Difluorodeoxyuridine plasma concentrations after low-dose gemcitabine during chemoradiation in head and neck cancer patients

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Abstract

Purpose: The aim of this study was to investigate whether relevant plasma levels of dFdU could be detected during concurrent chemoradiation (CRT) with low doses of dFdC administered in patients with head and neck cancer and to assess the toxicity related to dose.

Methods: dFdC was administered at doses of 5 mg/m² twice weekly or 10, 50 or 100 mg/m² weekly. Plasma concentrations of dFdU were determined daily for 7 days after the first administration and before each administration, thereafter. A high-performance liquid chromatographic method was used. During CRT, skin and mucosal toxicity were scored weekly according to the RTOG toxicity scoring system.

Results: Eight patients were sampled at the 10 and 50 mg/m² dose and nine at the 5 and 100 mg/m² dose. dFdU levels were in the micromolar range, inducing RS *in vitro*.There was a strong correlation between the area under the curve of dFdU and the dose of dFdC ($r = 0.803$, $p < 0.001$) and a weak correlation between trough concentrations and total dose of dFdC ($r=0.408$, $p=0.017$). Duration of severe mucositis correlated with dFdC dose.

Conclusions: During CRT with 10-100 mg/m^2 of dFdC weekly or 5 mg/m^2 twice weekly, dFdU remains detectable at potentially radiosensitizing concentrations.

Introduction

Gemcitabine (2',2'-difluorodeoxycytidine; dFdC) is a fluorinated pyrimidine nucleoside analogue (1) with antitumor activity against a wide variety of tumors, including head and neck cancer (2). Gemcitabine is a prodrug that requires several steps of phosphorylation to yield the active metabolites difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate (dFdCTP), which target DNA and RNA and are presumably responsible for the cytotoxic effect (1, 3, 4). dFdCTP is incorporated into DNA and inhibits DNA polymerase processing, whereas dFdCDP interferes with the enzyme ribonucleotide reductase, causing depletion of deoxynucleotide triphosphate necessary for DNA synthesis. The rate limiting step is the phosphorylation of gemcitabine into difluorodeoxycytidine monophosphate by deoxycytidine kinase (5-7). In addition to its cytotoxic effects, gemcitabine has potent radiosensitizing properties, as shown both in preclinical and clinical settings, with *in vitro* radioenhancement factors up to three, depending upon schedule and concentration (8). The current evidence suggests that accumulation in the S phase of the cell cycle, depletion of dATP pools, reduction of apoptotic threshold, inhibition of DNA synthesis and reduction of DNA repair might contribute to, or might even be essential for gemcitabine-mediated radiosensitization (9-13).

The radiosensitizing properties of gemcitabine have been exploited in patients with pancreatic cancer (8, 14, 15), other gastrointestinal cancers (8), lung cancer (8), cancer of the uterine cervix (16, 17) and head and neck cancer (18-20). In patients, gemcitabine is rapidly cleared from the plasma with a half-life of only a few minutes (21, 22). It is deaminated by deoxycytidine deaminase to its main metabolite difluorodeoxyuridine (dFdU) which has little cytotoxic activity at clinically relevant levels (23). However, dFdU has clear radiosensitizing properties *in vitro* when given in micromolar ranges (23) and has the additional advantage of a prolonged half-life (21, 22). Our hypothesis was that the radiosensitizing potential of dFdU could be of clinical importance even when very low doses of gemcitabine are given, when this metabolite would be continuously available in plasma in concentrations that in vitro are inducing

radiosensensitization. We therefore measured dFdU concentrations in plasma in patients with tumors in the head and neck region who were treated with low dose gemcitabine during chemoradiation.

Patients and methods

Study objectives

The aims of this study were to investigate whether relevant plasma levels of dFdU could be detected during concurrent chemoradiation with low doses of gemcitabine administered in patients with cancer in the head and neck region and to assess the acute toxicity related to gemcitabine dose.

