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ORIGINAL ARTICLE

Phase II study with the combination of gemcitabine and DTIC in patients with advanced soft tissue sarcomas

R. Losa · J. Fra · A. López-Pousa · M. Sierra · A. Goitia · E. Uña · R. Nadal · J. García del Muro · M. Gión · J. Maurel · P. Escudero · E. Esteban · José M. Buesa

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Abstract *Purpose*: Based on the promising results of a Phase I study with a combination of gemcitabine and DTIC performed in advanced soft tissue sarcoma (ASTS) patients, and due to the limited efficacy of second or third line therapies in those patients, we designed a Phase II study to determine the activity of this new regimen. *Methods*: Patients with ASTS, measurable disease, pretreated with chemotherapy, received gemcitabine 1,800 mg/m^2 infused over 180 min followed by DTIC 500 mg/m^2 (one cycle), every 2 weeks. The pharmacokinetics (PK) of gemcitabine and 2′,2′-difluorodeoxyuridine (dFdU), and the accumulation of gemcitabine triphosphate (dFdCTP) by peripheral blood mononuclear cells were studied. The influence of the sequence of administration on those parameters was examined to exclude potential drug interactions. *Results*: Twenty-six patients received a total of 158 cycles (mean four cycles, range 1–18). Grade 3–4 anemia (23% of patients), granulocytopenia (46%) or thrombocytopenia (12%), and grade 3 increase in AST (18%), ALT (21%), or γ-glutamyltransferase (9%) were noted. Response rate in 23 patients was 4% (95% CI: 0–24%), and in 8 of 11 patients stable disease lasted > 6 months. Progression-free rate (PFR) at 3 and 6 months was, respectively, 48 and 28%, and median overall survival 37 weeks. Pooled data from the Phase I and Phase II studies showed clinical benefit in patients with leiomyosarcomas (LMS) (57%) and malignant fibrous histiocytomas (MFH) (33%). The sequence of administration did not influence PK of gemcitabine or dFdU. There was a trend ($P = 0.11$) toward a lower accumulation of dFdCTP when DTIC preceded gemcitabine. *Conclusions*: Although the remission rate was low, PFR figures indicate that this regimen has activity in patients with ASTS. It should be compared with DTIC, or other gemcitabine-containing combinations, in patients with LMS or MFH, to determine whether this combination offers advantages in PFR or in overall activity.

Keywords Gemcitabine · DTIC · Soft tissue sarcomas · Pharmacokinetics

J. Fra and R. Losa contributed equally to this work.

R. Losa · J. Fra · M. Sierra · A. Goitia · E. Uña · M. Gión · E. Esteban · J. M. Buesa (✉)
Servicio de Oncología, Hospital Central de Asturias, 33006 Oviedo, Spain
e-mail: jmbuesa@hca.es

A. López-Pousa · R. Nadal
Hospital de Sant Pau, Barcelona, Spain

J. G. del Muro
Instituto Catalán de Oncología, Hospitalet, Barcelona, Spain

J. Maurel
Hospital Clínic, Barcelona, Spain

P. Escudero
Hospital Universitario Lozano Blesa, Zaragoza, Spain

Introduction

Patients with an advanced soft tissue sarcoma (ASTS) progressing to schedules of doxorubicin and ifosfamide have limited therapeutic options [4]. Dacarbazine (DTIC) is a drug which induces an 18% remission rate in this clinical situation [6], whereas gemcitabine has

shown an activity that varies from 3 to 18% in several Phase II studies, activity that seems limited to patients with leiomyosarcoma (LMS) or angiosarcoma [21, 23, 32]. ET-743, a marine-derived compound, has disclosed a 4–10% remission rate when given as second- or third-line to ASTS patients and is under active research [37]. The combination of taxotere and gemcitabine, on the other hand, has shown a 43–53% activity, especially in patients with LMS [13, 17].

