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Phase I clinical and pharmacokinetic study of trabectedin and doxorubicin in advanced soft tissue sarcoma and breast cancer

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ABSTRACT

The combination of trabectedin (T) and doxorubicin (D) was brought into clinical development in soft tissue sarcoma (STS) and advanced breast cancer (ABC) because of its *in vitro* and *in vivo* additive anti-tumour effect, the fact that there are no overlapping toxicities and the anti-tumour activity of T in those tumours. Feasibility and anti-tumour activity of T+D administered every 3 weeks were evaluated in 38 patients (STS=29, ABC=9) untreated for advanced disease. D was given at 60 mg/m^2 and T at escalating doses from 600 to 800 μg/m^2, which was the maximum tolerated dose due to dose-limiting febrile neutropenia and asthenia. The recommended dose - given to 18 patients in total - was 700 μg/m^2 T with 60 mg/m^2 D. The pharmacokinetic profile of T and D at cycle 1 was analysed in 20 patients. The most common toxicities included a severe but reversible ASAT/ALAT increase (94%), nausea/vomiting, neutropenia, asthenia/fatigue, stomatitis. Partial response and stable disease were assessed in 18% and 56% of STS patients and in 55% and 33% of ABC patients. No pharmacokinetic interaction between T and D was observed. The lack of cumulative toxicity and related complications and the promising activity in STS support further development of T+D.

1. Introduction

Trabectedin (T) (formerly known as ET-743) is a tetrahydroisoquinoline alkaloid isolated from the Caribbean ascidian *Ecteinascidia turbinata* that binds in the minor groove of DNA, forming adducts at the N2 position of guanine.[1] The DNA structural changes that T induces in DNA - with a bending of the minor groove towards the major groove - is different from that induced by any other DNA-interacting agent investigated so far[2] and this possibly explains the unique biological properties of T in relation to DNA repair[3–5] and transcription regulation.[6–8]

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Adult soft tissue sarcomas (STS) are a pathologically heterogeneous group of rare malignant tumours which account for less than 1% of all malignant neoplasms. They arise from a variety of connective tissues, including blood vessels, muscles and Schwann cells, and encompass high-grade and low-grade tumours; histological subtype and grade are the most important prognostic factors.[9]

The results so far achieved with chemotherapy in advanced STS are limited due to an overall intermediate chemosensitivity,[10] heterogeneity of the biological features and of the clinical behaviour of the different subtypes.

The most active single agents are anthracyclines (doxorubicin (D) or epirubicin)[11] and alkylating drugs (mainly ifosfamide)[12]; in combination, a 30% response rate can be achieved with standard doses,[13,14] increasing up to 70% when high doses with colony stimulating factor (CSF) support[15,16] are administered; responses, however, are of short duration and the identification of new active compounds in STS is of high priority.

Long lasting objective responses to T were noted in four out of 20 sarcoma patients resistant to standard chemotherapy, treated across the phase I programme at the recommended doses. The antitumour activity in STS was subsequently confirmed in phase II studies[17–19] and T was approved by the EU regulators as therapy for patients with advanced STS resistant to or relapsed after anthracyclines and ifosfamide or to those cases not suitable for conventional chemotherapy. The observation of very good efficacy of the combination in xenografts that were poorly sensitive to either D or T[20] and the potential efficacy of T in advanced breast cancer, resistant to anthracyclines and taxanes,[21] provided further rationale to investigate this combination. Here we report the clinical and pharmacological results of a Phase Ib study, the primary objectives of which were the definition of the Maximum Tolerated Dose (MTD) of the combination of T and D and the definition of the least toxic sequence of administration of the two drugs. The sequence of administration of the two drugs was investigated because of the potential acute liver impairment caused by T and the consequent pharmacokinetic interaction with anthracyclines.[22,23] In addition, in STS cell lines HT-1080 and HS-18, a sequence dependent enhancement of cytotoxicity by T given 24 h before D had been reported.[24]

A 3-h duration of infusion of T was selected because of good tolerability, the long half-life of T[25] and the ease of administration shown in Phase II[17,18]; a starting dose of T of 600 $\mu g/m^2$, corresponding to about 50% of the recommended dose (RD), was selected because of the expected neutropenia of the combination; D was given at 60 mg/m^2 corresponding to the dose more commonly used in combinations.

