Dacarbazine in solitary fibrous tumor: a case series analysis and preclinical evidence vis-à-vis temozolomide and antiangiogenics

Author: S. Stacchiotti¹, M. Tortoreto², F. Bozzi³, E. Tamborini³, A. Morosi⁴, A. Messina⁴, M. Libertini¹, E. Palassini¹, D. Cominetti², T. Negri³, A. Gronchi⁵, S. Pilotti³, N. Zaffaroni², P. G. Casali¹

¹Adult Sarcoma Medical Oncology Unit, Department of Cancer Medicine, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy
²Molecular Pharmacology Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy
³Experimental Molecular Pathology Unit, Department of Pathology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy
⁴Department of Radiology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy
⁵Department of Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

Presented at the 17th Annual Meeting of the Connective Soft Tissue Oncology Society (CTOS), Prague, November 2012

Correspondence to: Dr. S. Stacchiotti, MD, phone +390223902803; fax +390223902404; Fondazione IRCCS Istituto Nazionale dei Tumori, via Venezian 1, 20133 Milan Italy; e-mail: silvia.stacchiotti@istitutotumori.mi.it

Disclosures

Casali PG — Pfizer: advisory, honoraria, research funding. Glaxo Smith-Kline: advisory, honoraria, research funding.

**Running title:** Dacarbazine in solitary fibrous tumor
ABSTRACT

Purpose: To explore the value of triazines in solitary fibrous tumor (SFT).

Experimental design: We retrospectively reviewed 8 cases of patients with SFT treated with dacarbazine (DTIC) (1200 mg/m2 every 3 weeks) as from January 2012. Then we studied a dedifferentiated-SFT subcutaneously xenotransplanted into SCID mice. DTIC, temozolomide, sunitinib, bevacizumab and pazopanib were administered at their reported optimal doses for the mouse model when mean tumor volume (TV) was about 80 mm³; each experimental groups included 6 mice. Drug activity was assessed as TV inhibition percentage (TVI%). DTIC was tested according to two different schedules of administration. 120 days after treatment interruption, mouse tumor samples were analyzed.

Results: Among the 8 patients treated with DTIC, best RECIST responses were 3 partial response, 4 SD, 1 progression. Two responsive patients had paraneoplastic hypoglycemia that disappeared after 10 days from starting DTIC. In the dedifferentiated-SFT xenograft model, DTIC and temozolomide showed the highest antitumor activity (about 95% TVI), confirmed pathologically. Sunitinib and pazopanib were only marginally active (52% and 41% TVI, respectively), whereas bevacizumab caused a 78% TVI. No tumor re-growth was observed up to 100 days from end of treatment with temozolomide and DTIC, while secondary progression followed sunitinib, pazopanib and bevacizumab interruption.

Conclusions: DTIC as single agent has antitumor activity in SFT. Our preclinical results suggest a cytotoxic effect of temozolomide and DTIC, as compared to a cytostatic role for sunitinib, pazopanib and bevacizumab. A phase II study on DTIC in advanced SFT is planned.
Statement of relevance

This study is the first report showing that dacarbazine (DTIC) as single agent has antitumor activity in patients with progressive pre-treated advanced solitary fibrous tumor (SFT). The clinical evidence is supported by preclinical results obtained on a human high-grade dedifferentiated SFT xenotransplanted into SCID mice. When singly administered in the mouse model, the two triazene compounds DTIC and temozolomide were found to have a high and superimposable antitumor activity, and to induce an almost complete tumor volume inhibition, which was maintained after treatment interruption and confirmed pathologically. By contrast, the anti-angiogenic agents sunitinib, pazopanib and bevacizumab were found to be less active, with tumor re-growth appreciable immediately after drug withdrawal. Clinical prospective studies are needed to compare DTIC and temozolomide in advanced SFT patients and to correlate their activity with tumor aggressiveness.
INTRODUCTION

Solitary fibrous tumor (SFT) is a rare soft tissue sarcoma (STS), marked by the presence of a recurrent NAB2-STAT6 gene fusions, which was recently identified (1,2). “Typical” SFT can be cured in the majority of cases by complete surgical resection, while they have a 10-15% risk of metastases in the “malignant” presentations (MSFT), or higher in the pleomorphic/dedifferentiated (DSFT) variant (1). A medical treatment is required in case of locally advanced or metastatic disease.