Eligibility criteria

Included were patients who were considered fit enough by the multidisciplinary team of dedicated head and neck surgeons, radiotherapists and medical oncologists to receive gemcitabine-based concurrent chemoradiaton for tumors located in the head and neck region, with no restrictions regarding the site of the tumor, the histology, the disease setting (either after induction chemotherapy or as definitive chemoradiation or adjuvantly after surgery), age, performance status, or organ functions (serum creatinine, liver enzymes). Patients were informed about the pros and cons of gemcitabine-based chemoradiation and were to give informed consent.

Treatment

Concurrent chemoradiation was given either as definitive treatment alone or after induction chemotherapy or in the postoperative setting. The planned irradiation dose was 66-70 Gy in 33-35 fractions of 2 Gy over 6.5-7 weeks. Gemcitabine was administered over 30 minutes within 2 hours before irradiation at doses of 5 mg/m² twice weekly or 10, 50 or 100 mg/m² weekly along with radiation therapy.

Blood sampling and storage

Ten milliliter blood was drawn into a lithium heparin tube every 24 hours for 7 days, starting 24 hours after the first administration of gemcitabine, and before each

administration thereafter. Hundred microliter of a tetrahydrouridine (THU) solution 10 % was added. The mixture was vortexed immediately at 3941 rounds per minute for 15 minutes and the plasma was stored at minus 20 °C.

Analysis of dFdU in plasma

A high-performance liquid chromatographic (HPLC) method was used and validated for the determination of dFdU in human plasma. Floxuridine (5-flouro-2'-deoxyuridine was used as an internal standard. Tetrahydrouridine (THU) was used to prevent the deamination of gemcitabine to dFdU after sampling. Standard samples of blanc plasma were spiked with dFdU (50 ng-50.000 ng) and extracted in the same way as the other samples and used for a calibration curve. Separation was achieved isocratic on a Chrompack Sperisorb ODS-2 column (5 µm, 4,6 x 250 mm). The mobile phase was Pic B7 (Water Corporation) in a 10 % methanol solution (pH 3,1) with a flow rate of 1,0 ml/min. The analytes were detected by ultra violet detection at 270 nm. The limit of detection was about 50 ng/ml for dFdU. Within-run and between-run precisions were less than 10 % and average accuracies were between 90 and 110 %.

Plasma Extraction

For the sample pretreatment procedure, 100 µl internal standard work solution and 50 µl of a THU solution (10 mg/ml) were added to 200 µl of plasma. After vortexing, the sample was treated with 6 ml of iso-propanol (15 %) in ethyl acetate and mixed thoroughly. After centrifugation, the organic phase was transferred to a glass tube and evaporated till dry by vacuum centrifugation. The residue was redissolved in 1 ml of the mobile phase (a 5 x dilution) and filtered over a 0.45 µm PVDF HPLC-filter (Acrodisc, Waters Corporation) for HPLC injection (20 µl).

Calculations and toxicity scoring

The dFdU area under the curve during the first week of treatment was calculated using the trapezoid method. The mean trough concentration of dFdU for each patient and the median of the mean trough concentrations for each dose level were calculated.

The acute mucosal and skin toxicity during the chemoradiation were scored weekly according to the RTOG Acute Radiation Morbidity Scoring Criteria (24) by the same very

experienced radiotherapist who was blinded to the gemcitabine dose. The sum of the number of weeks with grade 3 or 4 toxicity during the treatment was calculated for each patient.

Statistics

Correlations were calculated using the Spearman's rho test. Means were compared using the one way ANOVA after performing a Levene's test in order to exclude significant variances within groups. All statistical analyses were calculated using Statistical Package for Social Sciences (SPSS) version 15.

Results

Patient population

Thirty-four patients were included in the study between October 2006 and June 2008. Patient, tumor and treatment characteristics are summarized in table 1. The results are summarized in tables 2 and 3. Eight of the 34 patients received chemoradiation in the postoperative setting, five in the 5 mg/m^2 dose group and one each in the other dose groups. Therefore, the patients in the 5 mg/m^2 dose group received proportionally a lower radiation dose than the other three groups. The area under the concentration-time curve (AUC) of dFdU by dose level of dFdC during the first week of treatment is shown in figure 1. We found a clear correlation between the AUC of dFdU and the gemcitabine dose level (Spearman's rho = 0.805; $p < 0.0001$).

