The Oncologist
Review and Commentary

Gemcitabine: Future Prospects of Single-Agent and Combination Studies

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Key Words. *Gemcitabine · Cisplatin · Taxanes · Non-small cell lung cancer · Pancreatic cancer · Breast cancer · Antimetabolites · Deoxycytidine kinase*

ABSTRACT

Gemcitabine (2′,2′-difluorodeoxycytidine, Gemzar) is a deoxycytidine analog with excellent antitumor activity against a number of solid tumors. Gemcitabine needs to be activated by deoxycytidine kinase and other kinases to its triphosphate, gemcitabine triphosphate, which can be incorporated into RNA and DNA. The latter effect is considered to be responsible for its antitumor effect and causes masked chain termination and inhibition of DNA repair. This effect may be of importance for combination with DNA interacting agents. In phase I trials daily, twice weekly, weekly and every two weeks schedules have been evaluated. At the weekly schedule of 1,000-1,250 mg/m^2 significant antitumor activity was observed in bladder, breast, ovary, and pancreatic cancer, non-small cell lung cancer (NSCLC), and small cell lung cancer of 31%, 33%, 22%, 11%, 22% and 27% total response rates, respectively. Gemcitabine also showed considerable improvement in clinical symptoms, while toxicity was not severe with mild myelosuppression. Due to its ability to inhibit DNA replication, combination studies were initiated with DNA damaging agents. For the various combinations with cisplatin in phase II studies on NSCLC, response rates varied from 42%-54%, with a median survival of generally more than 12 months. Also, combinations with taxanes, etoposide, doxorubicin and vindesin seem promising. Gemcitabine is an important agent for the management of several relatively chemoresistant cancer types, both with respect to antitumor activity and clinical benefit. Future research on combination studies deserves high priority considering the high response rates in NSCLC and bladder cancer.
The Oncologist *1997;2:127-134*

INTRODUCTION

Gemcitabine (Gemzar, 2′,2′-difluorodeoxycytidine, dFdC) is one of the most promising agents for the treatment of solid tumors. The drug has good activity as a single agent and excellent activity in combination therapy against several resistant tumors such as non-small cell lung cancer (NSCLC) and pancreatic cancer. Gemcitabine is a deoxycytidine analog with structural resemblance to cytarabine (ara-C) which is the most effective agent in adult acute leukemias [1], but which does not show activity against solid tumors as does gemcitabine. This might be due to the multiple mechanisms of action of gemcitabine [2]. Gemcitabine membrane transport is mediated by the facilitated nucleoside transporter [3]. Subsequently, it is phosphorylated to its mononucleotide by deoxycytidine kinase [4] and by nucleotide kinases to its active metabolite, gemcitabine triphosphate (dFdCTP). A strong correlation between the activation of gemcitabine, the extent of dFdCTP formation, its incorporation into DNA, and its inhibition of DNA synthesis was found [3, 5-7]. Furthermore, gemcitabine can be incorporated into RNA [6] and can induce apoptosis [8]. Other effects of metabolites of gemcitabine include inhibition of ribonucleotide reductase and dCMP deaminase [9] enhancing the incorporation of dFdCTP into DNA. Gemcitabine exhibits excellent preclinical activity against xenografts of lung, breast, head and neck, colon, and ovarian cancers. This activity was very

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schedule-dependent [10-15], which could be a key issue for further clinical development, when translated properly.

Phase I Studies

In phase I trials, several schedules with gemcitabine have been studied. The daily × 5 schedule [16] with a maximally tolerated dose (MTD) of 9 mg/m^2 caused fever, a flu-like syndrome, and life-threatening hypotension. In the twice-weekly schedule [17], the maximum tolerated dose of gemcitabine was 150 mg/m^2, and flu-like symptoms with fever, rigors, and malaise were observed. Neither schedule was recommended for phase II studies. In other phase I studies, the drug was administered as a weekly or every two weeks 30 min infusion, in which the drug was extremely well tolerated, with dose-limiting toxicity appearing to be mainly myeloid; however, lethargy, mild flu-like symptoms, and a reversible skin rash were also found [18-20]. The MTD of gemcitabine in the weekly schedule was 790 to 1,500 mg/m^2. The every two weeks schedule was not recommended for phase II studies, although in several combination studies (e.g., with paclitaxel [21]), gemcitabine was given every two weeks. The initial schedule for phase II studies was a 30 min infusion of 800 mg/m^2 (days 1, 8, 15 every four weeks) which was increased to 1,000-1,250 mg/m^2 due to lack of toxicity in most patients.

