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Combination Therapy with Gemcitabine in Non-small Cell Lung Cancer

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The nucleoside analogue, gemcitabine, has shown activity as a single agent in the treatment of metastatic non-small cell lung cancer (NSCLC), producing consistent response rates of 20% and above. Because of its unique mechanism of action and its non-overlapping toxicity with other active agents, gemcitabine is an attractive candidate for trials in combination with other cytotoxic agents. In preclinical models, the cisplatin–gemcitabine combination suggested synergy between the two drugs. In phase I–II studies, response rates are as high as 54% when gemcitabine is combined with cisplatin, both in stage III and IV NSCLC. The combination of gemcitabine and ifosfamide is also being explored with an overall response rate of 32%. The gemcitabine-containing regimens showed a favourable safety-efficacy profile and compared well with standard regimens used in NSCLC. These preliminary results must be validated by large randomised trials comparing gemcitabine-containing regimens with NSCLC reference chemotherapy regimens.

Key words: non-small cell lung cancer, combination chemotherapy, gemcitabine, review, clinical trials
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INTRODUCTION

GEMCITABINE (2',2'-difluorodeoxycytidine), a pyrimidine antimetabolite, is a deoxycytidine analogue, with two fluorine substitutes for the two hydrogen atoms in the 2' position of deoxyribose sugar. Although there are structural similarities to the antimetabolite cytarabine (ara-C), gemcitabine is substantially different in its cellular pharmacology and mechanism of action: it has greater membrane permeability, and higher affinity for the deoxycytidine kinase (their activating enzyme), as well as longer intracellular retention, with a prolonged inhibition of DNA synthesis. In addition, gemcitabine has significantly greater activity against a wide range of solid tumour models [1].

After entering the cell, gemcitabine is phosphorylated to the active forms (gemcitabine diphosphate and triphosphate). Gemcitabine mimics the structure of the natural nucleoside, deoxycytidine, and thus is inserted into DNA in the deoxycytidine sites. After gemcitabine is incorporated on the end of the DNA strand, one more natural nucleoside is added, masking gemcitabine from the DNA repair mechanism that might excise it; thereafter, the DNA polymerases are unable to proceed. This unique process is termed "masked DNA chain termination" [2]. Gemcitabine not only acts on the DNA, but is also incorporated into RNA [3].

Multiple 'self-potentiation' mechanisms potentiate the activity of gemcitabine both by increasing formation of the active forms (gemcitabine diphosphate and triphosphate) and decreasing elimination of gemcitabine (Table 1).

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In preclinical studies, gemcitabine has shown antitumour activity across a range of tumour xenografts, including lung, ovarian, breast, head and neck, and colon cancer [4]. Based on experiences in a large number of phase I trials, the weekly schedule of gemcitabine i.v. injection over 30 min for 3 weeks followed by 1 week of rest provides activity with minimal non-haematological toxicity; the maximum tolerated dose (MTD) was defined at 2800 mg/m^2, with short-lived myelosuppression being the dose-limiting side-effect [5, 6]. This weekly schedule was tested in a broad phase II programme which confirmed gemcitabine's efficacy as a single agent in lung, breast, ovarian and pancreatic cancer.

Phase II studies of single-agent gemcitabine in NSCLC

Eight phase II studies have been performed in advanced non-small cell lung cancer (NSCLC) in 535 assessable patients with a consistent, reproducible response rate in the range of

Table 1. Mechanistic rationale for combining gemcitabine with other agents

(1)	Self-potentiation (higher intracellular gemcitabine)
(2)	Cytotoxicity due to:
	Inhibition of ribonucleotide reductase (essential for DNA synthesis and repair)
	Reduced incorporation of deoxycytidine nucleotide
	Increased incorporation of gemcitabine nucleotide
(3)	Masked DNA chain termination
	Inhibition of DNA excision repair
	Lower potential for resistance

20–23% in 7 out of 8 trials. The toxicity profile in all these studies using the same gemcitabine weekly schedule (i.v. infusion over 30 min for 3 weeks followed by 1 week of rest), at doses ranging from 800 to 1250 mg/m^2, was modest, consisting mainly of a short-lasting decrease in leucocytes and thrombocytes, skin reactions (5–8%), peripheral oedema and transient increase in transaminase levels [7, 8].

Phase II studies of combination therapy with gemcitabine

Because of its unique mechanism of action and its non-overlapping toxicity with other active agents, gemcitabine is an attractive candidate for trials in combination with other cytotoxic agents and also with radiotherapy.

A recent meta-analysis using updated individual patient data from randomised trials, both published and unpublished, evaluating the impact of combination chemotherapy on survival compared to locoregional treatment or best supportive care in advanced NSCLC, has demonstrated that cisplatin-containing chemotherapy is favoured, obtaining a small but significant 10% survival benefit at 1 year versus best supportive care [9]. Accordingly, cisplatin-containing regimens are currently considered as the best chemotherapy treatment of advanced NSCLC, although still experimental because of treatment morbidity and marginal impact on survival.

