Cancer Therapy: Clinical

Dacarbazine in Solitary Fibrous Tumor: A Case Series Analysis and Preclinical Evidence vis-à-vis Temozolomide and Antiangiogenics


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 (1,200 mg/m2 every 3 weeks) as from January 2012. Then, we studied a dedifferentiated-SFT subcutaneously xenotransplanted into severe combined immunodeficient (SCID) mice. Dacarbazine, 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 tumor volume inhibition percentage (TVI%). Dacarbazine was tested according to two different schedules of administration. One hundred twenty days after treatment interruption, mouse tumor samples were analyzed.

Results: Among the eight patients treated with dacarbazine, best response evaluation criteria in solid tumors responses (RECIST) were three partial responses, 4 stable disease, 1 progression. Two responsive patients had paraneoplastic hypoglycemia that disappeared after 10 days from starting dacarbazine. In the dedifferentiated-SFT xenograft model, dacarbazine 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 regrowth was observed up to 100 days from end of treatment with temozolomide and dacarbazine, whereas secondary progression followed sunitinib, pazopanib, and bevacizumab interruption.

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

Introduction

Solitary fibrous tumor (SFT) is a rare soft-tissue sarcoma (STS), marked by the presence of a recurrent NAB2-STAT6 gene fusion, which was recently identified (1, 2). "Typical" SFT can be cured in the majority of cases by complete surgical resection, whereas 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 +/− 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 such as sorafenib, sunitinib, bevacizumab plus temozolomide, pazopanib, insulin-like growth factor (IGF)-1R inhibitors was described (8–15). In all cases, responses were mostly nondimensional. In particular, researchers from the University of Texas MD Anderson Cancer Center (Houston, TX) observed 2 partial responses (PR) and 12 stable diseases...
Translational Relevance

This study is the first report showing that dacarbazine as single agent has antitumor activity in patients with progressive pretreated advanced solitary fibrous tumor (SFT). The clinical evidence is supported by preclinical results obtained on a human high-grade dedifferentiated-SFT xenotransplanted into severe combined immunodeficient (SCID) mice. When singly administered in the mouse model, the 2 triazine compounds dacarbazine 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. In contrast, the antiangiogenic agents sunitinib, pazopanib, and bevacizumab were found to be less active, with tumor regrowth appreciable immediately after drug withdrawal. Clinical prospective studies are needed to compare dacarbazine and temozolomide in patients with advanced SFT and to correlate their activity with tumor aggressiveness.

(SD) by response evaluation criteria in solid tumor (RECIST; ref. 16) in 14 patients treated by temozolomide and bevacizumab. The median progression-free survival (PFS) was about 10 months (8). Temozolomide is an imidazotetrazine derivative of the alkylating agent dacarbazine. The drug is approved for treatment of glioblastoma multiforme and anaplastic astrocytoma (17), but is not approved for STS, whereas the use of dacarbazine is authorized for advanced STS cases in several European Union Member States, including Italy.

We report hereafter on the activity of dacarbazine 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 dacarbazine, temozolomide, sunitinib, pazopanib, and bevacizumab.

Materials and Methods

Patients

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

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 (PD) before starting treatment, Eastern Cooperative Oncology Group performance status (ECOG PS) ≤3 and an adequate bone marrow and organ function.

Histologic diagnosis was centrally reviewed in all cases. The diagnosis was rendered according to the last World Health Organization classification (1).

Qβ-methylguanine-DNA-methyltransferase gene methylation assessment. Qβ-methylguanine-DNA-methyltransferase (MGMT) gene methylation assessment was retrospectively conducted in all patients treated with dacarbazine. DNA was extracted from formalin-fixed paraffin-embedded selected tumor samples using the QiAmp DNA Mini Kit (Qiagen) and bisulphate treated (Methylation Kit; Zymo Research). PCR amplification was conducted using the following primers: MGMT Met Fw: 5′-cgaatatataaaaacaacccgc -3′; MGMT Met Rev: 5′-gtatttttcgggagccagt-3′; MGMT UnMet Fw: 5′-ccaatataaaaacaacccaca-3′; MGMT UnMet Rev: 5′-igtatttttcgggagccagt-3′ (18). The analysis was conducted twice in all cases.