Figure 2 shows the dFdU concentrations versus gemcitabine dose for each day during the first week of treatment. Of notice, in the 5 mg/m^{2}dose group, patients received the second administration after the trough concentrations measured on day 4. Figure 3 shows the means and the 95 % confidence intervals of all dFdU trough concentrations by dFdC dose (mg/m²). Mean dFdU trough concentrations were not significantly different between the four different dose levels. Difluorodeoxyuridine remained detectable in plasma of the majority of patients even after doses as low as 10 mg/m² once weekly or 5

mg/m² twice weekly. However, there was a wide interpatient variation as illustrated by the wide confidence intervals.

Skin and mucosal toxicity is summarized in table 4. The majority of patients developed grade 3 or 4 mucositis and grade 2 radiodermatitis, regardless of the dFdC dose. However, there was a correlation between the sum of the number of weeks with grade 3 or 4 mucositis during the treatment and the gemcitabine dose given during irradiation (figure 4; Spearman's rho: 0.592; $p < 0.0001$). Hematologic toxicity is summarized in table 5. Grade 3 or 4 hematological toxicity was rare and occurred only in patients who had received prior induction chemotherapy before the concurrent use of low-dose gemcitabine and radiation.

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Discussion

Difluorodeoxyuridine remained detectable in plasma of our patients, even after administration of doses of gemcitabine as low as 10 mg/m² once weekly or 5 mg/m² twice weekly. There were substantial interpatient variations at the four studied dose levels, reflecting the wide variation in pharmacokinetics and pharmacodynamics of the drug (25-28). Indeed gene polymorphisms were reported for several enzymes involved in dFdC metabolism, including deoxycytidine kinase and cytidine deaminase (29-31). Difluorodeoxyuridine was previously shown to be a very potent radiosensitizer in vitro in multiple cell lines after exposure for only 24 hours, even at a concentration of 10 µM (23). In most of our patients, we measured trough concentrations within the micromolar range. Moreover, if we take into account that the elimination of dFdU after the second and subsequent administrations follows the same pattern as after the first administration, considerably higher levels of dFdU were probably present for several days during the radiation in the majority of patients, even at these low gemcitabine dosages. Although multiple factors most likely are responsible for the radiosensitizing effect of dFdC (9-13), the sustained presence of dFdU in the blood could at least partly explain the often dramatic radioenchancement which is observed after administration of gemcitabine, despite short half life of the parent drug.

We are fully aware of the limitations of this study due to the low number of patients in each treatment cohort and the differences in age, tumor type, tumor site, and tumor stage. However, with these limitations in mind, we observed no correlation between the dose or dose level and the maximum grade of skin and/or mucosal toxicity. In contrast, when we took into account the duration of grade 3 or 4 toxicity during the treatment, a significant correlation was observed between the weeks spent with grade 3 or 4 mucositis and dose level. The heterogeneity of the patient and treatment characteristics precludes any evaluation of the long term toxicity related to gemcitabine dose.

Concomitant chemoradiation with low doses of gemcitabine induces little hematologic toxicity, even after prior induction chemotherapy. Renal toxicity is extremely rare, even at standard cytotoxic doses (32). When used as a single agent, gemcitabine does not cause neurotoxicity or ototoxicity (32). These characteristics of the compound can be extremely useful in patients previously treated with cisplatin (e.g. after induction chemotherapy), or in patients with preexisting neurophathy, hearing loss or impaired renal function. Nevertheless, caution is warranted when patients are treated with severe impairment of the renal function, as elimination of dFdU is dependent on renal excretion and can accumulate in patients with renal failure (33, 34).

The parameters in this study, as summarized in table 2, are of course not sufficient to discuss the complete pharmacokinetic profile of gemcitabine and dFdU.

We conclude that during chemoradiation with 10-100 mg/m^2 of gemcitabine weekly or 5 mg/m^2 twice weekly, dFdU remains detectable at potentially radiosensitizing concentrations. Such gemcitabine doses are significantly correlated with the AUC of dFdU and the duration of severe mucositis.