Because no good alternatives existed for this group of patients, we decided to explore the efficacy of a combination of DTIC and gemcitabine, two agents with different mechanisms of action and moderate toxicity. No preclinical studies were available and, as this regimen had not been studied before in the clinic, we first performed a Phase I study to determine its toxicity and the recommended dose [5]. Single agent gemcitabine is usually delivered at 1,000–1,250 mg/m^2 as a 30 min infusion on a 3 out of 4-week schedule [28]; however, on day 14 the dose has to be frequently omitted due to toxicity, what has derived in the administration of fortnightly regimens. The results of some of those studies indicated that 2,200 mg/m^2 of gemcitabine infused over 30 min every 2 weeks caused mild toxicity and no dose reductions were necessary [25, 34]. In ASTS patients DTIC is usually given either at 250 mg/m^2/day for 5 consecutive days [10] or at 1,200 mg/m^2 every 3 weeks [6], while doses of 750 mg/m^2 are applied when combined with other agents [27, 39]. With those antecedents, and although no prior studies had been conducted with DTIC administered every 2 weeks, a fortnightly schedule was selected to combine DTIC and gemcitabine.

Gemcitabine is a nucleoside that has to be phosphorylated to its diphosphate and triphosphate (dFdCTP) forms to exert its biologic effect. This agent is deaminated by cytidine deaminase generating 2′,2′-difluorodeoxyuridine (dFdU), a metabolite that is considered inactive [26]. Both activation and deamination pathways take place intracellularly, with plasma deaminase activity contributing minimally to gemcitabine catabolism. Data on dFdCTP kinetics have been obtained in peripheral blood mononuclear cells (PBMCs) and, in vivo, it has been shown that gemcitabine activation by cytidine kinase, the limiting step in this pathway, is optimal with gemcitabine plasma concentrations of 26 μM [1, 12]. This concentration is achieved when gemcitabine is infused at a fixed dose-rate (FDR) of 10 mg/m^2/min [12], so that this form of administration, a more rational way of delivering this agent than the usual 30 min infusion, has been applied in our studies. The clinical relevance of this, however, has to be demonstrated, and a randomized trial that compared both types of administration in patients with advanced pancreatic adenocarcinoma did not demonstrate a higher efficacy of FDR infusion [33].

In the Phase I study, the dose of gemcitabine ranged from 800 to 2,160 mg/m^2 while that of DTIC was fixed at 500 mg/m^2. Liver toxicity, characterized by an increase in ALT and γ-glutamyl-transferase values, was dose-limiting, and doses recommended for subsequent studies were gemcitabine 1,800 mg/m^2 infused over 3 h and DTIC 500 mg/m^2, both repeated every 2 weeks. In this study, a promising 26% remission rate was observed in 19 assessable patients [5]. Theoretically, when gemcitabine is combined with another agent, an interaction could occur that would influence gemcitabine pharmacokinetics (PK) or activation as it has been shown, respectively, for combinations of gemcitabine with vinorelbine [8] or paclitaxel [15]. In this respect, preliminary data obtained during the Phase I study compared with previously published reports, suggested that the administration of DTIC immediately after the infusion of gemcitabine did not influence gemcitabine PK nor the synthesis of dFdCTP by PBMC.

The purpose of the present Phase II study was to further evaluate the activity and the toxicity of this new combination delivered at the recommended dose, information that could constitute the basis for an eventual Phase III trial. Another objective was to exclude potential drug interactions, dependent of the sequence of administration, between gemcitabine and DTIC.

Materials and methods

Patients

Eligible patients should have: histologic diagnosis of ASTS; locally advanced or metastatic, progressive disease; measurable disease; performance status ≤ 2 (WHO); adequate bone marrow (leukocytes ≥ 3.5×10^9/l, granulocytes ≥ 1.5×10^9/l, platelets ≥ 100×10^9/l), liver (serum bilirubin ≤ 1.5-fold and AST and ALT < 2.5-fold upper normal limits), and renal (serum creatinine ≤ 1.5 mg/dl) function. Prior therapy with doxorubicin or ifosfamide was required for protocol inclusion. Those patients who had received gemcitabine at doses lower than those of the present study, or a protracted oral temozolomide regimen, were also eligible. Patients younger than 18 years, with severe associated diseases, active infection, or CNS metastases were excluded. Ethics Committee of participating institutions approved the study and informed patients signed a consent form.