The study was approved by the Ethics Committee of each participating institution and all enrolled patients gave their informed consent before starting any study-related procedures.

2. Patients and methods

2.1. *Eligibility*

Eligibility criteria were a diagnosis of STS or advanced breast cancer (ABC). STS patients could have received only prior adjuvant chemotherapy while breast cancer patients could have received a maximum of one prior chemotherapy for advanced disease. ECOG performance status (PS) $\leqslant 1$, measurable disease, $\geqslant$ 55% LVEF by echocardiogram or MUGA scan, adequate haematological, renal and liver function (alkaline phosphatase (AP), total serum bilirubin, ALT, AST within upper normal limit (UNL), $\leqslant 1.5 \times$ UNL in case of liver metastases; if total AP $\geqslant$ UNL, the liver fraction had to be within UNL). In patients with STS no prior chemotherapy for advanced disease was allowed, only chemotherapy with adjuvant intent and completed > 6 months before starting the study. A maximum cumulative dose of D (or D equivalents) $\leqslant 280\ mg/m^2$ was allowed.

Exclusion criteria were serious cardiac disease (e.g. congestive heart failure or angina pectoris, even if medically controlled, documented myocardial infarction within 1 year prior to study entry, uncontrolled hypertension or arrhythmia), chronic active hepatitis or cirrhosis, symptomatic brain metastases or leptomeningeal disease.

2.2. *Ethics*

The protocol was approved by the local Ethics Committee of each participating centre and patients had to sign a written informed consent.

2.3. *Treatment and study design*

In the first part of the study (dose finding and least toxic sequence definition) patients with STS received increasing doses of T in combination with 60 mg/m^2 of D; Three to six patients per dose level were treated according to toxicity.

The starting dose of T of 600 $\mu g/m^2$ was escalated by 100 $\mu g/m^2$ increments up to the MTD, which was defined as the dose at which at least two out of six patients treated with the least toxic sequence experienced a dose limiting toxicity (DLT); the RD was fixed one dose level below.

For the definition of the least toxic sequence, consecutive patients were assigned to receive, at cycle 1, sequence A (T followed by D) or sequence B (D followed by T); in absence of DLT, the same doses with the opposite sequence were given at cycle 2, until the least toxic sequence was determined. After a total of 12 cycles had been administered to the first six patients (six with each sequence) the criteria for DLT were used to define the least toxic sequence; in absence of DLT, the total number of toxic events experienced by each patient was utilised to define the least toxic sequence.

If a patient presented a DLT at cycle 1, the dose of T was reduced by one dose level and the patient continued with the same sequence, while three additional patients had to be treated with the same sequence at cycle 1; if one or more DLTs occurred, the MTD for that sequence was established; in case of no more DLT, three patients were treated with the reverse sequence at the same dose level and in case of no DLT the dose was escalated.

The RD of the least toxic sequence was planned to be tested in the second part of the study (expansion part) in which the endpoint was the assessment of the anti-tumour activity of the combination in patients with STS and ABC. A total of 20 patients was originally planned.

DLTs were the occurrence of febrile neutropenia (NCI-CTC definition), Grade 4 neutropenia lasting >7 days (prophylactic colony stimulating factor was not allowed), Grade 4 thrombocytopenia or anaemia, lack of recovery from haematological toxicities by day 35, Grade 3 increase of AP, any grade increase of bilirubin and/or ASAT/ALAT and /or AP not recovering to baseline values by day 28, > Grade 2 stomatitis for >3 days, ⩾ Grade 3 non haematological toxicity (excluding liver function tests, stomatitis and nausea/vomiting responding to anti-emetic treatment), and ⩾ Grade 2 non haematological toxicity not recovered by day 28.

In addition, the dose was to be reduced in case of AP or bilirubin elevation of any Grade.