Treatment. All patients provided a written informed consent to the treatment. Patients received dacarbazine 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 dacarbazine was reduced to 1,000 and then to 800 mg/m². Granulocyte colony stimulating factors (GCSF) were administered in case of grade 3–4 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 imaging (MRI) of the sites of disease, and a whole body bone scan. CT/MRI were repeated after 4 to 8 weeks of treatment then every 2 to 3 months.

Response to treatment was assessed applying RECIST (16).

PFS and overall survival were estimated with Kaplan–Meier method (19). Failure for PFS was progressive disease according to RECIST or death.

Experimental model and pharmacologic 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 4 years after the primary tumor and was consistent with DSFT (Supplementary Fig. S1).

A fresh tumor specimen was collected at the time of the local relapse surgical resection, aseptically dissected, cut into small fragments (3 × 3 × 3 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...
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Age at time of CT (y)</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 dacarbazine: 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>

*aY/n, yes/no.*
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: \( TV = \frac{d \times D}{2} \), 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 Amp-FISTR Identifiler PCR Amplification Kit (Applied Biosystems, PN4322288).

**Platelet-derived-growth-factor-receptor β and VEGF receptor 2 expression/activation.** Platelet-derived-growth-factor-receptor β (PDGFRB) expression was assessed by immunohistochemistry on fixed material using the rabbit anti-PDGFRB antibody (Cell Signalling Technology, cat: SC 432, Santa Cruz Biotechnology, Inc.) or anti-VEGFR2 (Amersham Biosciences) and specific anti-PDGFRB (cat: #4564), diluted 1:100. The reaction was carried out using the Bench Mark Ultra automatic staining apparatus (Ventana).

PDGFRB and VEGF receptor 2 (VEGFR2) activation profile was analyzed by means of phospho-RTK arrays (ARY001, R&D Systems; ref. 14) and by immunoprecipitation (IP)/Western blotting (WB). For the immunoprecipitation 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 Biotechnology, Inc.) or anti-VEGFR2 (cat: #5168, Cell Signaling Technology). Western blotting was carried out using antiphosphotyrosine antibody (05-321) to detect the activation/phosphorylation of the two receptors. The filters were stripped and incubated with the specific PDGFRB (cat: SC 432, Santa Cruz Biotechnology, Inc.) and VEGFR2 (cat: #2479, Cell Signaling Technology) antibodies to evaluate the degree of expression of the two receptors.

**O'-methylguanine-DNA-methyltransferase gene methylation assessment.** MGMT methylation assessment was conducted in the human tumor specimen from which the model was derived and in the xenograft, as described earlier.

**Xenograft treatments.** Treatments with the different drugs started when xenotransplanted tumors were approximately 80 mm³ (early-stage tumor). Dacarbazine was also delivered to mice bearing late-stage tumors (approximately 250 mm³). Six mice for each experimental group were used. Sunitinitib (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 × 5d/w × 4w) at the dose of 40 mg/kg and of 100 mg/kg, respectively. Bevacizumab (Avastin, Roche) was delivered intraperitoneally twice a week for 4 weeks (q3-4d/w × 4w) at the dose of 4mg/kg. Temozolomide was delivered by gavage 3 times per week for 4 weeks (q2-3d/w × 4w) at the dose of 50 mg/kg. Dacarbazine (Sanofi-Aventis) was delivered intraperitoneally using two different schedules: (i) 3 times per week for 4 weeks (q2-3d/w × 4w) at the dose of 70 mg/kg; (ii) every 7 days for 4 times (q7d × 4) at the dose of 210 mg/kg (20, 21).