Treatment

Patients received gemcitabine 1,800 mg/m^2 immediately followed by DTIC 500 mg/m^2, every 2 weeks. This constituted one cycle of therapy. Gemcitabine was given at a rate of 10 mg/m^2/min, and DTIC was infused over 20 min, protected from light exposure. The dose of both agents was diluted in 250 ml of normal saline. Antiemesis consisted of anti-HT_3 plus dexamethasone at standard doses, although dexamethasone was omitted when gemcitabine kinetics were studied [38]. Cycles were repeated on schedule if transaminase or γ-glutamyl-transferase values were < 2.5 its upper normal value, granulocytes $\geq 1.0 \times 10^9$/l, and platelets $\geq 100 \times 10^9$/l. Otherwise, the cycle was delayed by 1 week or until reaching those values, and the dose of gemcitabine reduced to 1,500 mg/m^2. If toxicity recurred, the dose of DTIC was also reduced to 400 mg/m^2.

Study parameters

Patients were seen weekly during the first two cycles, with BCC and serum chemistries performed, to determine and grade both analytical and clinical toxicity according to CTC NCI version 2.0 [7]. Measurable lesions were evaluated every 2 months (or after four cycles of therapy), and response to therapy was assessed by WHO criteria [22]. Time to progression was the time elapsed from inclusion until progressive disease was first detected. Progression-free rate (PFR) was the proportion of patients without progression at a given time. Progression-free survival was equal to zero in patients with progressive disease at the time of the first evaluation, and equal to the interval from inclusion to first evidence of progression in responders and in those patients with disease stabilization [36].

The dose intensity per patient was calculated by dividing the total dose given (mg/m^2) by the time elapsed from the first to the last dose, plus 2 additional weeks. Relative dose intensity was the ratio of received to projected dose intensity.

Pharmacokinetic analysis

In order to compare the possible influence of the sequence of administration of gemcitabine and DTIC on gemcitabine kinetics, some patients received DTIC immediately followed by gemcitabine and the opposite sequence in two consecutive cycles. Blood samples were collected in heparinized tubes containing tetrahydrouridine (a cytidine deaminase inhibitor). Time 0 was the beginning of gemcitabine infusion, and samples were obtained at baseline, at 60, 120 min, just before completion of infusion, and hourly during 8 h. Samples were placed on ice, centrifuged, and plasma stored at −26°C. PBMCs were isolated through a Ficoll-Hypaque gradient, and preserved at −70°C. Gemcitabine and dFdU plasma concentrations and dFdCTP levels in PBMCs were determined by reverse phase HPLC, according to published methods [18, 19]. The lowest limit of quantification for gemcitabine and dFdCTP were, respectively, 0.36 and 0.174 μg/ml.

Gemcitabine concentration at steady state (C_{ss}) was the mean of values after equilibrium was reached. C_{max} was the highest concentration detected for dFdU and dFdCTP. The area under the concentration–time curve ($AUC_{0-8\,h}$) for gemcitabine, dFdU, and dFdCTP was obtained by applying the linear trapezoidal rule from time 0 until 8 h from start of gemcitabine infusion. AUC_{inf} was the accumulation of dFdCTP in PBMCs during gemcitabine infusion. Total body clearance for gemcitabine was obtained from the relation Dose/AUC.

Statistics

Sample size was calculated according to the two-stage optimal design of Simon, with $\alpha = 0.05$ and $\beta = 0.20$, $P_0 = 10\%$ and $P_1 = 25\%$ [29]. If less than two remissions occurred in the first 22 patients, the study should be interrupted because objective activity would be lower than 10%. Time events were estimated according to the method of Kaplan and Meier [14].

Mean ± standard deviation (sd) of the different PK parameters were determined, and data on day 14 were pair-compared with those on day 1 by the Wilcoxon signed ranks test. The correlation between PK and analytical parameters was examined by means of the Spearman's coefficient. All P values presented are two-sided.

Results

From January 2003 to November 2004, 26 patients were included in the study (Table 1). Five patients had been pretreated with a combination of doxorubicin (60 mg/m^2 on day 1) and gemcitabine (800 mg/m^2 on days 1 and 8), 17 had received doxorubicin plus ifosfamide, 2 temozolomide, and 2 other combinations containing doxorubicin. Three patients whose lesions were not measured were not assessable for objective activity, although were included in PFR analysis as progressing at time 0. A total of 158 cycles were delivered with a median of four cycles per patient (range 1–18).

