuropathy	CTC 1	7 (26%)	8 (20%)
	CTC 2		1 (2%)
	CTC 3		
Alopecia	CTC 1	8 (30%)	11 (41%)
	CTC 2	1 (4%)	1 (2%)
Ototoxicity	CTC 1	4 (15%)	10 (24%)
	CTC 2	2 (7%)	5 (12%)
	CTC 3	1 (4%)	1 (2%)

stomatitis, and dizziness. Ototoxicity occurred in 23 patients (34%) and was dose limiting on the two-weekly schedule. On the highest levels reached (cisplatin 97.5 mg/m^2 at level 10 and 105 mg/m^2 at level 11), 6 of 11 patients had ototoxicity CTC grade 2 (3 at each level). In addition, 1 patient developed ototoxicity CTC grade 3 at level 11. This toxicity, generally manifesting itself as hearing loss of the higher tones, was not reversible after cessation of treatment. At the MTD, established at level 9, 1 patient had ototoxicity grade 2, and the other 5 patients had ototoxicity grade 1, which was not considered dose limiting. Other nonhematological toxicities at the MTD were nausea/vomiting CTC grade 1–2 in 5 of 6 patients, fatigue CTC grade 2 in 3 of 6 patients, and neuropathy CTC grade 1 in 2 of 6 patients.

Responses. Sixty of 73 patients were evaluable for response. Twenty-four patients achieved an objective partial response (40%), 11 patients a minor response (18%), and 19 patients (32%) had stable disease. The remaining 6 patients (10%) showed disease progression. After treatment with gem-

Table 5 Responses
Number of patients per response group and percentage.

	PR[a]	SD	PD
Total (n = 60)	24 (40%)	30 (50%)	6 (10%)
Weekly schedule (n = 28)	6 (23%)	15 (58%)	5 (19%)
Two-weekly schedule (n = 34)	18 (53%)	15 (44%)	1 (3%)
Tumor stage IIIB (n = 23)	13 (57%)	9 (39%)	1 (4%)
Tumor stage IV (n = 36)	11 (31%)	20 (56%)	5 (14%)
PS 0 (n = 26)	11 (42%)	12 (46%)	3 (12%)
PS 1 (n = 26)	12 (46%)	12 (46%)	2 (8%)
PS 2 (n = 8)	1 (13%)	6 (75%)	1 (13%)

[a] PR, partial response; SD, stable disease; PD, progressive disease.

Table 6 Number of patients who achieved a partial response (and number of patients evaluable for response) per dose level

Dose level	Weekly schedule	Two-weekly schedule
1	1/3	1/3
2	0/2	0/2
3	2/3	2/3
4	1/3	2/3
5	1/2	1/3
6	1/3	3/3
7	0/3	2/3
8	0/3	0/3
9	0/3	3/5
10		2/4
11		2/3

citabine and cisplatin, patients with stage IIIB disease were further treated with radiotherapy if they had achieved a partial response on induction therapy. Three of these patients are alive at 2, 2.5, and 3 years, respectively, without evidence of disease. In stage IV patients who were responding, gemcitabine single agent was continued. Table 5 outlines response rates for subsets of patients, and in Table 6, the responses are given according to dose level. When comparing the two administration schedules, a difference in response rate was seen (53% on the two-weekly schedule *versus* 23% on the weekly schedule). However, part of this effect could be attributable to differences in patient characteristics (Table 2).

Gemcitabine Pharmacokinetics. Pharmacokinetic sampling was performed in all but 3 patients. Gemcitabine was detectable (>0.1 μg/ml) until 1.5 h after the end of infusion. The pharmacokinetic parameters C_{max} and AUC increased linearly with increasing doses ($r = 0.7$, $P < 0.01$ for C_{max} and $r = 0.69$, $P < 0.01$ for AUC). Furthermore, gemcitabine had a half-life of 13 min (±3.4), a clearance of 195 liters/h (±54), and a volume of distribution of 42 liters (±13). For the metabolite dFdU, samples were obtained until 48 h after the end of infusion. Additionally for this metabolite, the C_{max} as well as the AUC showed a good positive correlation with the dose ($r = 0.74$, $P < 0.01$ and $r = 0.41$, $P = 0.01$, respectively). Peak plasma levels of dFdU were reached ~10 min after the end of infusion, and dFdU had a terminal half-life of 22 h (±8.3). The AUC of dFdU levels in plasma correlated with the peak plasma concentrations of gemcitabine ($r = 0.44$, $P < 0.01$). Over the first 24 h, 5.5% (±2.7) of the total dose of gemcitabine was excreted unchanged in urine. Over 48 h, an additional 79% (±34) of the total dose was excreted as dFdU. The peak levels of dFdCTP in WBC showed wide interpatient variability (88% at the MTD of 1350 mg/m^2). Overall, peak levels of 75–1390 pmol/10^6 cells were measured at 2–4 h postinfusion. These could not be correlated with the dose or AUC of gemcitabine.