No prospective studies on the activity of chemotherapy in SFT are available. Few retrospective series showed a low response rate (RR) to standard anthracycline-based chemotherapy, ranging between 0 and 20% (3, 4). Few responses in patients treated with anthracycline plus dacarbazine (DTIC) +/- ifosfamide are also reported (5, 6). A higher RR can be observed in histologically more aggressive DSFT cases (7).

In the last years, the activity of new targeted agents like sorafenib, sunitinib, bevacizumab plus temozolomide, pazopanib, IGF1R inhibitors was described (8-15). In all cases, responses were mostly non-dimensional. In particular, researchers from the MD Anderson Cancer Center, US, observed 2 partial responses (PR) and 12 stable diseases (SD) by Response Evaluation Criteria in Solid Tumor (RECIST) (16) in 14 patients treated by temozolomide and bevacizumab. The median PFS was about 10 months (8). Temozolomide is an imidazotetrazine derivative of the alkylating agent DTIC. The drug is approved for treatment of glioblastoma multiforme and anaplastic astrocytoma (17), but is not approved for STS, while the use of DTIC is authorized for advanced STS cases in several European Union Member States, among which Italy.
We report hereafter on the activity of DTIC as a single agent in a series of 8 patients with advanced, progressive SFT, whom we treated as from January 2012. Given the evidence of responses, we carried out preclinical experiments on a human high-grade DSFT xenotransplanted into SCID mice, exploring the activity of DTIC, temozolomide, sunitinib, pazopanib and bevacizumab.
MATERIAL AND METHODS

1. Patients

From January to December 2012, we have started treating with DTIC as single agent 8 patients carrying a locally advanced/metastatic SFT at Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy (INT).

Patient characteristics are listed in Table 1 (male/female: 3/5; mean age: 61 years; pretreatment with ≥1 medical treatment: 8; ECOG PS ≥3: 1). All patients had evidence of progressive disease before starting treatment. Eastern Cooperative Oncology Group performance status (ECOG PS) ≤3 and an adequate bone marrow and organ function.

Histological diagnosis was centrally reviewed in all cases. The diagnosis was rendered according to the last WHO classification (1).

O6-methylguanine-DNA-methyltransferase (MGMT) gene methylation assessment

O6-methylguanine-DNA-methyltransferase (MGMT) gene methylation assessment was retrospectively performed in all patients treated with DTIC. DNA was extracted from formaline-fixed paraffin-embedded selected tumor samples using the QIAmp DNA Mini Kit (Qiagene) and bisulphate treated (Methylation KIT-Zymo Research). Polymerase chain reaction (PCR) amplification was conducted by using the following primers: MGMT Met Fw: 5’-cgaatatactaaaacaacccgcg-3’; MGMT Met Rev: 5’-gtatatttttctggagcgaggc-3’. MGMT UnMet Fw: 5’-ccaaatatactaaaacaacccaca-3’; MGMT UnMet Rev: 5’-gtatatttttttctggagctggt-3’ (18). The analysis was performed twice in all cases.

All patients provided a written informed consent to the treatment.
Treatment

Patients received DTIC as single agent, at the total dose of 1,200 mg/m2 per cycle, divided in 2 doses on day 1 and 2, infused intravenously over 60 minutes and repeated every 3 weeks, till toxicity or progression. In all cases chemotherapy was administered together with steroids (dexamethasone) and antiemetic (metoclopramide and ondansetron). Cycles were not started unless the granulocyte count was >1,000/µL and platelets were >100,000/µL. If counts were not at these levels, the treatment was postponed for a week. If grades (G) 3 to 4 (defined according to the National Cancer Institute Common Toxicity Criteria, version 3.0) thrombocytopenia, G4 neutropenia, or febrile neutropenia occurred, the dose of DTIC was reduced to 1,000 and then to 800 mg/m2. Granulocyte colony stimulating factors (GCSF) were administered in case of G3-4 neutropenia.

Clinical assessment

Full blood cell count and biochemistry were assessed at baseline and before every administration. Adverse events were recorded. Disease status was assessed at baseline by a whole body computed tomography scan (CT), a CT or magnetic resonance (MRI) of the sites of disease, and a whole body bone scan. CT/MRI were repeated after 4-8 weeks of treatment then every 2-3 months.