Trabectedin (YONDELIS®) was supplied by PharmaMar (Madrid, Spain) as lyophilised powder concentrate for solution for infusion, in two strengths of 0.25 mg and 1 mg to be reconstituted with 5 ml or, respectively, 20 ml of sterile water for injection.

The calculated amount of T was given through a free flowing intravenous (IV) line as a 3-h infusion; commercially available D was administered as a 5 min IV bolus. The administration of T and D were separated by a 60 min interval and were repeated every 3 weeks.

Steroid premedication (dexamethasone) was given; antiemetic prophylaxis for T with IV 5HT3 antagonists and metoclopramide up to 48 h after T was mandatory.

2.4. *Treatment assessment*

During treatment at least weekly clinical controls with full chemistry were performed, until the definition of the MTD; AP, ASAT/ALAT, bilirubin were also done on day 4; complete blood count was done at least twice weekly or more often in case of toxicity. Tumour assessment by radiological imaging was performed within 4 weeks before starting and repeated every two cycles. ECG was repeated at the end of each cycle and LVEF after the 4th and the 6th cycles.

Responders continued treatment for four cycles after confirmation of partial response (PR) or up to relapse; a maximum cumulative dose of D of 480 mg/m^2 could be administered after which treatment with single agent T at 1500 μg/m^2 could be continued. Patients with stable disease (SD) could continue treatment up to six cycles, or up to progressive disease (PD) according to the investigator's judgement.

NCI-CTC version 2 criteria for toxicity and modified RECIST criteria for the definition of response were applied.[26]

2.5. *Sample collection and pharmacokinetic analysis*

Blood samples (4 ml) were collected in Li-heparine at baseline, 1 h, just before the end of infusion and 0.5, 1,1.5, 2, 3, 5, 8, 20, 44 and 68 h after the infusion of T; 0, 5, 10, 30 min and 1, 2, 4, 7, 24, 48, 72 h after D bolus. Blood samples were immediately centrifuged at 2500 g for 10 min at 4 °C and, after separation by specific filters, plasma was transferred in two or four screw capped polypropylene tubes and stored frozen at –30 °C.

T was measured in plasma by a liquid chromatography coupled with electrospray ionisation tandem mass spectrometry (LC-MS/MS) method.[27]

D and its major metabolite, doxorubicinol, were measured following a HPLC–Fluorescence method characterised by on-line plasma sample extraction.[22]

Analytical data were analysed by a non-compartmental model. The area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0\text{-inf}}$) was determined by calculating the $AUC_{0\text{-last}}$ from time zero to the last sampling time by log-trapezoidal rule and then adding the area relative to the extrapolated region; the terminal half-life ($T_{1/2}$) was derived by the formula $T_{1/2}$= 0.693/Ke where Ke was the slope of the linear equation that best fitted the last three or four concentration-time data; CL_{TB} was calculated as dose/$AUC_{0\text{-inf}}$.

3. Results

3.1. *Patient characteristics*

Thirty-eight patients were treated in three centres (23 in the dose finding and 15 in the expansion part); all were evaluable for safety; 29 patients had progressive STS and nine had

Table 1 – Patient characteristics.

Study part	Dose finding part	Expansion part (T 700 / D 60)	All patients enrolled at RD (T 700 / D 60)
No. of pts	23	15	18
Soft tissue sarcoma (grade III)	17 (8)	12 (3)	15 (4)
Uterine leiomyosarcoma	4 (0)	3 (0)	4 (0)
Leiomyosarcoma	3 (1)	1 (0)	1 (0)
Liposarcoma	-	3 (1)	3 (1)
Malignant schwannoma	1 (1)	1 (0)	2 (1)
Other	9 (6)	4 (2)	5 (2)
Advanced breast cancer (ABC)	6	3	3
M/F	3/20	7/8	7/11
ECOG Performance Status 0/1	21/2	13/2	16/2
Median age (range)	51 (38–75)	55 (32–68)	49.5 (32–68)
Prior treatment:			
CT (including anthracyclines)[a]	3 (2)	4 (4)	7 (6)
RT	8	3	3

Legend: RD = recommended dose; T = trabectedin; D = doxorubicin; M = male; F = female; CT = chemotherapy; RT = radiotherapy.
a Administered as adjuvant/neo-adjuvant therapy in patients with STS.

chemo-naïve ABC (Table 1). Liver tumour involvement was present in six patients with STS and in one with ABC. Although according to the study design patients with ABC were to be treated in the expansion part only, enrolment of patients with this tumour type started already at the highest tested dose of the dose finding part.