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Table 1: Patient, tumor and treatment characteristics

			Weekly gemcitabine dose (mg/m²)			
			2 x 5	10	50	100
			N	N	N	N
Total			9	8	8	9
Male			7	6	6	7
Median age in years (range)			57 (51-85)	62 (60-74)	63 (50-73)	67 (49-78)
Median WHO performance status (range)			1 (0-3)	1 (0-1)	1 (0-2)	1 (0-1)
Median creatinine clearance (range)*			96 (38-125)	93 (67-98)	94 (38-154)	72 (45-128)
Median total bilirubine (range)**			0.4 (0.1-0.6)	0.4 (0.2-0.8)	0.45 (0.3-0.6)	0.3 (0.2-0.5)
Tumor site						
	oropharynx		1	3	1	4
	hypopharynx		1	1	1	3
	nasopharynx			2		
	larynx		1		3	1
	oral cavity		1	2		
	maxillary sinus				1	1
	skin		2			
	salivary gland		2		1	
	other		a		b	
Tumor stage						
	T1	N2		1		
		N3				1
	T2	N1			1	1
		N2	1	1	1	2
		N3				1
	T3	N2	1	2		
	T4	N0		1	3	1
		N1				1
		N2	2	1	1	1
		N3		1		
Recurrent disease					1	
Prior treatment						
	Induction chemotherapy		4	7	1	6
	Surgery		5	1	1	1
Radiotherapy cumulative dose					1	
	66 Gy		4	2		
	70 Gy		5	6	7***	9

a: unknown primary; b: tumor of vestibulum nasi; *: ml/min, calculated using the Modified Cockcroft-Gault formula;**: mg/dl; ***: radiotherapy was stopped after 42 Gy in 1 patient due to toxicity

Table 2: First-week dFdU AUC and dFdU trough concentrations

	weekly gemcitabine dose (mg/m²)			
	2 x 5	10	50	100
Median AUC (ng.hour/ml)	46371	48840	161884	205908
Trough conc. (ng/ml), median of mean*	418	248	613	527
Trough conc. (ng/ml), range of mean	118-559	25-2987	384-2201	211-1118

*for each patient; AUC = area under the concentration-curve

Table 3: Relevant correlations

Independent variable	Dependent variable	r	p
gemcitabine dose (mg/m²)	mean trough concentration	.339	.05
gemcitabine dose (mg/m²)	AUC	.805	< .0001
gemcitabine dose (mg/m²)	weeks grade 3/4 mucositis	.535	.001
total gemcitabine dose	AUC	.803	< .0001
total gemcitabine dose	mean trough concentration	.408	0.017
total gemcitabine dose	weeks grade 3/4 mucositis	.592	< .0001
total dose divided by creatinine clearance	mean trough concentration	.364	.034
total dose divided by creatinine clearance	AUC	.818	< .0001
creatinine clearance	AUC	-.305	.073

r = Spearman's rho correlation factor; AUC = area under the concentration-curve

Table 4: Non-hematologic toxicities

Patients who received no induction chemotherapy

gemcitabine dose (mg/m²)	N	Pharyngitis				Dermatitis				Mucositis			
		grade 0	grade 1	grade 2	grade 3	grade 0	grade 1	grade 2	grade 3	grade 1	grade 2	grade 3	grade 4
2 x 5	**5**		1	4			3	2			2	3	
10	**1**			1			1					1	
50	**6**			5	1	1	1	3	1			6	
100	**3**				3			2	1			3	

Patients who received induction chemotherapy

gemcitabine dose (mg/m²)	N	Pharyngitis				Dermatitis							
		grade 0	grade 1	grade 2	grade 3	grade 0	grade 1	grade 2	grade 3	grade 1	grade 2	grade 3	grade 4
2 x 5	**4**			2	2		1	3				3	1
10	**7**			4	3		3	3	1			6	1
50	**2**			1	1	1		1				2	
100	**6**	*		1	4		1	5				5	1