Response to treatment was assessed applying RECIST (16).

Progression-free survival (PFS) and overall survival (OS) were estimated with Kaplan-Meyer method.(19) Failure for PFS was progressive disease according to RECIST, or death.
2. **Experimental model and pharmacological studies**

A SFT sample suitable for mouse implantation was obtained from the local recurrence of a patient previously surgically resected for a MSFT of the pelvis. The recurrence occurred four years after the primary tumor and was consistent with DSFT (Supplementary Fig.1).

A fresh tumor specimen was collected at the time of the local relapse surgical resection, aseptically dissected, cut into small fragments (3x3x3 mm) and grafted subcutaneously into the right flank of 6 to 8 week-old female SCID mice (Charles River, Calco, Como). Twenty-four hours after inoculum, 100 µl of Matrigel Basement Matrix (BD Biosciences) were injected intratumorally. Mice were housed in a pathogen-free facility with food and water available ad libitum. Tumor growth was followed by biweekly measurement of tumor diameters with a Vernier calliper. Tumor volume (TV) was calculated according to the formula: \[ TV (\text{mm}^3) = \frac{d^2 \times D}{2}, \]
where \( d \) and \( D \) are the shortest and the longest diameter, respectively. After the third passage in mice, and based on growth characteristics, the tumor-line was considered established. The xenograft origin was authenticated through microsatellite analysis by the AmpFISTR Identifiler PCR amplification kit (Applied Biosystems, PN4322288).

**Platelet-derived-growth-factor-receptor beta (PDGFRB) and vascular-endothelial-growth-factor-receptor 2 (VEGFR2) expression/activation**

PDGFRB expression was assessed by immunohistochemistry on fixed material using the rabbit anti-PDGFRB antibody (Cell Signalling, cat: #4564), diluted 1:100. The reaction was carried out using the Bench Mark Ultra automatic staining apparatus (Ventana).

PDGFRB and VEGFR2 activation profile was analysed by means of phospho-RTK arrays (ARY001, R&D Systems) (14) and by immunoprecipitation (IP) / western blotting (WB).
the IP analysis, equal amounts (1 mg) of protein lysates were precipitated by incubation with Protein A Sepharose (Amersham Biosciences) and specific anti-PDGFRB (cat: SC 432, Santa Cruz) or anti-VEGFR2 (cat: #5168, Cell Signaling). WB was carried out by using antiphosphotyrosine antibody (05-321, Upstate) to detect the activation/phosphorylation of the two receptors. The filters were stripped and incubated with the specific PDGFRB (cat: SC 432, Santa Cruz) and VEGFR2 (cat: #2479, Cell Signaling) antibodies to evaluate the degree of expression of the two receptors.

\[ O^6\text{-methylguanine-DNA-methyltransferase (MGMT) gene methylation assessment} \]

\textit{O}^6\text{-methylguanine-DNA-methyltransferase (MGMT) gene methylation assessment} was performed in the human tumor specimen from which the model was derived and in the xenograft, as described above.

\textit{Xenograft treatments.}

Treatments with the different drugs started when xenotransplanted tumors were approximately 80 mm\textsuperscript{3} (early stage tumor). DTIC was also delivered to mice bearing late stage tumors (approximately 250 mm\textsuperscript{3}). Six mice for each experimental group were used. Sunitinib (Sutent, Pfizer) and pazopanib (Votrient, Glaxo Smith-Kline) were dissolved in 0.5 \% carboxymethylcellulose and delivered by gavage 5 days a week for 4 weeks (qd x 5d/w x 4w) at the dose of 40 mg/kg and of 100 mg/kg, respectively. Bevacizumab (Avastin, Roche) was delivered i.p. twice a week for 4 weeks (q3-4d/w x 4w) at the dose of 4mg/kg. Temozolomide (Teva) was delivered by gavage 3 times per week for 4 weeks (q2-3d/w x 4w) at the dose of 50 mg/kg. DTIC (Sanofi-Aventis) was delivered i.p. using two different schedules: \textit{i}) 3 times per week for 4 weeks (q2-3d/w x 4w) at the dose of 70 mg/kg; \textit{ii}) every 7 days for 4 times (q7d x 4) at the dose of 210 mg/kg (20, 21).
The efficacy of drug treatment was assessed in terms of TV inhibition percentage (TVI%) in treated versus control mice expressed as TVI% = 100 – [(mean TV treated / mean TV control) X 100]. The toxicity of drug treatment was determined as body weight loss and lethal toxicity. Deaths occurring in treated mice before the death of the first control mouse were ascribed to toxic effects.