Prior adjuvant chemotherapy had been given in a total of seven patients, including anthracyclines in six with STS.

3.2. *Dose escalation, DLT, MTD and least toxic sequence*

Table 2 reports per dose level the number of patients treated and the DLTs observed. At dose level 1 (T 600 μg/m^2/ D 60 mg/m^2) the first patient treated with sequence A did not have toxicity, while the first patient treated with sequence B developed a DLT (febrile neutropenia); additional patients were then treated, three with sequence B and five with sequence A without toxicity. No DLT was observed in the three patients treated at dose level 2 (T 700 μg/m^2/D60 mg/m^2) while two patients in each sequence at dose level 3 (T 800 μg/m^2/ D60 mg/m^2) had DLT and defined the MTD. The RD for the expansion part was thus defined at T 700 μg/m^2/D60 mg/m^2.

In the dose finding part, 13 patients were treated at cycle 1 according to sequence A and 10 according to sequence B, without evidence of differences in the respective toxicity profiles. Due to the need for frequent dose reductions at cycle 2 (even in absence of DLT), there were too few reverse sequences at cycle 2 to permit an analysis of toxicity using patients as their own control; sequence A was then chosen for the expansion part based on its convenience for patients because steroid medication and anti-emetics required for T could act as an anti-emetic prophylaxis for D as well. A total of 18 patients were treated at the RD, of whom three (all with STS) were in the dose finding part and 15 (12 with STS and three with ABC) in the expansion part. Accrual of ABC patients in the expansion part was stopped at three patients because of the extremely slow recruitment rate, which was probably a result of changes in oncology practices in this patient setting since the original protocol was written. Two patients in the expansion part presented a toxicity at cycle 1, corresponding to the DLT definition, thus confirming the tolerability of the dose identified as RD.

3.3. *Safety/toxicity*

Table 3 reports per dose level the number of patients treated and the number of patients with the most frequent non haematological drug-related toxicities.

The safety profile described hereafter refers to the 18 patients treated at the RD. Overall, 22% of the patients (4/18) required a reduction of the dose of T.

The most common non haematological toxicity was hepatic, consisting of a severe but reversible dose-dependent increase in transaminases which occurred in 94% of patients; ASAT/ALAT changes peaked during the first week of the cycle and recovered within the following week without a cumulative effect during the subsequent cycles. The increase of AP and bilirubin was less frequent and of Grade 1 in all cases ex-

Table 2 – Number of patients and occurrence of DLT per dose level (dose finding part).

Dose level		No. of patients	No. of patients by sequence	Dose limiting toxicities	
T (μg/m^2)	D (mg/m^2)				
600	60	10	6A / 4B	*1 DLT sequence B*:febrile neutropenia	
700	60	3	1A / 2B	None	→ RD
800	60	10	6A / 4B	*2 DLTs sequence A*:>7 days G4 neutropeniafebrile neutropenia	→ MTD
				2 DLTs sequence B:asthenia G3febrile neutropenia	

Legend: RD = recommended dose; MTD = maximum tolerated dose; DLT = dose limiting toxicity; T = trabectedin; D = doxorubicin.

Table 3 – Non haematological toxicity – Incidence by patient and dose level.