A better understanding of the clinical pharmacology and the good tolerability of gemcitabine in most phase I studies have led to a second generation of phase I trials that attempted to increase dose intensity, either by escalating the dose or by an increase of the infusion duration. For most of these studies, the weekly schedule of gemcitabine was used. In general, the dose-escalating studies have demonstrated that gemcitabine doses of 1,250 to 2,800 mg/m^2 can be safely administered to chemotherapy-naive patients in good overall condition, with myelosuppression as the main toxicity at the higher doses; however, additional toxicities such as reversible increase in hepatic transaminases and proteinuria were also observed [22]. A randomized study comparing 1,250 with 2,500 mg/m^2 gemcitabine (days 1, 8, 15) will elucidate whether dose escalation will result in improved efficacy in NSCLC. It is, however, possible that at 1,250 mg/m^2 saturation of gemcitabine triphosphate will be observed, although from preclinical studies it may be concluded that for certain diseases, dose escalation might be effective [15]. An increase of infusion time to 24 h was studied by *Anderson et al*. in patients with NSCLC [23]. The MTD was 180 mg/m^2 with dose-limiting neutropenia and lethargy. In this phase I study, 21% of patients (95% confidence interval: 7% to 42%) achieved a partial response. This interesting result is in accordance with an in vivo study in murine colon tumors [15], which suggests better antitumor activity of a weekly 24-h infusion of gemcitabine compared to a bolus administration. However, the disadvantage of this schedule is a considerable dose reduction (1,000 to 180 mg/m^2 in patients and 120 to 15 mg/kg in mice), which may result in a too low gemcitabine triphosphate accumulation.

In two studies, the effect of increasing the duration of infusion with a constant dose of gemcitabine was studied following the finding that in most human tumor cell lines and xenografts, accumulation of gemcitabine triphosphate was dependent on the total time of exposure [2, 3, 9, 24, 25]. In one study, the dose was kept constant and only the duration of infusion was prolonged up to 270 min in patients with solid tumors [26]. The MTD was reached with weekly doses of 300 mg/m^2 for a 270 min infusion, with myelosuppression as the dose-limiting toxicity. In a study in which only the dose rate was kept constant, the duration of infusion was prolonged up to 480 min (constant infusion rate: 10 mg/m^2/min) every three weeks in leukemia patients [27] with an MTD of 4,800 mg/m^2. At these gemcitabine doses, gemcitabine triphosphate levels reached the saturation limits of mononuclear and leukemia cells. Unfortunately, the antileukemia activity in this study, as determined by reduction of the percentage of blasts in the marrow, was disappointing. However, this might have been a result of reduction of the intensity of the chemotherapy. A more focused schema in which gemcitabine is infused at more frequent intervals will further elucidate the activity of gemcitabine in leukemia.

Phase II Studies

Most phase II studies have been performed utilizing the weekly schedule (days 1, 8 and 15) with a 30 min infusion, repeated every four weeks at 800 to 1,250 mg/m^2 (Table 1). Excellent activity has been found in several tumors usually not responsive to chemotherapy. In NSCLC, several studies

Table 1. Antitumor activity of gemcitabine in phase II studies

Tumor type	No. evaluable patients	Mean RR (%)	Reference
Bladder	99	31	[20, 29, 30, 31]
Breast	93	33	[32, 33, 34]
SCLC	29	27	[35]
NSCLC	338	22	[22, 36, 37, 38]
Cervix	28	18	[28]
Ovary	111	22	[39, 40, 41]
Head & neck	61	13	[42]
Renal	55	8.1	[43, 44]
Pancreas	139	11	[45, 46, 47]