Table 1 Patient characteristics

Number	26
Male/female	17/9
Age (median, range)	51 (24–75)
Performance status:	
0	10
1	12
2	4
Histologic type:	
Malignant fibrous histiocytoma	6
Leiomyosarcoma	4
Rhabdomyosarcoma	3
Liposarcoma	2
Neurosarcoma	2
Other or unclassified	9
Grade of malignancy:	
1	3
2	3
3	20
Primary site:	
Trunk and limbs	14
Retroperitoneum	5
Uterine	4
Other	3
Disease-free interval:	
≤ 12 months	16
> 12–24 months	2
> 24 months	8

Toxicity

Hematologic toxicity is summarized in Table 2. Grade 3–4 toxicity on granulocytes was noted in 46% of patients, and grade 3–4 lymphopenia in 96%. On day 8 (data available from 36% of cycles), there was grade 3 and 4 neutropenia in 16 and 14% of cycles, respectively, grade 3–4 lymphopenia in 78%, grade 3 increase of AST values in 12% and of ALT in 33%. By day 14 there was grade 3 toxicity on granulocytes (2%) and on ALT values (2%), with grade 3 and 4 toxicity on lymphocytes still present in 45 and 7% of cycles, respectively.

One patient previously treated with two 6-weekly cycles of temozolomide and grade 3 toxicity on platelet count, developed grade 4 toxicity on hemoglobin and platelets on day 12 of the first cycle and abandoned the study due to excessive toxicity. Another patient pretreated with ifosfamide and doxorubicin, had grade 2 and 3 toxicity on platelets during the first and the second cycle of protocol therapy, respectively, with recovery by day 14, but developed persistent grade 4 thrombocytopenia after cycle 3 requiring multiple platelet transfusions.

Liver toxicity was reversible and no cumulative (Table 3). Overall, seven patients (27%) had some type of grade 3 hepatic toxicity. All other side-effects were moderate, with only asthenia and vomiting reaching grade 3 in 20 and 4% of patients, respectively (Table 4).

Fourteen percent of cycles were delayed a median of 1 week (range 1–3) due to toxicity. Median (range) dose intensity was 869 (500–973) mg/m^2/week for gemcitabine and 241 (155–259) mg/m^2/week for DTIC, with a median relative dose intensity of 96% (55–1.08%) for gemcitabine and 96% (62–1.03%) for DTIC.

Table 2 Hematologic toxicity (% of patients)

	NCI CTC grade				
	0	1	2	3	4
Hemoglobin	8	42	27	15	8
Leukocytes	19	15	19	35	12
Lymphocytes	–	–	4	44	52
Granulocytes	27	8	19	15	31
Platelets	42	27	19	4	8

Efficacy

In 23 assessable patients, 1 complete remission, 11 stabilizations, and 11 progressions were noted (remission rate 4%, 95% CI: 0–24%). The complete response was observed in a patient with a malignant fibrous histiocytoma (MFH) previously responding to gemcitabine

Table 3 Liver toxicity (% of patients)

	NCI CTC grade			
	0	1	2	3
AST	18	50	14	18
ALT	21	33	25	21
γ-Glutamyl-transferase	36	32	23	9
Alkaline phosphatase	39	44	17	–
Bilirubin	88	–	12	–

Table 4 Non-hematologic toxicity (% of patients)

	NCI CTC grade			
	0	1	2	3
Alopecia	50	23	27	–
Asthenia	12	32	36	20
Anorexia	50	31	19	–
Nausea	70	11	19	–
Vomiting	69	15	12	4
Diarrhea	84	12	4	–
Stomatitis	69	23	8	–
Esophagitis/dysphagia	85	11	4	–
Flu-like syndrome	77	19	4	–
Fever	58	34	8	–
Pruritus	81	15	4	–

and doxorubicin. In Fig. 1 we present the lung CT scans of this patient. The duration of stable disease was > 6 months (median 40 weeks) in 8 out of 11 patients. Four of the five patients pretreated with doxorubicin (60 mg/m^2) plus gemcitabine (800 mg/m^2) were evaluable for objective activity. One patient with complete remission and another with partial remission achieved now a second complete remission and a stabilization of the disease lasting > 6 months, after an interval between both treatments of, respectively, 7 and 9 months; another two patients, one with disease stabilization and the second in progression under doxorubicin and gemcitabine, progressed to present therapy.