Dose level T μg/m^2 / D mg/m^2 No. of patients	600 / 60 10		800 / 60 10		700 / 60 RD 18[a]	
	Any Grade	Grade 3–4	Any Grade	Grade 3–4	Any Grade	Grade 3–4
Alopecia	6	-	9	-	15 (83%)	-
Nausea	5	-	10	-	15 (83%)	-
Stomatitis	4	-	5	1	1 (5.5%)	-
Mucosal inflammation	1	-	4	1	7 (39%)	-
Asthenia	4	-	4	-	9 (50%)	-
Fatigue	3	-	5	2	2 (11%)	-
↑ ASAT / ALAT	7	3	10	7	17 (94%)	8 (44%)
↑ Alkaline Phosphatase	4	-	4	-	6 (33%)	-
↑ Bilirubin	3	1	2	-	4 (22%)	-

Legend: T = trabectedin; D = doxorubicin; RD = recommended dose; G = NCI-CTC grade.
a Three patients treated in the dose finding part and 15 in the expansion part.

ept for one which reported Grade 2 bilirubin elevation. The other more frequent non haematological side effects were nausea and/or vomiting (83% of patients), asthenia and/or fatigue (56%, not correlated with liver toxicity), stomatitis and/or other mucosal disorders (39%). No significant decrease of the LVEF values was observed.

Table 4 reports the haematological toxicity observed; non cumulative neutropenia occurred in all patients at the RD, of Grade 4 in 67%. Median time to neutrophil nadir at cycle 1 was 15 days with a median time to recovery to $\geqslant 1.5 \times 10^9$/l of 7 days. No Grade 4 thrombocytopenia was reported; one patient presented Grade 3 thrombocytopenia.

At the RD, four patients (22%) had to decrease the dose of T: in three cases due to neutropenia, one of which was complicated by infection, and due to bilirubin increase in one further case. No D dose reductions were required.

3.4. *Anti-tumour activity*

Ten patients achieved a PR (five with STS and five with ABC) and 16 had SD. Table 5 reports the anti-tumour activity; the number of responses per dose level and tumour type in Table 5A and the duration of partial response per tumour type (and histological sub-type for STS) in Table 5B; all responders were female, one with STS had neo-adjuvant chemotherapy. In responders with STS the median number of cycles was six (5–9) and the median duration of response (calculated from treatment start to disease progression or last available assessment) was 12.5 months; in patients with ABC the median number of cycles was 8 (4–9) and the median duration of response was 9.2 months. After achievement of the D maximum cumulative dose of 480 mg/m^2, four patients with ongoing PR and four with disease stabilisation continued treatment with T as single agent for at least one further cycle. The responses in STS were reported in patients with different histological sub-types, the longest one still ongoing after more than 5 years from the start of treatment in a patient with malignant schwannoma. This 44 year old woman had her first elbow exeresis followed by surgical radicalisation 1 month later. At the start of treatment she had two tumour lesions in the lung (the largest one being 4 cm in its longest diameter) and one mediastinal lymph node of 3.5 cm. After eight cycles the mediastinal lymph node's size had become 1.5 cm and both lung lesions showed a CR; at the first follow-up assessment performed 5 months after treatment discontinuation the CT-scan provided a completely negative picture, still confirmed at the last radiological assessment 58 months after T+D discontinuation, in absence of any kind of further local or systemic anti-tumour treatment.

The overall progression-free survival rate of STS patients at 6 months was 49.8%.

3.5. *Pharmacokinetics*

The pharmacokinetic profiles of T and D were analysed in 20 patients over a total of 22 cycles. Table 6 reports the mean values of the main pharmacokinetic parameters for T by dose level. Fig. 1 shows an example of the plasma pharmacokinetic profile of T (panel A) or D (panel B) in the same patients receiving the two drugs either according to sequence A or to sequence B. The curves are overlapping, indicating that the plasma disposition of T was not altered by the concomitant administration of D, whether D was given before T or after it. Also, the pharmacokinetics of D were not modified by concomitant T administration (data not shown).

4. Discussion

The development of T in combination with an anthracycline is of high priority due to the evidence of the anti-tumour activity of T in STS[17–19,28,29] and ovarian cancer[30] and reported pre-clinical data indicating a synergism between T and D in STS cell lines.[24] The finding that the synergism could be related to the ability of T to down-regulate the transcription of genes involved in the resistance to D, e.g. MDR1,[31] made the combination of the two drugs particularly attractive, especially for tumours that are not very sensitive to current therapies, like sarcomas.