The efficacy of drug treatment was assessed in terms of tumor volume inhibition percentage (TVI%) in treated versus control mice expressed as TVI% = 100 - [(mean tumor volume treated/mean tumor volume control) × 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**

**Patients**

Eight patients were treated with dacarbazine, 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.

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

**Toxicity.** Overall treatment was well tolerated. Grade 3 neutropenia was observed in 1 case and required the administration of GCSF with improvement; grade 3 thrombocytopenia was noted in 3 patients and required treatment delay. No nonhematologic 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 SD, and 1/8 PD. Responses were confirmed at 3 months. All SD lasted more than 3 months. Figure 1 shows two tumor responses to dacarbazine. Three patients are still on treatment. Of interest, in all the 3 cases with RECIST PR, the best response was observed after 4 cycles or more 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 with grade 3 para-neoplastic hypoglycemia requiring steroids solved and steroids could be completely stopped after 2 weeks from first dacarbazine administration. In a third patient grade 2 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.

**Experimental model and pharmacologic studies**

**Comparison of human and xenograft tumor findings.** As shown in Supplementary data and Supplementary Fig. S1, 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 dacarbazine, at their reported optimal doses (refs. 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 dacarbazine with the q2-3d/w × 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 dacarbazine with the q7d × 4 schedule to rule out whether a more intensive schedule would be necessary.

### Table 2. Antitumor activity of Bevacizumab, Sunitinib, Temozolomide, Dacarbazine on DSFT xenograft

<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>Early-stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>4</td>
<td>q3-4d/w × 4w</td>
<td>i.p.</td>
<td>78 (108)</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>40</td>
<td>qd × 5d/w × 4w</td>
<td>p.o.</td>
<td>52 (115)</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>100</td>
<td>qd × 5d/w × 4w</td>
<td>p.o.</td>
<td>41 (60)</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>50</td>
<td>q2-3d/w × 4w</td>
<td>p.o.</td>
<td>96 (120)</td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>70</td>
<td>q2-3d/w × 4w</td>
<td>i.p.</td>
<td>95 (120)</td>
</tr>
<tr>
<td>Late stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>70</td>
<td>q2-3d/w × 4w</td>
<td>i.p.</td>
<td>82 (105)</td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>210</td>
<td>q7d × 4</td>
<td>i.p.</td>
<td>89 (105)</td>
</tr>
</tbody>
</table>

Abbreviation: p.o., per os.

<sup>1</sup>Maximum tumor volume inhibition % in treated versus control mice. In parentheses, the day on which it was assessed.
would have corresponded to a superior activity. In fact, in the clinical practice dacarbazine is usually administered every 3 weeks, whereas temozolomide is given daily. A slight increase in tumor growth inhibition (maximum TVI%: 89) was observed compared with 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 dacarbazine. 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 regrowth), post-temozolomide (Fig. 4, C) and post-dacarbazine (Fig. 4, D) tumor samples showed a marked cellular depletion. Conversely, no changes compared with baseline were observed in the post-sunitinib (Supplementary data and Supplementary Fig. S2C), post-bevacizumab (Supplementary data and Supplementary Fig SD), and post-pazopanib (Supplementary data and Supplementary Fig. S2E) 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 dacarbazine 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. S3). 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, whereas they were still under treatment (Fig. 2). Therefore, we decided to evaluate PDGFRB and VEGFR2 status also at the end of treatment with pazopanib, for example, after four weeks of administration. Of interest, VEGFR2 was phosphorylated (Supplementary Fig. S3).