Three patients had uterine leiomyosarcoma (ULMS): one was not evaluable for activity due to excessive toxicity (thrombocytopenia); another had received adjuvant doxorubicin, had stabilization of her intrabdominal metastases with temozolomide, and also had stable disease for 10 months with protocol therapy; the third patient had achieved a partial remission with doxorubicin plus gemcitabine, and now obtained a stabilization longer than 6 months. A patient with a retroperitoneal LMS who had progressed to doxorubicin had stable disease.

Median time to progression was 15.4 weeks, and PFR (±standard error) at 3 and 6 months in 26 patients was 48 ± 10 and 28 ± 9%, respectively. With a median follow-up of 145 weeks (range 114–152 weeks) for the patients alive, median overall survival for the whole group was 37 weeks. At the moment of this analysis eight patients were alive, five with active disease and three apparently disease-free (two after complete resection of the disease and one treated with radiotherapy after a marginal resection of the primary tumor).

Pharmacokinetic study

The influence of the sequence of administration on gemcitabine kinetics was studied in six patients who received DTIC followed by gemcitabine and the alternate sequence 14 days apart. Overall, there were not

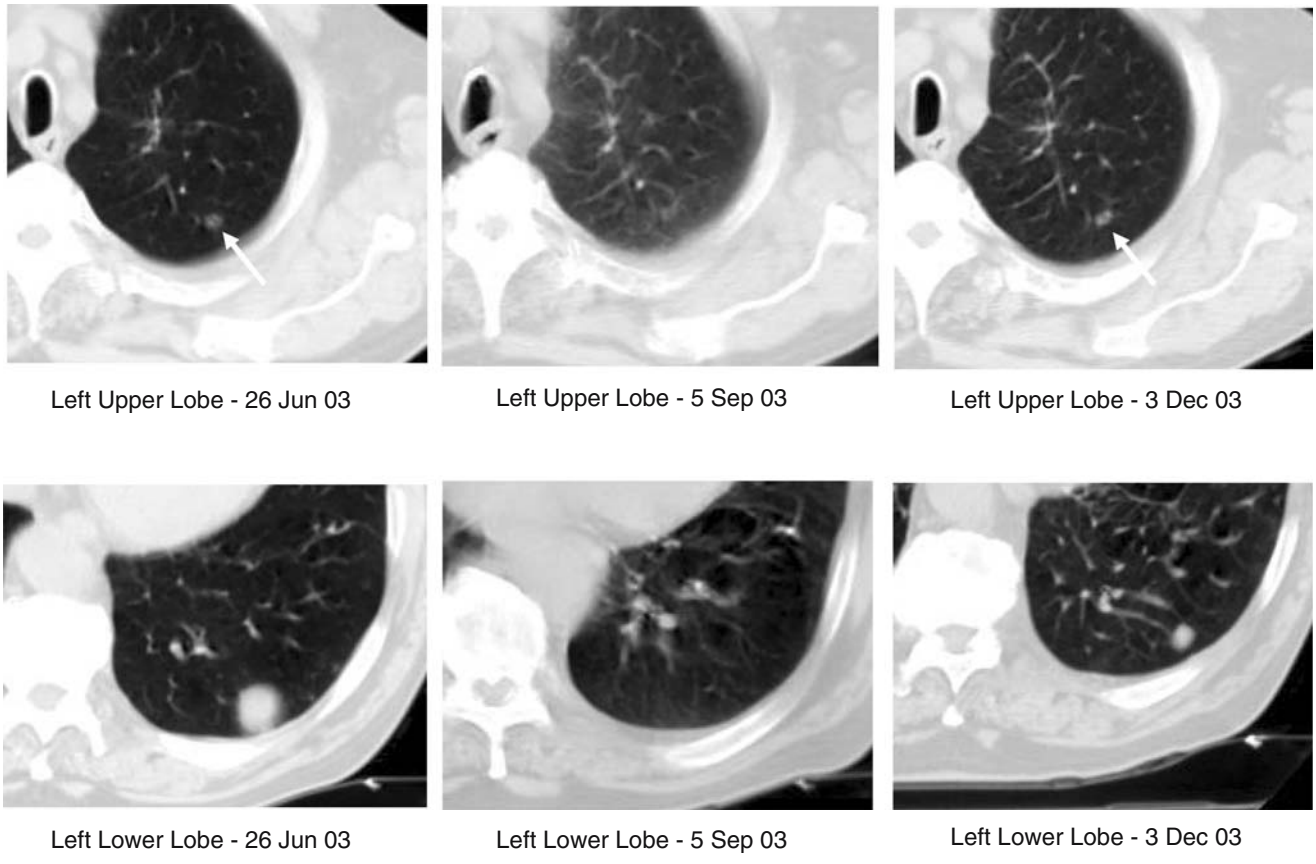

Fig. 1 From July 7th 2003 to October 15th 2003, this 68-year-old patient with a malignant fibrous histiocytoma and lung metastases received eight cycles of gemcitabine 1,800 mg/m^2 over 3 h and DTIC 500 mg/m^2, every 2 weeks. The first cycle was delivered in the sequence DTIC → gemcitabine and C_{max} of gemcitabine triphosphate (dFdCTP) in peripheral blood mononuclear cells (PBMCs) reached 180 pMol × 10^{-6} PBMC at 4.4 h from start of gemcitabine infusion; dFdCTP $AUC_{0-8\,h}$ and $AUC_{0-24\,h}$ were, respectively, 836 and 1,718 pMol h × 10^{-6} PBMC

Table 5 Pharmacokinetics of gemcitabine and its metabolites

Sequence of administration	DTIC → G	G → DTIC	*P*
Gemcitabine			
C_{ss} (μM)	21.4 ± 6.9	23.9 ± 7.4	0.23