The use of patient material in xenograft and all experiments were approved by the Ethics Committee for Animal Experimentation of INT, according to institutional guidelines that are in compliance with national and international laws and policies.
RESULTS

1. Patients

Eight patients were treated with DTIC, all evaluable for response. Among them, 3 are still on therapy. Median treatment duration was 5 (range: 2-9+) months.

Table 1 summarizes patients characteristics.

Morphologically 3 of 8 cases were classified as MSFT, 5 as DSFT.

MGMT gene methylation assessment

All patients were evaluable for MGMT methylation. MGMT gene resulted methylated only in one case (n.7, Table 1) while in the other 7 patients it was not methylated.

Toxicity

Overall treatment was well tolerated. G3 neutropenia was observed in 1 case and required the administration of GCSF with improvement; G3 thrombocytopenia was noted in 3 patients and required treatment delay. No non-hematologic G>2 toxicity were observed. Nobody stopped his treatment for toxicity.

Response

By RECIST the best response was partial response (PR) in 3/8 cases, 4/8 stable disease (SD) and 1/8 progressive disease (PD). Responses were confirmed at 3 months. All SD lasted more than 3 months. Fig.1 shows two tumor responses to DTIC. Three patients are still on treatment. Of interest, in all the 3 cases with RECIST PR, the best response was observed after >4 cycles of treatment, following initial tumor stabilization. RECIST PR occurred in one patient with a MSFT and in 2 with DSFT. A symptomatic improvement was observed in 3 of 4 cases with symptoms at baseline. In particular, in two cases G3
paraneoplastic hypoglycemia requiring steroids solved and steroids could be completely
stopped after 2 weeks from first DTIC administration. In a third patient G2 dyspnea fully
recovered in 2 weeks.
The median PFS was 7 (range 2-12) months. All patients are alive at the time of the
present analysis.

2. Experimental model and pharmacological studies

Comparison of human and xenograft tumor findings
As shown in Supplementary data and Supplementary Fig.1, at each passage the DSFT
xenograft maintained a morphology comparable with the human sample. Both human and
xenograft samples showed a strong expression and activation of PDGFRB along with
VEGFR1 activation.

MGMT gene methylation assessment
MGMT gene resulted methylated both in human sample and in the DSFT xenograft.

Antitumor activity studies
In the first experiment, the antitumor activity of sunitinib, bevacizumab and temozolomide,
singly administered at their reported optimal doses \(22,23\) was tested against early stage
DSFT xenografts. An additional experiment was successively carried out to investigate the
efficacy of pazopanib against the same tumor model. Although at a different extent, tumor
growth inhibition was observed during treatment with all the different agents. The antitumor
effect was maximum for temozolomide and less pronounced for sunitinib, pazopanib and
bevacizumab (Fig.2, Table 2). Tumor growth was promptly resumed following sunitinib,
pazopanib and bevacizumab withdrawal. In the case of sunitinib, treatment reiteration was
able to stabilize tumor volume for the duration of treatment but tumor restarted to growth
after drug withdrawal (Fig.2). Conversely, following temozolomide treatment, a stabilization of tumor volume was observed without any evidence of tumor regrowth until 120 day from drug withdrawal (Fig.2). No therapeutic advantage was observed when mice were treated with temozolomide in combination with bevacizumab (Fig.1). No sign of toxicity was registered following treatment with the different agents. A remarkable and superimposable tumor growth inhibition (maximum TVI%: about 95 for both agents) was observed treating two groups of early stage DSFT xenografts with temozolomide and with DTIC, at their reported optimal doses (22,23) (Fig.3A, Table 2). No evidence of tumor regrowth was appreciable 100 days after the end of treatments.

Also in late stage tumors, the administration of 70 mg/kg DTIC with the q2-3d/w x 4w schedule produced a remarkable tumor growth inhibition (maximum TVI%: 82), followed by stabilization of tumor volume after drug withdrawal (Fig.3B, Table 2), without any sign of toxicity.