N = number of patients; *: no score available for 1 patient

Table 5: Hematologic Toxicities

Patients who received no induction chemotherapy

gemcitabine dose (mg/m²)	N	Hemoglobin				Thrombocytes			ANC				
		grade 0	grade 1	grade 2	grade 3	grade 0	grade 1	grade 2	grade 0	grade 1	grade 2	grade 3	grade 4
2 x 5	**5**	3	1	1		3	2		5				
10	**1**		1			1			1				
50	**6**	3	3			3	3		3	2	1		
100	**3**		2	1		3			2	1			

Patients who received induction chemotherapy

gemcitabine dose (mg/m²)	N	Hemoglobin				Thrombocytes			ANC				
		grade 0	grade 1	grade 2	grade 3	grade 0	grade 1	grade 2	grade 0	grade 1	grade 2	grade 3	grade 4
2 x 5	**4**		2	2		2	2		4				
10	**7**		3	3	1	5	2		7				
50	**2**		1	1		1	1		1	1			
100	**6**			5	1	2	3	1	2	2	1		1

N = number of patients; ANC = absolute neutrophil count

Figure legends

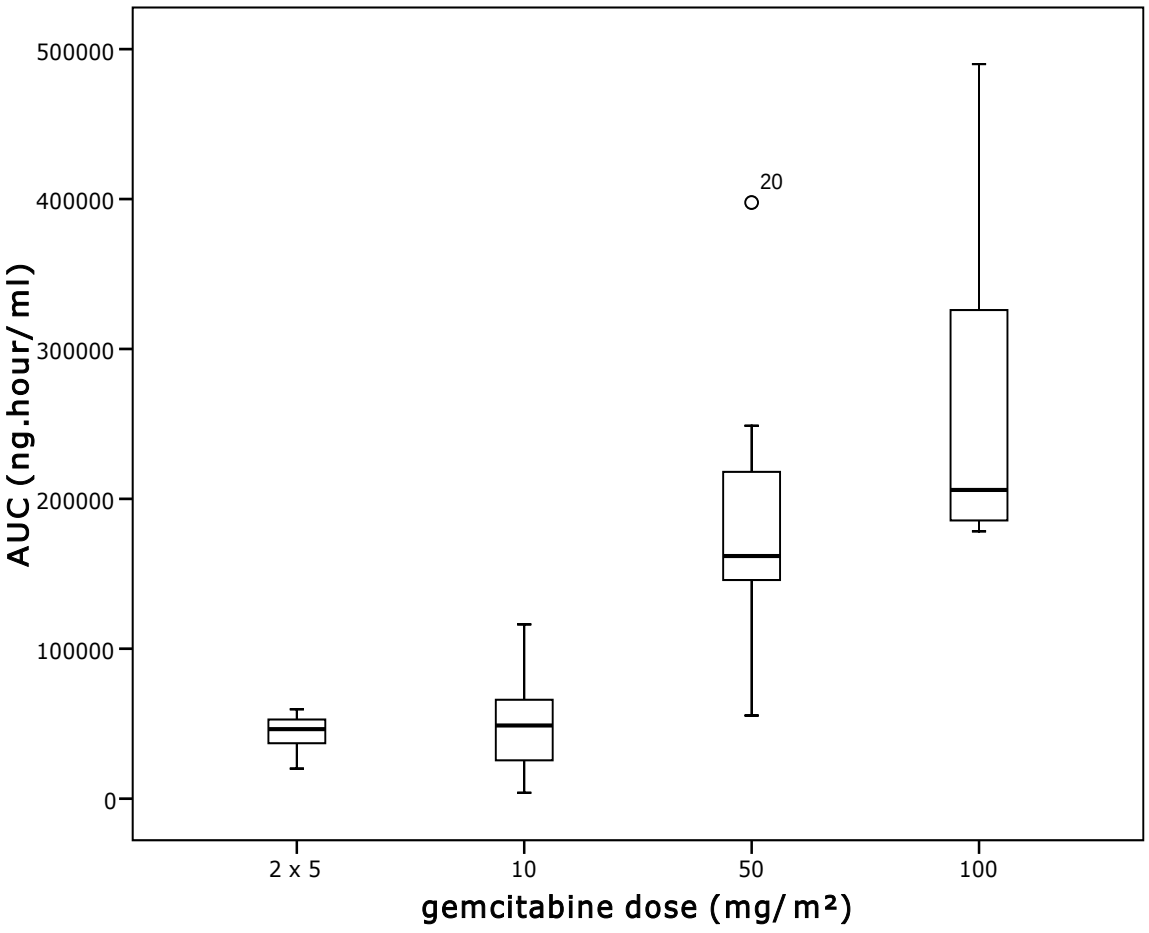
Figure 1: First-week dFdU area under the concentration-time curve (AUC) versus gemcitabine dose