AUC (μM h)	67.8 ± 23.3	68.1 ± 23.4	0.73
Cl (l/min)	3.0 ± 0.9	3.0 ± 1.0	0.86
dFdU			
C_{max} (μM)	136 ± 35	127 ± 42	0.61
V_{ac} (μM/h)	0.7 ± 0.2	0.6 ± 0.2	0.35
$AUC_{0–8\,h}$ (μM h)	651 ± 205	597 ± 164	0.73
$AUC_{0–8\,h}$ dFdU/AUC gemcitabine	9.9 ± 3.1	9.0 ± 1.4	0.17
dFdCTP			
C_{max} (pMol × 10^{-6} PBMC)	255 ± 63	300 ± 87	0.11
AUC_{inf} (pMol h × 10^{-6} PBMC)	423 ± 92	412 ± 107	0.75
$AUC_{0–8\,h}$ (pMol h × 10^{-6} PBMC)	1,520 ± 382	1,554 ± 365	0.60

Patients ($n = 6$) received DTIC (500 mg/m^2) immediately followed by gemcitabine (1,800 mg/m^2 over 180 min) on day 1, and the opposite sequence on day 15

Values represent mean ± SD. $P = P$ value of the Wilcoxon sign test for paired comparisons

C_{ss} concentration at steady state, *dFdU* difluorodeoxyuridine, C_{max} maximum concentration, V_{ac} rate of appearance of dFdU in plasma during gemcitabine infusion, $AUC_{0–8\,h}$ area under the curve for the first 8 h from start of gemcitabine infusion, *dFdCTP* gemcitabine triphosphate in peripheral blood mononuclear cells (PBMCs), *Cl* total body clearance, AUC_{inf} dFdCTP accumulation in PBMCs during gemcitabine infusion

significant changes in the PK of gemcitabine or dFdU, neither in the accumulation of gemcitabine triphosphate by PBMC in relation to the administration order (Table 5).

The sequence of administration did not influence the hematologic or hepatic toxicity observed in those six patients (data not shown). To increase the sample size, we grouped the PK data from six patients studied during the prior Phase I trial with those obtained in eight patients from the present study (those six receiving both sequences plus an additional two who received only one sequence). In this group of 14 patients, there was a positive correlation between the AUCs ratio of dFdU to gemcitabine and the γ-glutamyl-transferase ($r = 0.50$, $P = 0.02$) or alkaline phosphatase ($r = 0.52$, $P = 0.02$) absolute values. No consistent relations were found between the hematologic toxicity and gemcitabine, dFdU or dFdCTP parameters.