SCLC: small cell lung cancer
NSCLC: non-small cell lung cancer
RR: response rate

have been performed varying the gemcitabine dosage among a total of 361 patients. In an early phase II study in the United States, the results were disappointing, with a response rate of 3%. However, this was mainly due to underdosing of these patients since the mean and median doses delivered were both under 750 mg/m^2 [48]. In later phase II studies, response rates of 20% to 22.5% with a mean survival time (MST) of 7.6 to 12.7 months [22, 36-38] have been observed, placing gemcitabine among the most active single agents in this notoriously difficult-to-treat cancer type. Interestingly, the incidence of responders was somewhat higher in females (24%) than in males (18%), which may be related to the differences in metabolism of gemcitabine in males and females [49]. Moreover, most patients showed an improvement in performance and symptoms, which were better in this single-agent treatment than in most currently used combination regimens [50]. In a 90 mg/m^2 twice-weekly schedule phase II study, the response rate was comparable to the once-weekly schedule studies (19.7%) [51]. However, toxicity occurred in a higher incidence than with the once-weekly schedule; therefore, it was concluded that the once-weekly schedule should be used in preference to the twice-weekly schedule.

Not only in NSCLC were good response rates observed [52]. In patients with breast cancer treated with 725 mg/m^2 gemcitabine, a response rate of 25% was found (95% confidence interval: 12.7% to 41.2%) with an MST of 11.5 months [35]. In bladder cancer, response rates varying from 27%-38% were observed both in untreated and pretreated (MVAC or cisplatin) patients, while in ongoing studies with 1,200 mg/m^2, even higher responses of up to 46% were observed. Less but considerable activity has been documented in ovarian, head and neck, and renal cancers [39-44]. In a phase II study in ovarian cancer patients the response rate was 19%, but, more important, the responses were observed in a poor prognostic group of patients, characterized by primary platinum resistance [39]. In patients with NSCLC and pancreatic cancer a decrease in pain, weight gain and improvement in performance status were observed, suggesting improvement of quality of life [31, 45, 53]. In a study conducted by *Rothenberg et al.* [46] disease-related symptom relief was used as an endpoint in a phase II study in pancreatic cancer patients previously treated with 5-fluorouracil (5-FU). A clinical benefit response (CBR) (defined as a 50% or greater decrease in pain intensity, or a 50% or greater reduction in daily analgesic consumption, or a 20-point or greater improvement in Karnofsky status) was achieved in 27% of 63 patients. Subsequently, a randomized single-blind trial of gemcitabine versus 5-FU was performed showing a 23.8% versus 4.8% difference in CBR [54], and median survival of 5.7 versus 4.2 months, respectively. In colorectal and gastric cancer, no significant responses were observed with the applied schedule [55, 56] in contrast to the results in preclinical models. Overall, the drug was well tolerated. The most frequent toxic effects were myelosuppression, a mild flu-like syndrome, nausea, vomiting, and skin rash.

Altogether the phase II studies showed that at its recommended dose, gemcitabine was an active agent in several chemoresistant cancers. More importantly, gemcitabine was active in pretreated cisplatin-resistant tumors. Not only in pancreatic cancer, but also in NSCLC, an improvement in clinical symptoms was observed, very much in line with several preclinical studies [15]. These data suggest that the possibilities of gemcitabine as a single agent have not yet been fully explored.

Combination Studies with Gemcitabine

Gemcitabine, because of its inherent ability to inhibit DNA replication and repair, is an attractive candidate for combination with therapy that causes DNA damage, including radiation. Preclinical studies have demonstrated synergism between gemcitabine and cisplatin in ovarian, NSCLC, head and neck cancer, and colon cancer cell lines [57-61] which is likely to be related to increased formation of DNA platinum adducts [59]. Pretreatment with gemcitabine gave the best results. In vivo studies with head and neck xenografts and murine colon cancer also demonstrated a schedule-dependent, at least additive, and in some lines, a more than additive effect of gemcitabine and cisplatin [57, 60]. In other in vivo studies with lung xenografts, treatment with cisplatin after the last dose of gemcitabine also enhanced antitumor activity [61]. Altogether, these studies indicate that more schedules of gemcitabine and cisplatin might be synergistic. Furthermore, other studies demonstrated additivity and synergism in some lung and ovarian cancer cell lines for gemcitabine and etoposide [62], mitomycin [62], 5-FUdR [63] and LY231514 [62], the multitargeted antifolate, topotecan, and 5-FU (unpublished observations).