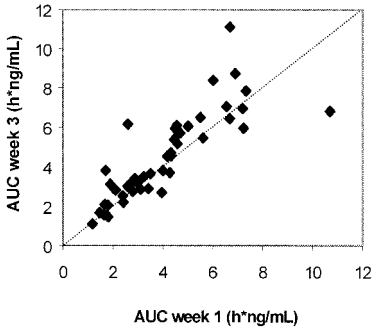

Fig. 2 Unbound platinum AUC in week 1 *versus* week 3. *Dotted line*, the line of identity.

Cisplatin Pharmacokinetics. For free Pt, the following pharmacokinetic parameters were calculated: $t_{1/2}$ of 53 min (±23), Cl of 3 liters/h (±0.9), and V of 92 liters (±32). Both C_{max} and AUC showed a strong positive correlation with the dose ($r = 0.91$, $P < 0.001$ and $r = 0.85$, $P < 0.01$). When comparing the 1st and 3rd week of treatment, no differences were observed in any of the pharmacokinetic parameters, as outlined for the AUC in Fig. 2, indicating the absence of carryover or an inducible effect of gemcitabine on unbound Pt pharmacokinetics. In addition, the dose of gemcitabine did not affect the clearance of Pt. Urinary excretion over the first 24 h was 34 ± 24% of the total dose.

The intracellular Pt-DNA intrastrand adducts Pt-GG and Pt-AG were measured in WBC. Peak levels of ≤3 fmol/μg DNA for Pt-GG and 0.3 fmol/μg DNA for Pt-AG were reached at the higher dose levels. Peak levels as well as AUAs for both types of adducts correlated to cisplatin dose, although interpatient variability was considerable (ranging from 6 to 66% throughout all dose levels). To investigate a possible interaction between gemcitabine and intracellular cisplatin pharmacology, Pt-DNA adduct levels corrected for cisplatin dose (by division) were plotted against gemcitabine dose (Fig. 3). As can be seen, levels of both GG and AG adducts were reduced by gemcitabine ($r = 0.4$, $P = 0.001$ for Pt-GG and $r = 0.39$, $P = 0.002$ for Pt-AG). This phenomenon was most pronounced for peak levels but occurred with AUAs as well. The ratio between GG and AG adducts was not influenced by the gemcitabine dose. There was no difference in these reductions between the weekly and two-weekly schedule (data not shown).

Pharmacokinetic-pharmacodynamic Analysis. Both the dose and AUC of gemcitabine and cisplatin were tested in singular as well as in a combined model (by logistic regression) for a correlation with hematological toxicity and response. Furthermore, C_{max} and AUA of cisplatin-DNA adducts were tested for such a correlation. No relationships could be established.