Discussion

The results of this study confirm the good tolerance of this combination of gemcitabine and DTIC administered every 2 weeks. Grade 3–4 toxicity noted by day 7 recovered in most patients by day 14 (except for lymphopenia), and only 14% of cycles had to be delayed, so that a median relative dose intensity of 96% was reached for both agents. However, caution must be taken with regards to thrombocytopenia, most likely secondary to DTIC, which may be irreversible in some patients. For this reason, if platelet toxicity reaches grade 3 or 4, it is advisable to omit DTIC in subsequent cycles. Also of note is the toxicity of this regimen on lymphocytes, with grade 3–4 lymphopenia still present in 52% of patients by day 14, with no episodes of opportunistic infections reported. Lymphopenia has not been systematically studied in patients treated with DTIC, but it is a well-known side-effect of temozolomide, a DTIC analog [31].

Although the 4% remission rate encountered was lower than the 36% observed during the Phase I study [5], it is to note that in 8 out of 11 patients (72%) disease stabilization lasted > 6 months, what points to a clinical benefit derived from therapy. In tumors as heterogeneous as STS, lack of reproducibility of efficacy data between studies is not uncommon. For this reasons, and in order not to discard potentially active agents, other criteria such as PFR have been applied to measure the biological effect of new agents in ASTS. For active drugs delivered as second-line therapy, PFR figures (±standard error) of at least 39 ± 5 and 14 ± 5% at 3 and 6 months, respectively, would be indicative of activity [35]. Despite some unfavorable features of this series, with a disease-free interval (DFI) shorter than 12 months in 61% of patients and a performance status equal to 2 in 4, PFR at 3 and 6 months were 48 and 28%, respectively, values similar to those encountered with new compounds probably active in second-line therapy [16]. However, PFR patterns proposed to measure the efficacy of new agents or combinations should be validated in prospective randomized trials where patients are stratified by factors such as performance status and DFI.

To explore the clinical benefit obtained with this combination by histologic subtype, we have pooled the data from the Phase I and Phase II studies (Table 6). Even though subgroups are small, the highest clinical benefit was observed in patients with MFH (33%) or LMS of any origin (57%). This profile of activity is similar to that of single agent DTIC, according to the results of the EORTC STBSG trial which showed objective remissions in patients with MFH (4/5 patients), gynecologic LMS (3/8), or liposarcoma (2/2). In this study, remissions were short-lived and only 1 of 13 stabilizations lasted longer than 6 months [6]. On the other hand, after single agent gemcitabine, remissions have been detected on angiosarcoma, ULMS and non-ULMS, and MFH [2, 11, 21, 23, 30, 32]. When gemcitabine was combined with docetaxel, a 54%

Table 6 Activity detected in the Phase I and Phase II studies by histologic subtype

	Complete remission	Partial remission	No change	Progressive disease	Clinical benefit[a]/total
Leiomyosarcoma	–	2	3 (2)[b]	2	4/7
Malignant fibrous histiocytoma	2	2	5 (1)	6	5/15
Fibrosarcoma	–	–	2 (1)	–	1/2
Liposarcoma	–	–	1 (1)	1	1/2
Carcinosarcoma	–	–	1 (1)	–	1/1
Rhabdomyosarcoma	–	–	–	3	0/3
Angiosarcoma	–	–	–	1	0/1
Synovial sarcoma	–	–	1	2	0/3
MPNST	–	–	–	2	0/2
Other[c]	–	–	2	1	0/3
Unclassified	–	–	2 (1)	1	1/3

MPNST malignant peripheral nerve sheath tumor

[a] Clinical benefit: objective remissions plus estabilizations > 6 months

[b] Number of patients. In brackets, patients with estabilization > 6 months

[c] Other: ganglioneuroblastoma (1), sarcoma phyllodes (2) activity in leiomyosarcomas: uterine: 2 stabilizations > 6 months; non-uterine: 2 partial remissions, 1 stabilization > 6 months, 2 progressions

response rate was observed in a pool of 46 patients with either ULMS or non-ULMS [13, 17]. Thus, gemcitabine plus docetaxel seems very active against LMS, a subtype also sensitive to DTIC plus gemcitabine.