The mild toxicity profile of gemcitabine at an active dose together with the nonoverlapping toxicity pattern of gemcitabine and cisplatin formed the basis for phase I/II studies of this combination in several malignancies. In all clinical studies utilizing different schedules of cisplatin and gemcitabine, an increased activity compared to each agent alone has been observed in NSCLC (Table 2). This is very much in line with the preclinical studies and indicates at least an additive effect. Gemcitabine preceding cisplatin [66] resulted in 26 partial responses (PR) and 1 complete response (CR), with an overall response rate of 58% (95% confidence interval; 44% to 72%) and manageable toxicity. In none of the phase II studies conducted so far, however, were response rates below 42% observed, and in all studies toxicity was primarily hematologic and easily manageable. It is of interest to note that although these studies were not

Table 2. Antitumor effect of several schedules of the combination of gemcitabine and platinum compounds in phase I and phase II studies in non-small cell lung cancer

Schedule		No. evaluable patients	RR (%)	Median survival (months)	Reference
Gemcitabine (mg/m^2)	Cisplatin (mg/m^2)				
1,000-1,800 (d 1,8,15)	30 (d 1, 8, 15)	47	30	6	[64]
1,000 (d 1,8,15)	100 (d 15)	43	42	10.2	[65]
1,000 (d 1,8,15)	100 (d 2)	48	54	13	[66]
1,000 (d 1,8,15)	100 (d 1)	26	42		[67]
1,000 (d 1,8,15)	100 (d 15)	50	52	13	[68]
1,200 (d 1,8,15)	100 (d 15 before dFdC)	40	48	10.7	[69]
	Carboplatin				
1,000 (d 1,8,15)	AUC 5.2 mg/ml/min	13	33	11.3	[70]

AUC: Area under the plasma concentration versus time curve.

randomized, altogether more than 50% of the patients were alive after 12 months, compared to six to nine months for most other regimens currently being employed. Gemcitabine with cisplatin is also being studied in other malignancies with encouraging results in pancreatic, ovarian, breast, and bladder cancers. Although the combination of gemcitabine with cisplatin is a very interesting treatment for patients with NSCLC, because of its high response rates and low toxicity, the optimal schedule is not clear. To study this schedule dependency in more detail in patients, a phase I study was conducted in which DNA platination and gemcitabine triphosphate accumulation were determined in white blood cells of patients treated with either gemcitabine (800 mg/m^2) four h before cisplatin (50 mg/m^2) or cisplatin four h before gemcitabine [71]. Because no clear differences were found comparing these treatments, an interval between drugs of 24 h is currently being studied. Because of the different toxicity profiles of carboplatin, several studies are currently being performed in which the targeted AUC was set at 6 mg/ml/min [70].

Several other combination studies with gemcitabine in NSCLC include combinations, e.g., with paclitaxel, etoposide, or vindesine (Table 3). Response rates in NSCLC in these ongoing studies are varying, from 21% (epirubicin) to 32% (ifosfamide). Gemcitabine in combination with 5-FU or hydroxyurea are currently being studied in phase I trials. A study with the combination of gemcitabine and doxorubicin is ongoing in advanced breast cancer, with a very promising response rate of 75% [77].

Gemcitabine has also shown excellent preclinical activity as a radiosensitizer in colon, pancreas, breast, and head and

Table 3. Phase I and II combination studies with gemcitabine

Schedule*		Cancer type	No. evaluable patients	RR (%)	Reference
Phase I	**MTD****				
Paclitaxel***	75-175 before dFdC#	Breast	42		[21]
Paclitaxel	135 (d 8) before dFdC	Ovarian	8	17	[72]
Vindesine	3 (weekly × 7)	NSCLC	32	27	[73]
Etoposide	80 (d 8, 9, 10)		28	15.6	[74]
Epirubicin	15 (d 1, 8, 15)	Breast	12	25	[75]
Phase II					
Ifosfamide	1,500 (d 8, 9, 10, 11, 12)	NSCLC	50	32	[76]
Doxorubicin	25 (d 1, 8, 15 after dFdC)	Breast	24	75	[77]

* Gemcitabine dosage: 1,000 mg/m^2 (d 1, 8, 15).
** Dosage in mg/m^2.
*** Paclitaxel and gemcitabine (q2wks), gemcitabine dosage: 1,500-3,500 mg/m^2.
# Dose-limiting toxicity (neutropenia and transaminase increase) 175/3,500 mg/m^2, recommended phase II doses 150/3,000 mg/m^2 (*M. Rothenberg*, personal communication).

neck cancer [78, 79] cell lines. These in vitro studies are being used as guidelines for development of clinical trials of gemcitabine with radiation therapy. However, initial studies in NSCLC showed grade 4 toxicity in seven out of eight patients, and the study was discontinued [80]. This was considered to be related to the high gemcitabine dose (1,000 mg/m^2) resulting in aspecific sensitization of normal tissues; therefore, in subsequent studies, the dose was reduced to 150-300 mg/m^2.