In late stage tumors, we also evaluated the antitumor activity of 210 mg/kg DTIC with the q7d x 4 schedule to rule out if a more intensive schedule would have corresponded to a superior activity. In fact, in the clinical practice DTIC is usually administered every 3 weeks while temozolomide is given daily. A slight increase in tumor growth inhibition (maximum TVI%: 89) was observed compared to the previous schedule (Fig.3B, Table 2). However, treatment induced 15% body weight loss and one toxic death.

Pathologic evaluation of drug-treated early and late stage xenografts.

Early stage DSFT xenografts were sacrificed 120 day after the end of the treatments with sunitinib, bevacizumab, temozolomide and DTIC. Mice treated with pazopanib were instead sacrificed soon after the end of treatment. In line with the macroscopic features (i.e. no evidence of tumor re-growth), post-temozolomide (Fig.4, panel C) and post-dacarbazine (Fig.4, panel D) tumor samples showed a marked cellular depletion.
Conversely, no changes compared to baseline were observed in the post-sunitinib (Supplementary data and Supplementary Fig.2, panel C), post-bevacizumab (Supplementary data and Supplementary Fig.2, panel D) and post-pazopanib (Supplementary data and Supplementary Fig.2, panel E) tumor specimens. This was consistent with the macroscopic evidence of progression in the post-sunitinib and in the post-bevacizumab xenografts. Similarly to what observed in early stage tumors, a marked cellular depletion was found after DTIC treatment also in late stage xenografts, irrespectively from treatment schedules (data not shown).

**PDGFRB and VEGFR2 expression/activation**

After five days of treatment sunitinib and pazopanib induced a decrease in PDGFRB activation, bevacizumab and pazopanib in VEGFR2 (Supplementary Fig. 3). These results are in line with tumor growth curves shown in Fig.2. Contrary to what was observed in mice treated with sunitinib and bevacizumab, the tumor regrowth was observed in xenografts treated with pazopanib while they were still under treatment (Fig.2). Therefore, we decided to evaluate PDGFRB and VEGFR2 status also at the end of treatment with pazopanib, e.g after four weeks of administration. Of interest, VEGFR2 was phosphorylated (Supplementary Fig.3). The unexpected VEGFR2 re-activation could represent a mechanism of resistance. Additional “in vivo” studies are ongoing to better understand the mechanisms underlying response and resistance to antiangiogenics in SFT.
DISCUSSION

In this retrospective case series analysis, 8 patients with a progressive pre-treated advanced SFT (3 MSFT, 5 DSFT) were treated with DTIC. We observed 3 PR, 4 SD (all lasting more than 3 months), and 1 PD, with a median PFS of 7 months. Then, we studied the antitumor effect of DTIC by using an *in vivo* model of dedifferentiated-SFT (DSFT). In order to put this in the context of other promising agents in SFT, we also analysed the effect of sunitinib, pazopanib, bevacizumab, and temozolomide as single agents, and bevacizumab in combination with temozolomide on the DSFT xenograft. All agents were shown to be active. However, temozolomide and DTIC were superior in terms of tumor shrinkage, time to progression after treatment interruption and pathologic response. Overall, temozolomide and DTIC showed a cytotoxic effect, while sunitinib, pazopanib and bevacizumab displayed a cytostatic role. In addition, DTIC activity was equivalent with a more or less frequent administration schedule.

SFT represents an exceedingly rare disease. In addition, many cases are “typical”, i.e. low-risk, while those with a higher metastatic potential are “malignant” or “dedifferentiated/pleomorphic”, thus breaking down further clinical series. No prospective studies focusing on chemotherapy are available. Responses to sunitinib (9-13), and bevacizumab in combination to temozolomide were reported (8), while no clinical nor pre-clinical data on temozolomide and bevacizumab as single agents are available. Thus, we retrospectively reviewed a small series of SFT patients who received single-agent DTIC at our institute and we evaluated the role of all those agents in a xenograft model of DSFT. DTIC is a triazene compound as temozolomide (24) and – unlike temozolomide - it is approved in Europe for the medical therapy of advanced STS. This is the reason why we could not treat a second group of patients with temozolomide. However, for the first time...
these data show that DTIC can be a well tolerated therapeutic option in strongly pretreated advanced SFT patients.