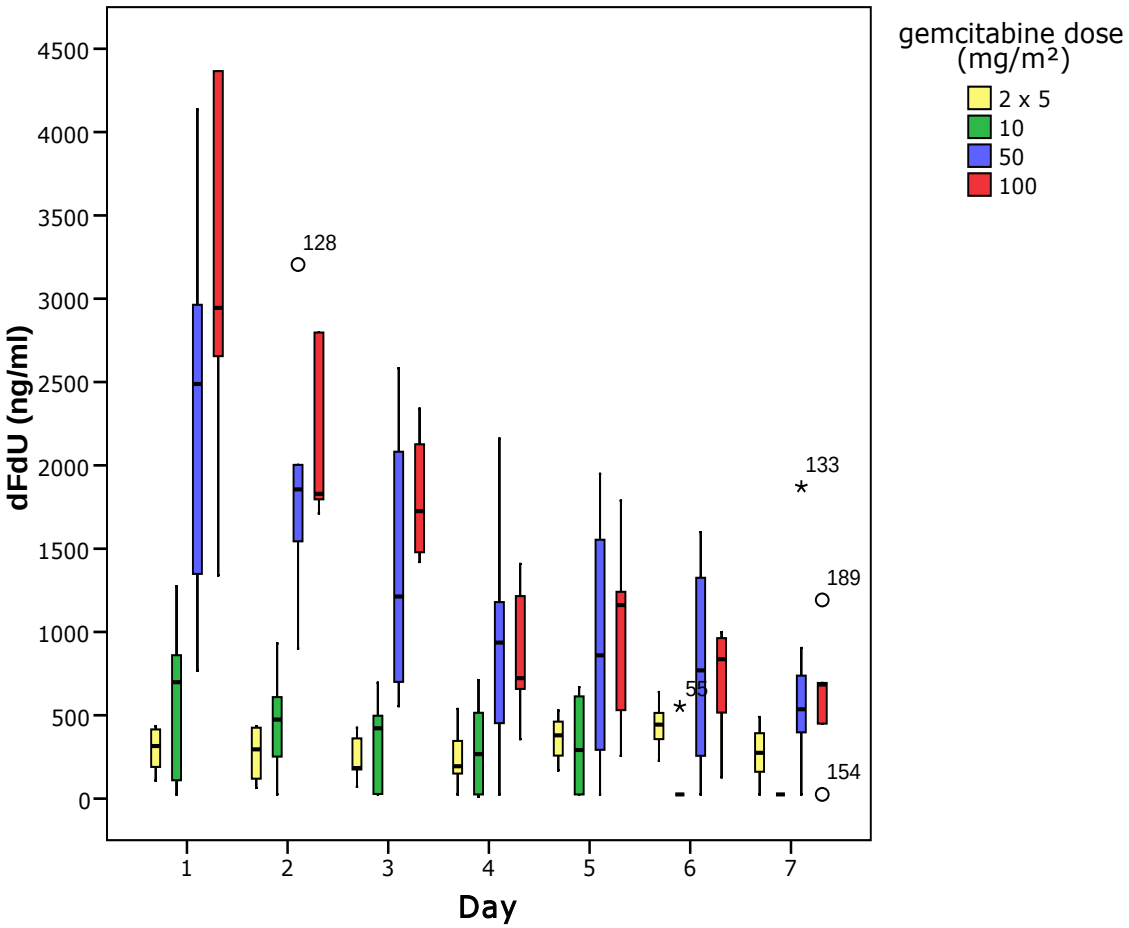
Figure 2: dFdU concentrations (median and range) in the first 7 days in the four different gemcitabine dose groups