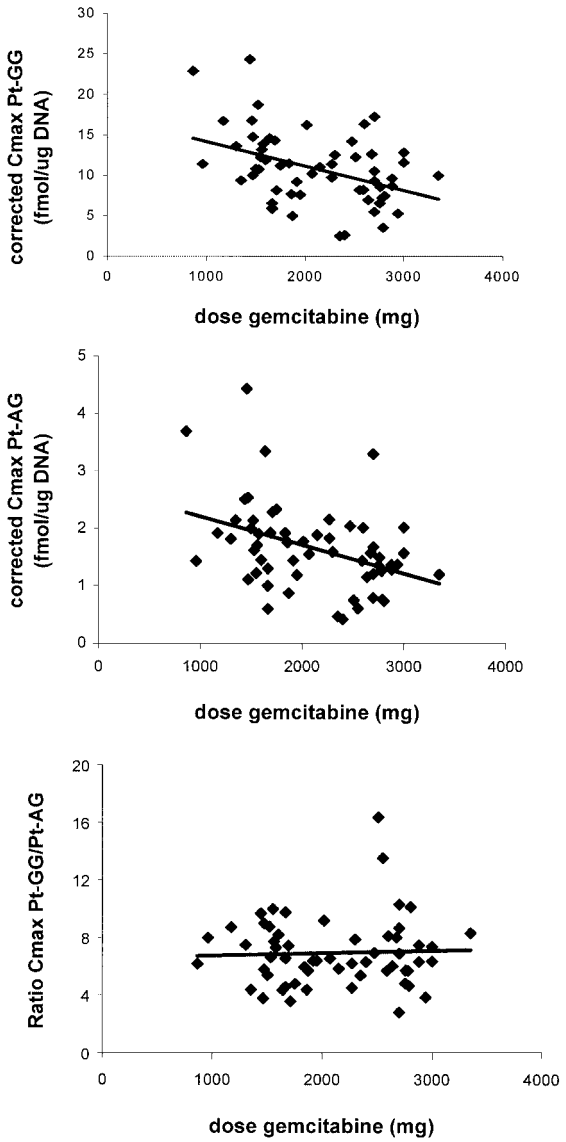

Fig. 3 Maximum adduct level of Pt-GG and Pt-AG (t = 1 h after end of infusion), and ratio of Pt-GG and Pt-AG adducts, corrected for cisplatin dose, *versus* dose of gemcitabine. Adduct levels are corrected for cisplatin dose by division.

Discussion

The efficacy of combined treatment with gemcitabine and cisplatin in patients with NSCLC has been established in several Phase II and III clinical trials (3, 4, 6, 26, 27). However, there is still debate 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 × 3, at doses of 1000–1500 mg/m^2. Next, the 21-day schedule was developed (4), which doses gemcitabine at 1250 mg/m^2 on days 1 and 8 and cisplatin at 70–100 mg/m^2 at day 2. In an attempt to improve the therapeutic index of this combination, we have studied the feasibility of increasing the dose intensity of cisplatin. This cannot be achieved by increasing the dose in the once every 21/28-day schedules, as 100 mg/m^2 is considered the MTD for a single cisplatin administration. However, we and others have previously shown that giving smaller doses more frequently is feasible (17, 28, 29). Furthermore, combining gemcitabine and cisplatin in each administration might allow full exploitation of the synergism ascribed to these agents. Our study reveals that the highest dose intensity of cisplatin, of 50 mg/m^2/week, could be reached on the two-weekly schedule. The weekly schedule was less well tolerated, and dose intensity could not be pushed further than 42 mg/m^2/week. In addition, the response rate in the weekly schedule was significantly lower than in the two-weekly schedule (Table 5), but this could have been attributable to small differences in patient characteristics between both randomization groups (relatively more patients with poorer PS at study entry in the weekly treatment arm). At least two other groups have explored weekly cisplatin administration in combination with gemcitabine. In a Phase II trial of Shepherd *et al.*, cisplatin was dosed weekly × 3 at 30 mg/m^2 in combination with gemcitabine at 1500 mg/m^2. Toxicity (fatigue) was prominent, and no indication of increased efficacy (response rate 26%) was obtained (20). Others reported better tolerability and an overall response rate of 40% (12). Our trial confirms the conclusions of Shepherd *et al.* When comparing toxicity in our two-weekly schedule with the Phase II trials executed to date, the profiles seem to be similar (3–6, 27, 30). However, given the Phase I nature of our trial using 11 different dose levels, subsequent evaluation in a Phase II study is warranted. In this perspective, the response rate of 53% on the two-weekly schedule appears promising but requires confirmation. Given the relatively low age of the patients in our study (median age 51–55 years in the different treatment groups), future studies in older patients, which generally make up the overall lung cancer population, are also required. Moreover, a Phase II study with the two-weekly schedule will help to determine whether increasing the dose intensity of cisplatin and combining the two agents in each administration leads to higher response rates in NSCLC. The role of dose intensity of chemotherapeutic agents has not been established in the palliative setting. However, we believe that increasing the dose intensity of cisplatin may aid to improve responses in the neo-adjuvant setting for patients with less advanced disease. In ovarian cancer, good results have been obtained with this approach (16), but for other tumor types, such as lung carcinoma, this remains to be determined.