Our preclinical results support clinical evidence. A limitation of our in vivo experiments is due to the intrinsic xenograft model characteristics, which specifically recapitulate the features of DSFT. In fact, SFT is a disease characterized by a broad spectrum of malignancy (1,25-27) and DFST represent a particular subset among different SFT subtypes, though it is probably more frequent than believed in advanced SFT series. Thus, our preclinical findings need to be confirmed in so-called, less aggressive “malignant” SFT. Another limitation is due to the fact that our data refer only to one model of DSFT. In principle, this can be not representative of all cases of DFSP. However, due to the difficulty to generate mouse models from such a rare and slowly growing sarcoma we could not implement the xenograft panel. As matter of fact, work is in progress in our lab to establish new SFT models for future studies. With these limitations, in our DSFT in vivo model, we confirmed that sunitinib, pazopanib, bevacizumab, temozolomide and DTIC as single agents are all active, though to a different extent. Of interest, we found that temozolomide and DTIC were superior in terms of tumor shrinkage, as shown by a maximum TVI of about 95% for both agents. Furthermore, temozolomide and DTIC antitumor effects lasted even after long treatment interruptions (i.e. no tumor progression observed at 120 day from treatment completion), while tumor regrowth was observed soon after withdrawing sunitinib, and bevacizumab. In case of pazopanib, tumor started regrowing earlier, when mice were still under therapy. This was confirmed by the pathologic evaluation of xenografts obtained when animals were sacrificed: 120 days after treatment interruption tumors treated with temozolomide and DTIC showed an almost complete pathologic response, while tumors treated with sunitinib, bevacizumab and pazopanib were completely viable.
These data are consistent with a cytotoxic effect of temozolomide and DTIC, compared to a cytostatic effects of antiangiogenics. As expected, temozolomide and DTIC were superimposable both in terms of tumor regression, treatment effect duration and pathologic response. Of note, DTIC is a prodrug that requires enzymatic conversion to its active form, 5-(3-methyltriazen-1yl)-imidazole-4-carboxamide (MTIC), while temozolomide is rapidly metabolized to MTIC at physiologic pH, without enzymatic involvement. This may not entail any difference in mice, since it is expected that DTIC conversion occurs better in mice than in humans (28). Thus, in principle, temozolomide might be advantageous in humans, though clinical evidence thereof is lacking. In addition, temozolomide could be superior to DTIC in case of CNS disease, whether for brain metastases or meningeal primaries (which is typical of a subgroup of SFT) given its ability to enter the cerebrospinal fluid (29). As a matter of fact, we saw convincing responses to DTIC in a small group of patients. A comparative study would be useful to confirm DTIC activity in a larger number of patients and to compare it to temozolomide.

Of notice, MGMT gene was shown to be methylated both in the DSTF xenograft and in the human tumor from which it was derived, while it was methylated only in one case among patients we treated with DTIC. In particular MGMT was methylated in one of the 3 cases who responded to DITC. On this basis a correlation between the antitumor activity of DTIC and MGMT status cannot be excluded. In fact, it is known that in glioblastoma the presence of MGMT promoter methylation – that enhances the response to alkylating agents by inhibiting DNA repair - is predictive of a better response to temozolomide in combination with radiotherapy (17) On the contrary, MGMT predictive role has not been definitively confirmed in other tumors responsive to temozolomide, such as melanoma.
The correlation between MGMT gene methylation status and response to DTIC and temozolomide can thus represent an interesting field of research in SFT.

We found that the temozolomide-bevacizumab combination did not induce a better therapeutic activity than temozolomide alone. A tentative explanation could be that the administration of antiangiogenic drugs may impair cytotoxic agent delivery by pruning tumor vessels and by reducing vascular permeability. Again, a comparative study on temozolomide or dacarbazine as single agents versus their combination with an antiangiogenic agent would be worth doing in principle.

In clinical practice, temozolomide is administered every day, while DTIC is given every 3 weeks. For this reason, we decided to run an additional experiment comparing two different DTIC schedules: DTIC at a lower dose every 3 days versus DTIC at a higher dose every 7 days, for an overall equivalent dose. Of interest, no remarkable difference could be noted in activity between the two regimens.