Our Phase I trial was designed to escalate the dose of gemcitabine, and objective remissions were observed in 1 of 7 patients treated at 800–1,200 mg/m^2 and in 4 of 12 receiving 1,500–2,160 mg/m^2 ($P = 0{,}36$), a sample too small to uncover whether there is a dose–response relationship for gemcitabine in ASTS. Up till now, such a relationship has not been proven in solid tumors in comparative studies, although in non-small-cell lung cancer patients treated with gemcitabine infused over 30 min, there was a lower response rate for patients receiving doses below 900 mg/m^2/week than in those treated at $\geq$ 1,200 mg/m^2/week [28], and a trend for an increased response rate was noted as the dose went from 1,000 to 2,800 mg/m^2/week [9]. The determinants of ASTS sensitivity to gemcitabine are presently unknown, but dFdCTP accumulation by tumor cells is considered one of the main factors. FDR infusion gemcitabine optimizes this accumulation by PBMC and, in spite of a wide interindividual variability, mean C_{max} and dFdCTP $AUC_{0–8\,h}$ values are higher after 180 min than after 80-min infusions (R. Losa, personal communication). This increased exposure to dFdCTP of PBMC, and perhaps also of tumor cells, would favor gemcitabine antitumor activity, an assertion that seems in conflict with some clinical data obtained from combination studies. In the current trial, the objective response did not improve in four assessable patients when they received DTIC plus gemcitabine at 1,800 mg/m^2 after progressing to doxorubicin plus 800 mg/m^2 gemcitabine. Furthermore, significant activity has been encountered in LMS patients treated with the low gemcitabine doses (675–900 mg/m^2 by FDR infusion on days 1 and 8) tolerated when this agent is combined with docetaxel at full doses [13, 17]. Then, in sensitive tumor types, it would appear that clinical response to combinations of gemcitabine would not strictly depend on the dose of gemcitabine administered. One might speculate that, among other factors, the therapeutic effect of the combination could be related to the capacity of tumor cells to accumulate and retain dFdCTP, what would probably be influenced by the combination partner, and to the efficacy of this partner. Presently, no dFdCTP data are available from patients exposed to gemcitabine plus docetaxel to know whether there is any interaction that would correlate with the clinical activity detected.

In the six patients studied, sequence of administration did not influence gemcitabine and dFdU kinetics, or the activation of gemcitabine to dFdCTP (Table 5). There was a trend toward a lower C_{max} of dFdCTP ($P = 0.11$) and higher values of the ratio $AUC_{0–8\,h}$ dFdU/AUC gemcitabine ($P = 0.17$) when DTIC preceded gemcitabine, suggesting that a higher proportion of gemcitabine is deaminated with this than with the opposite sequence. Therefore, it seems more convenient that gemcitabine precedes DTIC infusion if we pretend to achieve the highest possible dFdCTP concentration. The proportion of gemcitabine converted to dFdU, estimated by the ratio of their AUCs, correlated positively with the increase in γ-glutamyl-transferase ($P = 0.02$) and alkaline phosphatase ($P = 0.02$) values, indicating that dFdU is likely associated with

the liver toxicity observed. dFdU is not usually considered an active gemcitabine metabolite although, in in vitro studies, it has shown antitumor and radiosensitizing activity at concentrations achievable in the clinic [24]. This toxicity could be mediated through mitochondrial damage secondary to dFdU phosphorylation by thymidine kinase 2 [3]. Overall, these data support the concept that this gemcitabine metabolite is not as innocuous as usually considered.

In summary, the combination with gemcitabine probably improves the efficacy of DTIC measured in terms of PFR, and further studies would be necessary to expand the information on subtype specificity or to confirm the superiority of gemcitabine combinations (gemcitabine plus either DTIC or docetaxel) over single agents. A recent Multicenter Study has compared the objective activity of docetaxel plus gemcitabine versus gemcitabine at 1,200 mg/m^2 on days 1 and 8, with stratification by histology (LMS versus non-LMS) [20]. Taking a different approach, the Spanish Group for Research on Sarcomas (GEIS) is conducting a Phase III trial to confirm whether the present combination of gemcitabine and DTIC offers some advantage over single agent DTIC (1,200 mg/m^2 every 3 weeks) in terms of PFR in ASTS.

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