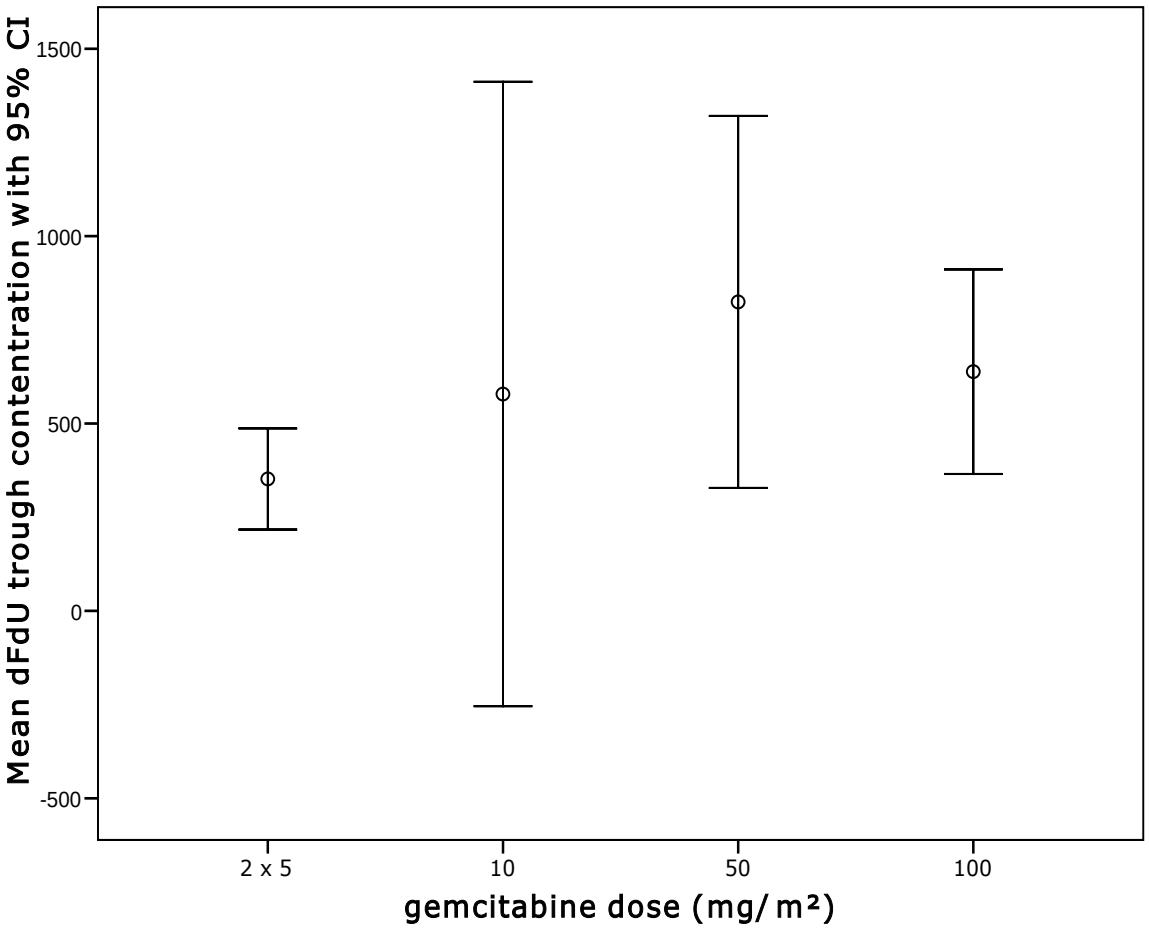
*°:Symbols and numbers represent outliers