Cisplatin acts by forming DNA–DNA cross-links (both intrastrand and interstrand) and DNA–protein cross-links; resistance to cisplatin is thought to be caused by excision repair of the affected DNA. Recent data from Peters and colleagues [10, 11] show that the interaction between cisplatin and gemcitabine can be multifactorial, with either synergism or an additive effect both *in vitro* and *in vivo*. Scheduling and drug exposure time seems to be relevant for this interaction. In cell culture, simultaneous addition of both drugs resulted in synergism when exposure time was longer than 4 h. This synergism was observed in cisplatin-resistant cell lines but not in gemcitabine-resistant cells. It seems likely that a certain extent of gemcitabine incorporation into DNA is required to achieve an interaction with cisplatin. As with ara-C, incorporation into DNA of the cytidine analogue enhances formation of cisplatin–DNA adducts [12]. Since the mechanism of action of cisplatin consists of formation of DNA adducts, and since tumour cells can become resistant to cisplatin because of a DNA excision-repair process, it has been postulated that gemcitabine inhibits this DNA-repair process when used in combination with cisplatin. Indeed, gemcitabine induces depletion of both deoxyribonucleotide and ribonucleotide pools, essential for good functioning of the DNA-repair mechanism.

Based on these considerations, several phase I–II trials were designed to evaluate gemcitabine in combination with cisplatin in advanced NSCLC. In all trials, gemcitabine was administered on a weekly basis, while cisplatin schedules varied.

In a Canadian phase I study [13], both drugs were given weekly for 3 weeks with 1 week rest. Gemcitabine doses were increased from 1000 to 2250 mg/m^2 per week with a fixed cisplatin dose of 30 mg/m^2 per week (except 7 patients at dose level 1 who received 25 mg/m^2). The MTD (maximum tolerated dose) over four cycles was 1500 mg; responses were seen at all dose levels with no evidence of a dose–response effect. 16 patients were stage III and 34 stage IV. In 47 patients evaluable for response, 14 partial responses were documented for an overall response rate of 30%. If the data were analysed according to the intention-to-treat principle, the overall response rate in all 50 enrolled patients would be 28%. Median duration of response was 20 weeks [13].

In a French–UK phase I–II study [14], gemcitabine was given weekly at a dose of 1000 mg/m^2, for 3 consecutive weeks, followed by 1 week rest. Cisplatin was administered on day 15 of each course at escalating doses from 60 to 100 mg/m^2. 66 patients were enrolled: 2 had stage IIIa, 41 stage IIIb, and 23 stage IV disease. The 10 patients at the top-dose level (cisplatin 100 mg/m^2) in the phase I study were included in the efficacy analysis along with the 50 patients enrolled in the phase II study, all of whom received 100 mg/m^2 of cisplatin. Of the 60 eligible patients, data were available for 52 evaluable patients who had received $\geq$2 courses of chemotherapy: 20 partial responses were observed, for a response rate of 38%. The incidence of combined worst WHO grade 3 and 4 toxicities per patient was: neutropenia 51%, thrombocytopenia 25%, transient increase in transaminase levels 2%, alopecia 2%, fever 4%, nausea and vomiting 50%. Neutropenia and thrombocytopenia were of short duration and uncomplicated [14].

In an identical study by Abratt and colleagues [15], gemcitabine was given at a dose of 1000 mg/m^2 weekly for 3 weeks followed by 1 week rest and cisplatin was given at a dose of 100 mg/m^2 on day 15. 38 patients were entered into the study: 11 were stage IIIa, 13 stage IIIb, and 14 stage IV disease. Of 38 patients, 35 were evaluable for response, having received at least one cycle of chemotherapy. A complete response was observed in 1 patient (3%) and a partial response in 15 patients (43%) for an overall response rate of 46%. A WHO grade 1 and 2 increase in serum creatinine occurred in 11% and 3% of patients, respectively. WHO grade 3 and 4 neutropenia occurred in 24% and 0%, and thrombocytopenia in 19% and 3% of patients, respectively.

In these three studies all responses were independently validated by an external Oncology Review Board.

The Hoosier Oncology Group in Indiana conducted another phase II study using cisplatin 100 mg/m^2 on day 1 and gemcitabine 1000 mg/m^2 weekly for 3 weeks followed by 1 week rest [16]. 30 patients were enrolled; 26 are currently evaluable for response and toxicity. 21 of 26 patients had stage IV and 5 had stage IIIb disease. There were 11 responses (1 complete response and 10 partial responses) in the 26 evaluable patients for an overall response rate of 42%. Toxicity was mainly haematological, with a frequent need to omit doses of gemcitabine on days 8 and/or 15 [16].