The unexpected VEGFR2 reactivation 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 pretreated advanced SFT (3 MSFT, 5 DSFT) were treated with dacarbazine. 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 dacarbazine using an in vivo model of DSFT. To put this in the context of other promising agents in SFT, we also analyzed 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 dacarbazine were superior in terms of tumor shrinkage, time to progression after treatment interruption and pathologic response. Overall, temozolomide and dacarbazine showed a cytotoxic effect, whereas sunitinib, pazopanib, and bevacizumab displayed a cytostatic role. In addition, dacarbazine activity was equivalent with a more or less frequent administration schedule.
SFT represents an exceedingly rare disease. In addition, many cases are "typical", that is low-risk, whereas those with a higher metastatic potential are "malignant" or "dedifferentiated/pleomorphic", thus breaking down further clinical series (1). No prospective studies focusing on chemotherapy are available. Responses to sunitinib (9–13), and bevacizumab in combination to temozolomide were reported (8), whereas no clinical nor preclinical data on temozolomide and bevacizumab as single agents are available. Thus, we retrospectively reviewed a small series of SFT patients who received single-agent dacarbazine at our institute and we evaluated the role of all those agents in a xenograft model of DSFT. Dacarbazine is a triazene compound as temozolomide (24) and unlike temozolomide it is approved in Europe for the medical therapy for 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 dacarbazine 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 DSFT 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 dedifferentiated solitary fibrous tumor. 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 laboratory 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 dacarbazine as single agents are all active, though to a different extent. Of interest, we found that temozolomide and dacarbazine were superior in terms of tumor shrinkage, as shown by a maximum TVI of about 95% for both agents. Furthermore, temozolomide and dacarbazine antitumor effects lasted even after long treatment interruptions (i.e., no tumor progression observed at 120 day from treatment completion), whereas 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 dacarbazine showed an almost complete pathologic response, whereas tumors treated with sunitinib, bevacizumab, and pazopanib were completely viable.

These data are consistent with a cytotoxic effect of temozolomide and dacarbazine, compared with a cytostatic effects of antiangiogenics. As expected, temozolomide and dacarbazine were superimposable both in terms of tumor regression, treatment effect duration and pathologic response. Of note, dacarbazine is a prodruk that requires enzymatic conversion to its active form, 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC), whereas temozolomide is rapidly metabolized to MTIC at physiologic pH, without enzymatic involvement. This may not entail any difference in mice, as it is expected that dacarbazine 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 dacarbazine in case of central nervous system 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 dacarbazine in a small group of patients. A comparative study would be useful to confirm dacarbazine activity in a larger number of patients and to compare it with temozolomide.

Of notice, MGMT gene was shown to be methylated both in the DSFT xenograft and in the human tumor from which it was derived, whereas it was methylated only in one case among patients we treated with dacarbazine. In particular MGMT was methylated in one of the 3 cases who responded to dacarbazine. On this basis, a correlation between the antitumor activity of dacarbazine 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 (30). The correlation between MGMT gene methylation status and the response to dacarbazine 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, whereas dacarbazine is given every 3 weeks. For this reason, we decided to run an additional experiment comparing two different dacarbazine schedules: dacarbazine at a lower dose every 3 days versus dacarbazine 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.

Using dacarbazine, 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 2 patients with a baseline pancreatic grade 3–4 hypoglycemia requiring steroids. As expected, dacarbazine 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 with DSFT (12). With the limitation of an external comparison among small retrospective series, it can be noted that the RR by RECIST was greater among patients treated with dacarbazine, for example, 3 of 8 cases, compared with what obtained with sunitinib. This suggests that dacarbazine could be more effective in achieving a tumor shrinkage compared with sunitinib, at least in more aggressive cases. Interestingly, most patients treated in this series received sunitinib before dacarbazine (Table 1): responses to dacarbazine and vice versa.

In conclusion, our results confirm that temozolomide and dacarbazine can be active as single agents in SFT. A prospective phase II study on dacarbazine in advanced SFT is due to start. Among others, subjects to be elucidated could include a comparison between dacarbazine 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.

Disclosure of Potential Conflicts of Interest
S. Stacchiotti has commercial research grant from Pfizer and Glaxo Smith Kline. P.G. Casali is a consultant/advisory board member in Pfizer. No potential conflicts of interest were disclosed by the other authors.

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