In addition to the doublets which have now been investigated extensively in various malignancies, a couple of logical triplets have entered clinical trials. These include combinations of gemcitabine, with a platinum and a taxane, etoposide, ifosfamide, or vinorelbine, or with radiation. These studies may represent additional problems since the sequence of the drugs can induce unexpected toxicities. The composition of the combinations will be determined by the disease, as in the use of gemcitabine, a platinum, and a taxane for NSCLC, SCLC, and ovarian cancer.

Future Prospects

From all studies, it is clear that gemcitabine is a very active drug in various malignancies. Considering the relatively low and easily manageable toxicity, it may be concluded that single-agent gemcitabine may have been underdosed. It is, however, possible that for certain diseases there is a maximal effective dose for gemcitabine. From preclinical studies, there is evidence that an increase in dose can result in a better antitumor activity in certain tumors but not in other tumors. This may depend on the drug penetration in solid tumors. Several clinical studies are under way which address this question for NSCLC. The efficacy of single-agent gemcitabine may also have been limited by its schedule. Again, from preclinical studies in cell lines and animals, there is evidence that prolongation of exposure may enhance its activity; in clinical studies in which the dose intensity is increased, prolongation of the infusion period up to five to six h may very well increase the accumulation of the active metabolite in the target tumor cell. With a more prolonged infusion the dose intensity has to be reduced, probably leading to a reduction of gemcitabine triphosphate.

Single agent gemcitabine, due to its favorable toxicity profile, is an attractive treatment regimen for several second-line treatments such as in cisplatin-resistant ovarian and lung cancers. Optimization of the schedule, however, can change the drug to a first choice in first-line treatment, especially when combined with another proper active drug. Randomized studies such as the ECOG trial comparing Taxol-cisplatin, taxotere-cisplatin, gemcitabine-cisplatin, and Taxol-gemcitabine may provide an answer on the questions which are the active regimens in NSCLC. Additional attractive treatment regimens include an alternation of doublets such as carboplatin-Taxol, carboplatin-gemcitabine, and cisplatin-topotecan in ovarian cancer, although the composition of the doublets and the length of cycles may be debatable.

Disease types in which gemcitabine combinations may result in a substantial increase in the therapeutic efficacy are pancreatic cancer and bladder cancer. In pancreatic cancer, the activity of single-agent gemcitabine warrants the study of combinations with cisplatin and 5-FU. Ongoing combination studies with cisplatin also indicate an increased response rate for the combination. The relatively high activity of single-agent gemcitabine in second-line treatment of bladder cancer (25%-30%) and the relatively high activity of gemcitabine-cisplatin in ongoing studies (45%-70%) warrant future studies in comparison with the MVAC schedule in this disease. Similarly, combinations with doxorubicin in breast cancer are warranted.

An important issue in the combinations with cisplatin is the scheduling. From the various clinical studies, it appears that more schedules are active; however, the schedule in which cisplatin is given one day after the last gemcitabine dose is less toxic. In most schedules in which cisplatin is given at day 1 or 2 or at days 1 and 7, the last dose at day 15 cannot be given due to increased toxicity. Omission of the last gemcitabine dose did not appear to decrease the response rate, and it may be advisable to change the gemcitabine-cisplatin schedule to gemcitabine at days 1 and 8 and cisplatin at day 1 or 2 and repeat this every three weeks.

In conclusion, gemcitabine is an important agent for improved management of several relatively chemoresistant cancer types, both with respect to antitumor activity and to clinical benefit and patients' tolerability. Further studies concerning the single agent as well as the combination with other anticancer drugs deserve high priority. These studies might well benefit from thorough knowledge of the metabolism, mechanism(s) of action, resistance profile, and the mode of interaction of gemcitabine with the other compounds.

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