Dose finding studies have recently been completed with T and D in patients with STS[29] and with pegylated liposomal D (PLD) in patients with advanced malignancies.[32] D was given at 60 mg/m^2 and PLD at 30 mg/m^2 followed by T. In all the studies with anthracyclines in combination, including the present one, T was given as a 3 h infusion every 3 weeks because of the ease of administration, the favourable toxicity profile and anti-tumour activity comparable to that of longer infusions observed in phase II studies.[21] In the present study, the RDs of the combination were 700 g/m^2 of T and 60 mg/m^2 of D respectively. Neutropenia was dose-limiting and it was of Grade 3–4 in 89% of the patients treated at the RD, but it was associated with infection in only one case. The most frequent clinically relevant non haematological toxicities were non cumulative increase of transaminases, which occurred in

Table 4 – Haematological toxicity – Incidence of NCI-CTC Grade 3–4 by patient and dose level.

Dose Level T μg/m^2 / D mg/m^2	600 / 60		800 / 60		700 / 60 RD	
No. of patients	10		10		18[a]	
	G3	G4	G3	G4	G3	G4
Neutropenia	1	9	1	9	4 (22%)	12 (67%)
Thrombocytopenia	1	-	-	-	1 (5.5%)	-
Anaemia	2	-	2	-	1 (5.5%)	-

Legend: T = trabectedin; D = doxorubicin; RD = recommended dose; G = NCI-CTC grade.
a Three patients treated in the dose finding part and 15 in the expansion part.

Table 5 – Anti-tumour activity. (A) Number of responses per dose level and tumour type. (B) Duration of complete and partial response by tumour type and histological sub-type.

Dose T μg/m^2 / D mg/m^2	600 / 60		800 / 60		700 / 60 RD		All Dose Levels		Median TTP (months)	
Tumour type	STS	ABC	STS	ABC	STS	ABC	STS	ABC	STS	ABC
No of evaluable patients	10	-	4	6	14	3	28	9	5.8 (N=28)	9.2 (N=9)
Partial response	2	-	1	2	2	3	5 (17.9%)	5 (55.5%)	12.5 (N=5)	9.2 (N=5)
Stable Disease	5	-	1	3	7	-	13 (46.4%)	3 (33.3%)	9.4 (N=13)	NA (N=3)

Legend: T = trabectedin; D = doxorubicin; RD = recommended dose; STS = soft tissue sarcoma; ABC = advanced breast cancer; TTP = time to progression; N = number of patients; NA = not assessable.

Patient number	Dose T μg/m^2 / D mg/m^2	Tumour Type	Histological Sub-type & Grade	Visceral Disease	Bulky Disease (⩾ 5 cm)	PS at Study Entry	No of Cycles	Response	Response duration[a] (months)
3	600 / 60	STS	Leiomyosarcoma GII	Lung	No	0	9 (T single agent from cy 7)	PR	12.5
9	600 / 60	STS	Carcinosarcoma GIII	None	No	0	6	PR	5.4
13	700 / 60	STS	Schwannoma GIII	Lung	No	0	8	PR on study ↓ CR after study withdrawal	64 +
36	700 / 60	STS	Liposarcoma GIII	None	Yes	0	6	PR	5.8
15	800 / 60	STS	Uterine adenosarcoma GIII	None	Yes	0	5 (T single agent from cy 4)	PR	3.9
16	800 / 60	ABC	-	None	No	0	9 (T single agent at cy 9)	PR	9.2
22	800 / 60	ABC	-	None	Yes	0	5	PR	4.9
24	700 / 60	ABC	-	Lung	No	0	9 (T single agent at cy 9)	PR	8.3
25	700 / 60	ABC	-	None	No	0	4	PR	3.3
27	700 / 60	ABC	-	Lung	No	0	8	PR	7.2

Legend: T = trabectedin; D = doxorubicin; RD = recommended dose; STS = soft tissue sarcoma; ABC = advanced breast cancer; PS = performance status.
a From first dose to disease progression.