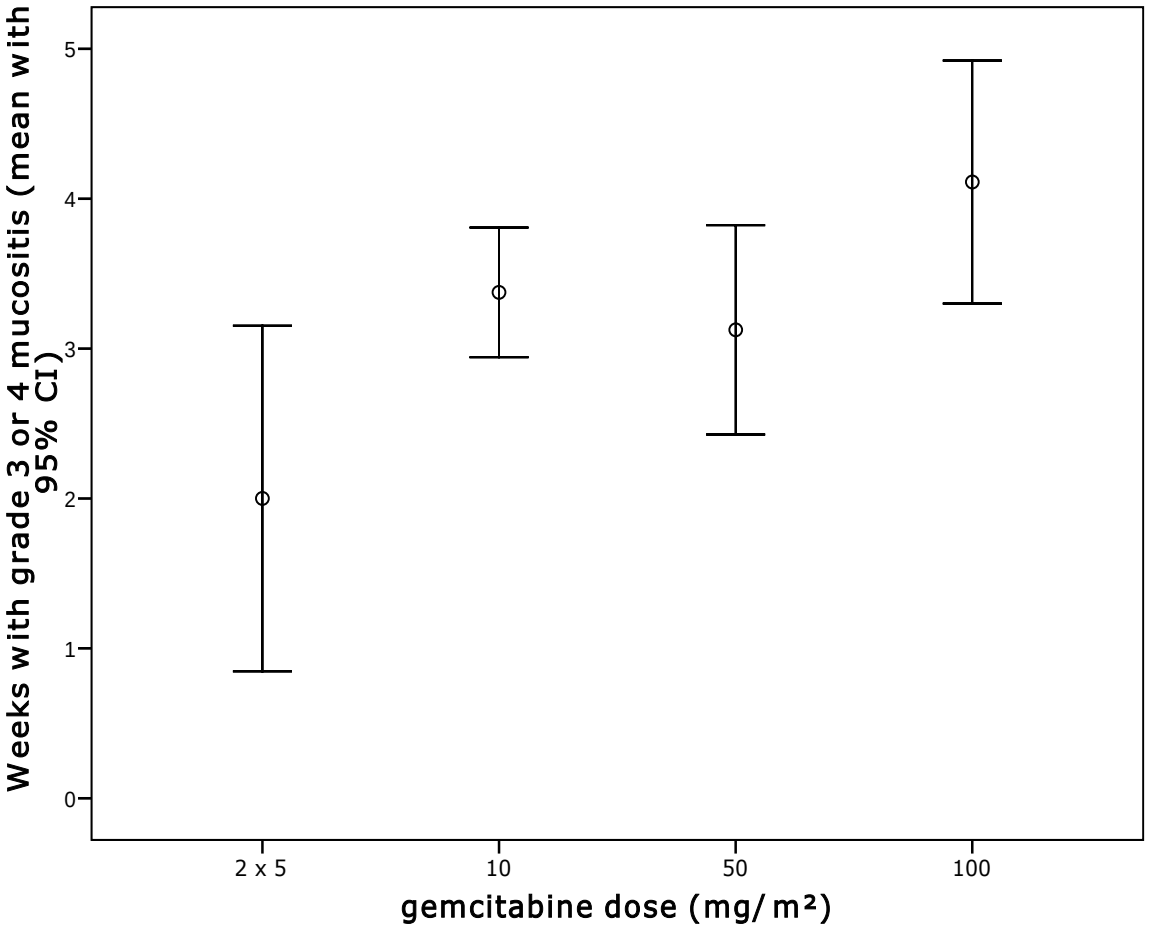
Figure 3: Mean of all dFdU trough concentrations (and 95% confidence intervals) in the four gemcitabine dose groups

Figure 4: Weeks with grade 3 or 4 mucositis versus gemcitabine dose




gemcitabine dose (mg/m²)
2 x 5
10
50
100
dFdU (ng/ml)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
128
133
189
154
55
1
2
3
4
5
6
7
Day


Mean dFdU trough contentration with 95% CI
1500
1000
500
0
-500
2 x 5
10
50
100
gemcitabine dose (mg/ m²)


Weeks with grade 3 or 4 mucositis (mean with 95% CI)
0
1
2
3
4
5
2 x 5
10
50
100
gemcitabine dose (mg/m²)