The interaction between gemcitabine and cisplatin has been studied extensively in preclinical experiments. Synergistic, additive, and antagonistic effects have been observed *in vitro*, depending on cell type and administration schedule (12, 15, 31, 32). *In vivo* studies in mice demonstrated that gemcitabine pretreatment decreased the intrastrand Pt-DNA adducts ~3-fold (33). Our data confirm the possibility of a negative effect of gemcitabine on intracellular cisplatin pharmacokinetics. The pharmacokinetic parameters we found for gemcitabine, dFdCTP, and free Pt were in good concordance with literature data (34, 35). However, there was a significant reduction in both types of Pt-DNA adducts with increasing doses of gemcitabine in WBC. This effect was highly significant and has led us to now explore the reversed administration sequence. To our knowledge, one other pharmacologic trial with cisplatin and gemcitabine has been performed, which did not find this de-

crease in adducts. However, an effect of gemcitabine on Pt-DNA adducts was observed, manifesting itself in a reduced GG:AG ratio (36). We have not found an influence of gemcitabine on this ratio. Currently, there is no explanation for the observed interaction. To study intracellular pharmacology, we used circulating WBCs, because of their easy accessibility. As we have shown previously, levels of adducts in WBC are a good marker for platination of DNA (17). However, the pharmacology of gemcitabine in these noncycling cells probably differs substantially from the pharmacology in tumor cells. Therefore, additional investigations of this interaction in other cell types are highly required. It is possible that cellular DNA repair mechanisms are involved (37), but again, more detailed investigations are warranted, because they could facilitate the design of the optimal clinical administration schedule for this combination.

In conclusion, we have demonstrated the feasibility of chemotherapy consisting of two-weekly gemcitabine in combination with cisplatin at a high dose intensity of 50 mg/m^2/week. In view of the observed pharmacologic interaction between the two agents, we will continue to explore the reversed administration scheme, also in two-weekly cycles.

References

1. Sweeney, C. J., and Sandler, A. B. Chemotherapy in non-small cell lung cancer. Investig. New Drugs, *18:* 157–186, 2000.

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

3. Abratt, R. P., Sandler, A., Crino, L., Steward, W. P., Shepherd, F. A., Green, M. R., Nguyen, B., and Peters, G. J. Combined cisplatin and gemcitabine for non-small cell lung cancer: influence of scheduling on toxicity and drug delivery. Semin. Oncol., *25:* 35–43, 1998.

4. Cardenal, F., Lopez-Cabrerizo, M. P., Anton, A., Alberola, V., Massuti, B., Carrato, A., Barneto, I., Lomas, M., Garcia, M., Lianes, P., Montalar, J., Vadell, C., Gonzalez-Larriba, J. L., Nguyen, B., Artal, A., and Rosell, R. 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., *17:* 12–18, 1999.

5. Rinaldi, M., Crino, L., Scagliotti, G. V., Mosconi, A. M., De Marinis, F., Gridelli, C., Selvaggi, G., Della Giulia, M., Darwish, S., Porrozzi, S., Novello, S., Cipri, A., Bartolucci, R., Calandri, C., and Tonato, M. A three-week schedule of gemcitabine-cisplatin in advanced non-small-cell lung cancer with two different cisplatin dose levels: a phase II randomized trial. Ann. Oncol. *11:* 1295–1300, 2000.

6. Soto-Parra, H. J., Cavina, R., and Antonelli, F. Superiority of three-week vs four-week schedule of cisplatin and gemcitabine: results of a randomized phase II study. Proc. Lung Cancer, *9:* 6, 2000.

7. Crino, L., Scagliotti, G., Marangolo, M., Figoli, F., Clerici, M., De Marinis, F., Salvati, F., Cruciani, G., Dogliotti, L., Pucci, F., Paccagnella, A., Adamo, V., Altavilla, G., Incoronato, P., Trippetti, M., Mosconi, A. M., Santucci, A., Sorbolini, S., Oliva, C., and Tonato, M. Cisplatin-gemcitabine combination in advanced non-small-cell lung cancer: a phase II study. J. Clin. Oncol., *15:* 297–303, 1997.

8. Jassem, J., Krzakowski, M., Roszkowski, K., Ramlau, R., Slominski, J. M., Szczesna, A., Krawczyk, K., Mozejko-Pastewka, B., Lis, J., and Miracki, K. A phase II study of gemcitabine plus cisplatin in patients with advanced non-small cell lung cancer: clinical outcomes and quality of life. Lung Cancer, *35:* 73–79, 2002.

9. Huisman, C., Giaccone, G., van Groeningen, C. J., Sutedja, G., Postmus, P. E., and Smit, E. F. Combination of gemcitabine and cisplatin for advanced non-small cell lung cancer: a phase II study with emphasis on scheduling. Lung Cancer, *33:* 267–275, 2001.