By using DTIC, we could observe dimensional responses in some patients, and tumor disease stabilization in others, both in MSFT and in DSFT. Responses were prolonged and corresponded to a significant improvement in symptomatic cases. In particular, we could note the rapid resolution of a hypoglycemic crisis in two patients with a baseline paraneoplastic G3-4 hypoglicemia requiring steroids. As expected, DTIC was very well tolerated.

We had already published a retrospective study on the activity of sunitinib in a series of 30 patients with advanced SFT. After sunitinib, we observed 2 RECIST PR and 16 SD, with a median PFS of 6 months, and a greater efficacy of sunitinib in MSFT compared to DSFT.
(12). With the limitation of an external comparison among small retrospective series, it can be noted that the response rate by RECIST was greater among patients treated with DTIC, e.g. 3 of 8 cases, compared to what obtained with sunitinib. This suggests that DTIC could be more effective in achieving a tumor shrinkage compared to sunitinib, at least in more aggressive cases. Interestingly, most patients treated in this series received sunitinib before DTIC (Table 1): responses to DTIC were observed in patients who progressed under sunitinib and vice versa.

In conclusion, our results confirm that temozolomide and DTIC can be active as single agents in SFT. A prospective phase 2 study on DTIC in advanced SFT is due to start. Among others, subjects to be elucidated could include a comparison between DTIC and temozolomide, the correlation of their antitumor activity with tumor aggressiveness (i.e., with whether the SFT is malignant or dedifferentiated), the value of MGMT gene methylation.
Funding

Supported by grant from Associazione Italiana per la Ricerca sul Cancro (AIRC): IG 10300.
REFERENCES


FIGURE LEGENDS

Figure 1

Response to dacarbazine. CT scan (arterial phase after contrast medium). Panel A shows a marked hepatomegaly due to multiple liver metastases from pelvic SFT at baseline. A dimensional response to treatment after 2 cycles of dacarbazine with disappearance of some lesions is shown in panel B. Tumor response and hepatomegaly improved further as confirmed by the CT scan after 8 cycles of therapy (panel C).

Figure 2

A Efficacy of oral Temozolomide (50 mg/kg, q2-3d/ w x 4w ), i.p Bevacizumab (4 mg/kg, q3-4d/ w x 4w ) -alone and in combination- and oral Sunitinib (40 mg/kg, qdx5d/ w x 4w ) against SFT xenotransplanted in SCID mice. The treatment duration is indicated by the grey bar.

B Efficacy of oral Pazopanib (100 mg/kg, qdx5d/ w x 4w ) against SFT xenotransplanted in SCID mice. The treatment duration is indicated by the grey bar.

Figure 3

A Efficacy of oral Temozolomide (50 mg/kg, q2-3d/ w x 4w ) and i.p. Dacarbazine (70 mg/kg, q2-3d/ w x 4w ) against SFT growing in SCID mice. The treatment duration is indicated by the grey bar.

B Efficacy of i.p. Dacarbazine against SFT xenotransplanted in SCID mice according to two different schedule: (70 mg/kg, q2-3d/ w x 4w ) or (210 mg/kg, q7d x 4). The treatment duration is indicated by the grey bar.

Figure 4
SFT xenotransplanted in SCID mice pathologic evaluation before and after treatment.

A and B Pre-treatment samples: high-grade sarcoma aspects mixed with cartilaginous (A) and osseous component (B).