Table 6 – Main pharmacokinetic parameters (mean ± SD of T) – Comparison between sequence A (cycle 1) and sequence B (cycle 2).

T μg/m^2	600		700		800	
Sequence	A	B	A	B	A	B
$AUC_{0\text{-last}}$ (nM•h)	22.7±2.2	19.4±6.4	7.6	15.8–13.0	17.0±7.0	18.8±4.6
$AUC_{0\text{-inf}}$ (nM•h)	26.4±5.1[a]	22.5±0.5	7.8	17.2–17.0	18.9±8.8	23.3±6.8
CL_{TB} (L/h/m^2)	30.7±5.7[a]	38.1±12.9	118.8	53.4–54.3	72.8±51.0	48.6±17.1
C_{max} (nM)	4.7±1.3	4.3±1.6	1.7	3.2–2.8	5.1±3.2	4.7±1.1
$T_{1/2\gamma}$ (h)	40.9±11.6[a]	38.6 ± 8.5	14.5	29.6–40.3	27.3±12.6	39.3±16.0
No. of patients	4	6	1	2	6	3

Legend: T=Trabectedin.
a No. of patients = 3.

94% of patients treated at the RD, and cumulative fatigue observed in 47%.

The lack of toxicity-related complications and cumulation of toxicities after repeated doses allowed continued treatment with T alone in eight patients treated across all dose levels who had achieved the maximum cumulative dose of 480 mg/m^2 of D.

The observation of one DLT (febrile neutropenia) and one further case of stomatitis leading to D dose reduction with the sequence B (D followed by T) at the first dose level impeded an adequate assessment of the least toxic sequence. Since at the MTD the two sequences were associated with the same number of DLTs, the more practical sequence A (T followed by D) was selected for the expansion part of the study.

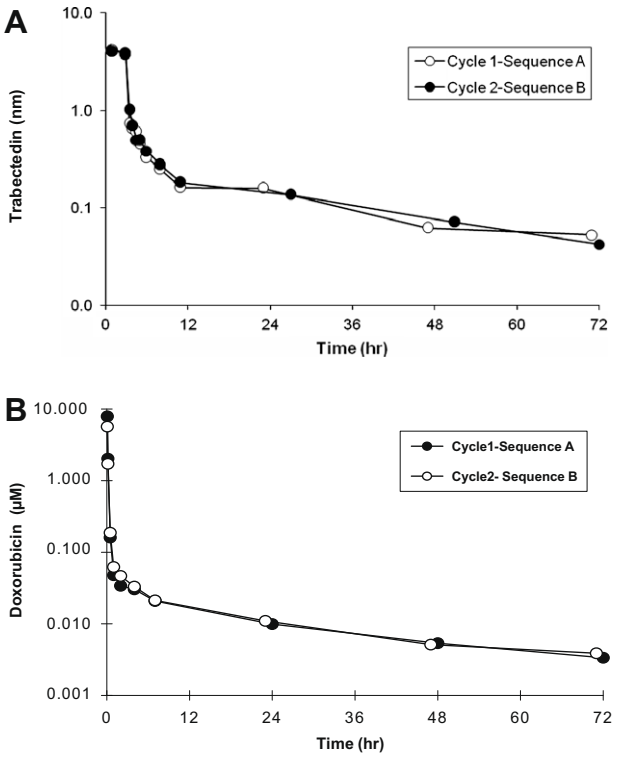

Fig. 1 – Patient 003: intra-patient comparison between sequence A (cycle 1) and sequence B (cycle 2) of plasma disposition. (A) Plasma disposition of trabectedin; (B) Plasma disposition of doxorubicin.