10. Fichtinger-Schepman A. M., van der Veer, J. L. den Hartog, J. H., Lohman, P. H., and Reedijk, J. Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation. Biochemistry, *24:* 707–713, 1985.

11. Huang, P., Chubb, S., Hertel, L. W., Grindey, G. B., and Plunkett, W. Action of 2′, 2′-difluorodeoxycytidine on DNA synthesis. Cancer Res., *51:* 6110–6117, 1991.

12. Bergman, A. M., Ruiz van Haperen, V. W. T., Veerman, G., Kuiper, C. M., and Peters, G. J. Synergistic interaction between cisplatin and gemcitabine *in vitro*. Clin. Cancer Res., *2:* 521–530, 1996.

13. Braakhuis, B. J. M., Ruiz van Haperen, V. W. T., Welters, M. J. P., and Peter, G. J. Schedule-dependent therapeutic efficacy of the combination of gemcitabine and cisplatin in head and neck cancer xenografts. Eur. J. Cancer, *31a:* 2335–2340, 1995.

14. Peters, G. J., Bergman, A. M., Ruiz. van Haperen, V. W. T., Veerman, G., Kuiper, C. M., and Braakhuis, B. J. Interaction between cisplatin and gemcitabine in vitro and in vivo. Semin. Oncol., *22:* 72–79, 1995.

15. van Moorsel, C. J., Pinedo, H. M., Veerman, G., Bergman, A. M., Kuiper, C. M., Vermorken, J. B., van der Vijgh, W. J., and Peters, G. J. Mechanisms of synergism between cisplatin and gemcitabine in ovarian and non-small-cell lung cancer cell lines. Br. J. Cancer, *80:* 981–990, 1999.

16. Kaye, S. B., Lewis, C. R., Paul, J., Duncan, I. D., Gordon, H. K., Kitchener, H. C., Cruickshank, D. J., Atkinson, R. J., Soukop, M., and Rankin, E. M. Randomised study of two doses of cisplatin with cyclophosphamide in epithelial ovarian cancer. Lancet, *340:* 329–333, 1992.

17. Schellens, J. H., Ma, J., Planting, A. S., van der Burg, M. E., van Meerten, E., de Boer-Dennert, M., Schmitz, P. I., Stoter, G., and Verweij, J. Relationship between the exposure to cisplatin, DNA-adduct formation in leucocytes and tumour response in patients with solid tumours. Br. J. Cancer, *73:* 1569–1575, 1996.

18. Planting, A. S., Schellens, J. H., Goey, S. H., van der Burg, M. E., de Boer-Dennert, M., Stoter, G., and Verweij, J. Weekly high-dose cisplatin in malignant pleural mesothelioma. Ann. Oncol. *5:* 373–374, 1994.

19. Planting, A. S. T., de Mulder, P. H. M., de Graeff, A., and Verweij, J. Phase II study of weekly high-dose cisplatin for six cycles in patients with locally advanced squamous cell carcinoma of the head and neck. Eur. J. Cancer, *33:* 61–65, 1997.

20. Shepherd, F. A., Cormier, Y., Burkes, R., Evans, W. K., Goss, G., Klimo, P., Feld, R., and Taylor, M. Phase II trial of gemcitabine and weekly cisplatin for advanced non-small cell lung cancer. Semin. Oncol. 24*:* S8–27–S8–30, 1997.

21. Guidelines for reporting of adverse drug reactions. Division of cancer treatment. Bethesda, MD: National Cancer Institute, 1988.

22. Plunkett, W., Chubb, S., Alexander, L., and Montgomery, J. A. Comparison of the toxicity and metabolism of 9-β-D-arabinofuranosyl-2-fluoroadenine and 9-β-D-arabinofuranosyladenine in human lymphoblastoid cells. Cancer Res., *40:* 2349–2355, 1980.

23. Freeman, K. B., Anliker, S., Hamilton, M., Osborne, D., Dhahir, P. H., Nelson, R., and Allerheiligen, S. R. Validated assays for the determination of gemcitabine in human plasma and urine using high-performance liquid chromatography with ultraviolet detection. J. Chrom. B, *665:* 171–181, 1995.

24. Ma, J., Stoter, G., Verweij, J., and Schellens, J. H. M. Comparison of ethanol plasma-protein precipitation with plasma ultrafiltration and trichloroacetic acid protein precipitation for the measurement of unbound platinum concentrations. Cancer Chemother. Pharmacol., *38:* 391–394, 1996.