C and D Post-temozolomide and post-dacarbazine samples: 120 days after the end of treatment with Temozolomide (C) and Dacarbazine (D) tumor lesions were removed. The samples showed an almost complete response to treatment, with almost no more evidence of tumor cells (C and D).
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Age at time of CT (years)</th>
<th>PS</th>
<th>Site of primary tumor</th>
<th>Site of relapse at the time of chemotherapy with DTIC</th>
<th>Diagnosis</th>
<th>Ki-67 expression</th>
<th>MGMT</th>
<th>Prior treatment (response y/n)</th>
<th>Response to DTIC: RECIST evaluation</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>74</td>
<td>1</td>
<td>pleura</td>
<td>locally advanced</td>
<td>MSFT</td>
<td>moderate</td>
<td>not methylated</td>
<td>sunitinib (y); gemcitabine (n)</td>
<td>PR</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>66</td>
<td>1</td>
<td>peritoneum</td>
<td>abdomen</td>
<td>MSFT</td>
<td>moderate</td>
<td>not methylated</td>
<td>sunitinib (n); cyclophosphamide (n)</td>
<td>SD</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>69</td>
<td>0</td>
<td>pelvis</td>
<td>liver, peritoneum</td>
<td>MSFT</td>
<td>moderate</td>
<td>not methylated</td>
<td>sunitinib (y); trabectedin (n)</td>
<td>SD</td>
<td>3+</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>69</td>
<td>0</td>
<td>peritoneum</td>
<td>liver</td>
<td>DSFT</td>
<td>high</td>
<td>not methylated</td>
<td>doxorubicin + ifosfamide (n); sunitinib (y);</td>
<td>PR</td>
<td>11+</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>59</td>
<td>1</td>
<td>pleura</td>
<td>local, lung</td>
<td>DSFT</td>
<td>high</td>
<td>not methylated</td>
<td>doxorubicin (n), ifosfamide (n), sunitinib (n), trabectedin (n)</td>
<td>PD</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>57</td>
<td>3</td>
<td>meninges</td>
<td>local, liver</td>
<td>DSFT</td>
<td>high</td>
<td>not methylated</td>
<td>doxorubicin + ifosfamide (not evaluable for response); gemcitabine (n)</td>
<td>SD</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>55</td>
<td>2</td>
<td>meninges</td>
<td>local, lung</td>
<td>DSFT</td>
<td>high</td>
<td>methylated</td>
<td>doxorubicin + ifosfamide (n); high-dose prolonged infusion ifosfamide (n); sunitinib (n)</td>
<td>PR</td>
<td>8+</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>39</td>
<td>0</td>
<td>meninges</td>
<td>local, lung</td>
<td>DSFT</td>
<td>nv</td>
<td>not methylated</td>
<td>sunitinib (n); trabectedin (n)</td>
<td>SD</td>
<td>4+</td>
</tr>
</tbody>
</table>
### Table 2

#### Early stage

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Route</th>
<th>Max TVI%&lt;sup&gt;1&lt;/sup&gt; (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>4</td>
<td>q3-4d/w x 4w</td>
<td>i.p.</td>
<td>78 (108)</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>40</td>
<td>qd x 5d/w x 4w</td>
<td>p.o.</td>
<td>52 (115)</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>100</td>
<td>qd x 5d/w x 4w</td>
<td>p.o.</td>
<td>41 (60)</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>50</td>
<td>q2-3d/ w x 4w</td>
<td>p.o.</td>
<td>96 (120)</td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>70</td>
<td>q2-3d/ w x 4w</td>
<td>i.p.</td>
<td>95 (120)</td>
</tr>
</tbody>
</table>

#### Late stage

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Route</th>
<th>Max TVI%&lt;sup&gt;1&lt;/sup&gt; (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacarbazine</td>
<td>70</td>
<td>q2-3d/ w x 4w</td>
<td>i.p.</td>
<td>82 (105)</td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>210</td>
<td>q7d x 4</td>
<td>i.p.</td>
<td>89 (105)</td>
</tr>
</tbody>
</table>
Figure 1

baseline

+2 mos

+8 mos

DTIC
Figure 2

(a) Tumor Volume (mm³)

- Control
- Temozolomide (T)
- Bevacizumab (B)
- Bevacizumab + Temozolomide
- Sunitinib (S)

Days after inoculum:
0 20 40 60 80 100 120 140 160 180 200

(b) Tumor Volume (mm³)

- Control
- Pazopanib (P)

Days after inoculum:
40 50 60 70 80 90 100
Figure 3

A

Control  
Temozolomide (50 mg/kg q2d p.o.)  
Dacarbazine (70 mg/kg q2dl.p.)

B

Control  
Dacarbazine (70 mg/kg ip)  
Dacarbazine (210 mg/kg ip)
Figure 4

Pretreatment

Post-Temozolomide

Post-Dacarbazine
Dacarbazine in solitary fibrous tumor: a case series analysis and preclinical evidence vis-à-vis temozolomide and antiangiogenics

Silvia Stacchiotti, Monica Tortoreto, Fabio Bozzi, et al.

Clin Cancer Res Published OnlineFirst July 25, 2013.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-0776

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/07/25/1078-0432.CCR-13-0776.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.