In an Italian phase II study, 48 previously untreated stage III–IV NSCLC patients were enrolled: a weekly schedule of gemcitabine 1000 mg/m^2 with cisplatin at a dose of 100 mg/m^2 on day 2 of each 28 day cycle was used [17]. There have been a few reports of renal failure of uncertain aetiology in patients receiving gemcitabine. Therefore, this schedule was adopted because of possible nephrotoxicity that could be potentiated by simultaneous exposure to cisplatin and because of the opportunity to assess separately gemcitabine's acute side-effects. At the same time, cisplatin was given on day 2 in order to maintain the synergistic effect by close administration of the two compounds. Although at the moment there is no clear evidence from experimental data of superiority for a particular schedule, it seems likely that sequential administration of gemcitabine and cisplatin, allowing some gemcitabine pre-incorporation into DNA, could be the most effective schedule [11]. Of 48 enrolled patients, 22 were unresectable stage III (21 stage IIIb and 1 stage IIIa) and 26 stage IV patients. Three patients did not completely satisfy the eligibility criteria: 2 because of unmeasurable disease and 1 because of previous chest

Table 2. Gemcitabine + cisplatin in NSCLC; efficacy summary

Study [Ref 1]	Cisplatin schedule administration	Response rate (%)
Italy [17]	day 2	54
U.K./France [14]	day 15	38
South Africa [15]	day 15	46
U.S.A. [16]	day 1	42
Canada [13]	weekly	30

radiotherapy. According to the 'intention-to-treat' principle, they are also included in the response rate calculation. Of 48 assessable patients, 1 (stage IV) had a complete response and 25 achieved a partial response for an overall response rate of 54%. 24 of 26 responses were validated by an independent Oncology Review Board. Median duration of response was 45.5 weeks, with 10 responses in excess of 60 weeks, 4 of which are currently ongoing. The median event-free survival time for all patients calculated as time to progression or time to death without evidence of disease progression was 34 weeks. Median duration of survival for all 48 assessable patients was 61.5 weeks.

Haematological toxicity was the limiting side-effect: thrombocytopenia WHO grades 3 and 4 was recorded in 52% of patients. Thrombocytopenia was mainly found after 2 weeks of treatment and caused the omission of the third gemcitabine administration in 50% of the courses; however, it was of short duration and no serious haemorrhagic event occurred. Neutropenia was recorded in 36% of patients and there was no associated infection.

The higher response rate (54%) and toxicity (mainly thrombocytopenia) when compared with the Canadian and European studies may possibly be explained by the sequential administration of gemcitabine and cisplatin. In some *in vivo* tumour models, sequential administration of gemcitabine and platinum was as effective as simultaneous administration, but toxicity was increased, especially at the 24 h interval [11].

Ifosfamide is another active drug in NSCLC. Gemcitabine and ifosfamide have different mechanisms of action and different toxicity profiles. Their combination was investigated in a German phase I–II trial [18]. In the phase I trial, gemcitabine was given at 1000 mg/m^2 weekly for 3 weeks followed by 1 week rest, whereas ifosfamide was escalated in sequential patient groups at 1200, 1500 and 1800 mg/m^2 on days 8–12 of each cycle. Based on these phase I data, the combination of gemcitabine (1000 mg/m^2) and ifosfamide (1500 mg/m^2) was chosen for the phase II study [19]. A total of 56 patients were entered: 50 are currently evaluable for response analysis. 16 partial remissions were documented for an overall response rate of 32%. Rapidly reversible myelosuppression was the predominant toxicity.

CONCLUSIONS

In conclusion, the gemcitabine-containing regimens have shown a favourable efficacy-safety profile comparing well with standard regimens in NSCLC. When gemcitabine is combined with cisplatin, response rates can be as high as 54% (Table 2) in both stage III and IV disease; these response rates are higher than with either drug used alone. The combinations are generally well tolerated; myelotoxicity, mainly thrombocytopenia, is the limiting side-effect, but hospitalisation or treatment with colony-stimulating factors are not required. These preliminary encouraging results must be validated by large randomised trials comparing gemcitabine-containing regimens with what is currently considered an active regimen for NSCLC, such as cisplatin-based combination chemotherapy. Quality of life and pharmacoeconomics should also be evaluated. In a recent, prospective randomised trial, activity and toxicity of three widely used cisplatin-containing regimens: MVP (mitomycin-C, vindesine and cisplatin), MIC (mitomycin-C, ifosfamide and cisplatin) and PE (cisplatin and etoposide) were evaluated. MIC and MVP achieved a better response rate; MIC showed a small but significant survival advantage versus PE at the Cox multivariate analysis [20]. Therefore, it is planned to evaluate in a phase III study the gemcitabine–cisplatin combination versus a three-drug regimen such as MIC (mitomycin-C, ifosfamide and cisplatin). This trial, which also contemplates a careful evaluation of the impact of the two regimens on the patients' quality of life, is now underway.

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