25. Van Warmerdam, L. J. C., van Tellingen, O., Maes, R. A. A., and Beijnen, J. H. Validated method for the determination of carboplatin in biological fluids by Zeeman Atomic Absorption Spectrometry. Fres. J. Anal. Chem., *351:* 1820–1824, 1995.

26. Pluim, D., Maliepaard, M., van Waardenburg, R. C., Beijnen, J. H., and Schellens, J. H. 32P-postlabeling assay for the quantification of the major platinum-DNA adducts. Anal. Biochem., *275:* 30–38, 1999.

27. Schiller, J. H., Harrington, D., Belani, C. P., Langer, C., Sandler, A., Krook, J., Zhu, J., and Johnson, D. H. Comparison of four chemotherapy regimens for advanced non-small-cell lungcancer. N. Engl. J. Med., *346:* 92–98, 2002.

28. Vogl, S. E., Berenzweig, M., Camacho, F., Greenwald, E., and Kaplan, B. H. Efficacy study of intensive cisplatin therapy in advanced non-small cell lung bronchogenic carcinoma. Cancer (Phila.), *50:* 24–26, 1982.

29. Lippe, P., Tummarello, D., Monterubbianesi, M. C., Silva, R. R., Giuliodori, L., Mari, D., Santo, A., Pasini, F., Cetto, G. L., Rossi, D., Porfiri, E., Cascinu, S., and Cellerino, R. Weekly gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase II study. Ann. Oncol., *10:* 217–221, 1999.

30. Sandler, A. B., Nemunaitis, J., Denham, C., von Pawel, J., Cormier, Y., Gatzemeier, U., Mattson, K., Manegold, C., Palmer, M. C., Gregor, A., Nguyen, B., Niyikiza, C., and Einhorn, L. H. 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, 2000.

31. Theodossiou, C., Cook, J. A., Fisher, J., Teague, D., Liebmann, J. E., Russo, A., and Mitchell, J. B. Interaction of gemcitabine with paclitaxel and cisplatin in human tumor cell lines. Int. J. Oncol., *12:* 825–832, 1998.

32. Voigt, W., Bulankin, A., Muller, T., Schoeber, C., Grothey, A., Hoang-Vu, C., and Schmoll, H. J. Schedule-dependent antagonism of gemcitabine and cisplatin in human anaplastic thyroid cancer cells. Clin. Cancer Res., *6:* 2087–2093, 2000.

33. van Moorsel, C. J., Pinedo, H. M., Smid, K., Comijn, E. M., Voorn, D. A., Veerman, G., Lakerveld, B., Van der Vijgh, W. J., Giaccone, G., Postmus, P. E., and Peters, G. J. Schedule-dependent pharmacodynamic effects of gemcitabine and cisplatin in mice bearing Lewis lung murine non-small cell lung tumours. Eur. J. Cancer, *36:* 2420–2429, 2000.

34. Vermorken, J. B., van der Vijgh, W. J. F., Klein, I., Gall, H. E., and Pinedo, H. M. Pharmacokinetics of free platinum species following rapid, 3-hr and 24-hr infusions of cis-diamminedichloroplatinum (II) and its therapeutic implications. Eur. J. Cancer Clin. Oncol., *18:* 1069–1074, 1982.

35. Abbruzzese, J. L., Grunewald. R., Weeks, E. A., Gravel, D., Adams, T., Nowak, B., Mineishi, S., Tarassoff, P., Satterlee, W., and Raber, M. N. A. phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J. Clin. Oncol., *9:* 491–498, 1991.

36. Moorsel, C. J. A., Kroep, J. R., Pinedo, H. M., Veerman, G., Voorn, D. A., Postmus, P. E., Vermorken, J. B., van Groeningen, C. J., van der Vijgh, W. J., and Peters, G. J. Pharmacokinetic schedule finding study of the combination of gemcitabine and cisplatin in patients with solid tumors. Ann. Oncol., *10:* 441–448, 1999.

37. Yang, L. Y., Li, L., Jiang, H., Shen, Y., and 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., *6:* 773–781, 2000.

Clinical Cancer Research

Randomized Phase I Clinical and Pharmacologic Study of Weekly *versus* Twice-Weekly Dose-intensive Cisplatin and Gemcitabine in Patients with Advanced Non-Small Cell Lung Cancer

Mirjam Crul, Nadja E. Schoemaker, Dick Pluim, et al.

Clin Cancer Res 2003;9:3526-3533.