The present study is the only one assessing the role of a potential pharmacokinetic interaction between T and D, which was of clinical concern because of the potential occurrence of acute liver toxicity due to T which could decrease the D clearance. As previously observed in pre-clinical systems,[20] no pharmacokinetic interaction between the two compounds was found in patients, and their pharmacokinetic profiles were comparable to those of the single agents.[25,33]

The observation of unmanageable neutropenia at the starting dose of 900 g/m^2 in the study of Blay prompted the introduction of prophylactic CSF, which allowed pursuit of the escalation of the dose of T with the definition of the RD at 1100 g/m^2. This can be an explanation for the higher incidence of thrombocytopenia and of less neutropenia - though associated with more frequent infections - in comparison to our study.

As regards the toxicity of the combination tested in the present study, it is necessary to point out that T at the doses tested does not induce unmanageable myelotoxicity *per se*, while D seems to have a crucial role in the development of such type of myelosuppression. In fact T caused early severe neutropenia, limited to the first week of cycle, but this toxic effect was prolonged by the concomitant administration of D up to the end of week 2. This observation invited us to speculate that tolerability might be improved by dividing the dose of T between d1 and d8 to limit the occurrence of severe neutropenia to week 2, when the combined myelotoxicity of T and D was expected. This hypothesis was tested in a subsequent Phase Ib study of the combination, the results of which will be reported separately.

The role of D in the development of neutropenia is also supported by the results of the phase I of T and PLD in which the severe neutropenia was first observed with T at 1100 μg/m^2; on the contrary the incidence of neutropenia and fatigue likely due to T was comparable, being 67% and 33% at a T dose of 600–750 μg/m^2. Apparently, more liver toxicity in terms of ASAT/ALAT was observed in our study but a meaningful comparison cannot be done since the time of assessment of liver function was not reported in the study of von Mehren.[32]

The overall response rate in patients with STS was 17.9% [CI 95%: 6.1–36.9]; objective responses were observed in patients with leiomyosarcoma, carcinosarcoma, liposarcoma, uterine adenosarcoma and malignant schwannoma, of more

than 5 years in the latter. The response rate is comparable to that reported with single bolus doses of D given every 3 weeks, which is still considered an appropriate first line chemotherapy option for advanced or metastatic STS,[11] while the higher rate and longer duration of disease stabilisation and the greater activity in selected histological subtypes could be distinctive features of T, which make a further development of the combination worthwhile.

The finding of a long-lasting complete remission of a metastatic schwannoma is particularly interesting considering that no effective second line therapies are available for patients affected by this disease.

Molecular pharmacology studies are warranted to investigate the molecular determinants of the sensitivity of schwannoma cells to T or T/D combination expression of particular genes that are down-regulated by the drugs.

The median time to progression of the 18 STS patients with PR and SD was of 5.8 months [CI 95%: 2.8–10.4]. Once again, T has shown to be an active agent in the treatment of STS; however, the heterogeneity of histological sub-types and the potential role of molecular features influencing the response to T do not allow us to draw any conclusions on the adequacy of the doses and schedule of the combination.

Five out of nine patients with ABC achieved a PR with a median duration of response (calculated from treatment start to disease progression) of 9.2 months. No conclusions could be drawn on the efficacy of the combination of T and D in ABC due to the small number of patients and their heterogeneity. Considering that these patients were chemotherapy-naïve, the limited duration of response and the availability of less toxic regimens do not recommend a further evaluation of the combination of T and D in ABC. However, in other tumour types like STS and ovarian cancer, in which T has shown activity as a single agent,[17–19,28,29] the lack of associated thrombocytopenia and neurotoxicity make the combination with D at least interesting and deserving refinement of the schedule, also with the inclusion of other anti-tumour agents. In this regard, the combination of weekly T and gemcitabine could be another promising regimen to be developed in STS because of the lower bone marrow and GI toxicity.[34] However, no objective responses were reported in the recently completed phase I study in solid tumours and the clinical development of this regimen is not supported by preclinical data of anti-tumour activity. A refinement of the schedule with the introduction of a 3-weekly dosing of T could allow the administration of higher and perhaps more effective doses of the marine compound.

Conflict of interest statement

J. Jimeno is Vice-President for Scientific Development at PharmaMar and owns stock of Zeltia (the holding Company); L. Gianni has acted as a paid consultant for PharmaMar; all other authors have no conflicts of interest to declare.

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