Temsirolimus in advanced leiomyosarcomas: patterns of response and correlation with the activation of the mammalian target of rapamycin pathway

Antoine Italiano[a], Michèle Kind[b], Eberhard Stoeckle[c], Natalie Jones[e], Jean-Michel Coindre[d] and Binh Bui[a]

Preclinical data have indicated that alteration of *PTEN* and activation of the mammalian target of rapamycin (mTOR) pathway play a crucial role in the oncogenesis of leiomyosarcoma. The objective of this exploratory study was to assess the clinical role of mTOR inhibition in patients with advanced leiomyosarcoma refractory to standard chemotherapy. Patients with advanced leiomyosarcoma were treated with temsirolimus and consented to retrospective collection of data from their medical records and analysis of archival tumor specimens. Tumor response was determined according to the response evaluation criteria in solid tumor (RECIST) and Choi criteria. Tumors were assessed for immunohistochemical evidence of PTEN loss of expression and mTOR activation. Six patients participated in the study. According to the RECIST, three patients had stable disease and three patients had progressive disease. The three patients with RECIST stable disease had partial response according to the Choi criteria. Partial response according to the Choi criteria was associated with clinical improvement and biological signs of temsirolimus antitumor activity. The immunohistochemical status of PTEN and phosphorylated S6 ribosomal protein was not predictive of the outcome. This exploratory study indicates antitumor activity of temsirolimus in leiomyosarcoma, possibly through a mechanism involving aberration of the *PTEN* gene. Further investigations of the phosphoinositide 3-kinases/PTEN/Akt/mTOR pathway are needed to explore the role of mTOR inhibitors, either alone or in combination, in patients with advanced sarcoma.

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Departments of [a]Medical Oncology, [b]Radiology, [c]Surgery, [d]Pathology and [e]INSERM U916, Institut Bergonié, Bordeaux, Cedex, France

Correspondence to Dr Antoine Italiano, Department of Medical Oncology, Institut Bergonié, 229 cours de l'Argonne, Bordeaux, Cedex 33076, France
Tel: +33 5 56 33 33 33; fax: +33 5 56 33 33 30;
e-mail: italiano@bergonie.org

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Introduction

Soft tissue sarcomas are a heterogeneous group of rare malignancies including more than 50 histological subtypes with variations in terms of biology and natural history [1]. On the basis of their genetic and molecular characteristics, sarcomas can be divided into two main groups: those with simple and recurrent molecular events (such as synovial sarcoma, myxoid liposarcoma, or well-differentiated/dedifferentiated liposarcoma) and those with variable complex genetic changes [2]. Sarcoma with complex genomics (SGC) is mainly composed of spindle cell/pleiomorphic tumors, corresponding to leiomyosarcomas and pleiomorphic tumors including myxofibrosarcomas, pleiomorphic liposarcomas, pleiomorphic rhabdomyosarcomas, and undifferentiated pleiomorphic sarcomas [1,2]. Leiomyosarcomas is one of the most frequent sarcoma among SGC subtypes. Several studies have shown that partial loss of chromosome 10q containing the *PTEN* gene is a frequent event in leiomyosarcomas and may be associated with poor prognosis [3–8]. *PTEN* is a tumor-suppressor gene encoding a phosphatase that negatively regulates the Akt–mammalian target of rapamycin (mTOR) pathway. Recent results obtained from a murine model have suggested that, in accordance with data obtained from the genomic analysis of human leiomyosarcomas, alteration of *PTEN* and activation of the mTOR pathway play a crucial role in the oncogenesis of leiomyosarcomas [9]. Altogether, these results suggest that the mTOR pathway is an ideal target for therapy in leiomyosarcomas.

Here, we report the clinical activity of temsirolimus, a highly specific inhibitor of mTOR, in a series of patients with advanced leiomyosarcomas and its correlation with pretreatment markers of mTOR activity.

Patients and methods

Patients

Patients treated in this series had to have progressive advanced leiomyosarcomas during the previous 2 months according to response evaluation criteria in solid tumor (RECIST) [10] and had to receive at least one line of chemotherapy containing anthracycline. A performance status (Eastern Cooperative Oncology Group) of less than or equal to 3 and an adequate bone marrow and organ function were also requested. All patients were aware

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that temsirolimus was used 'of label' as a compassionate treatment. For all patients, a histological review was carried out and the histological diagnosis of leiomyosarcomas was established according to the World Health Organization Classification of Tumors [11]. The histological grade was determined after central review as described earlier according to the French grading system [12]. This study was approved by our Institutional Review Board.

Treatment

Patients received 25 mg of temsirolimus once weekly, infused intravenously over 30 min on days 1, 8, 15, and 22. The treatment cycle lasted for 28 days. Treatment was continued until disease progression or unacceptable toxicity. Staging radiological studies were carried out after every two cycles. Response was assessed by RECIST and Choi criteria [10,13]. Toxicity was defined according to the National Cancer Institute Common Toxicity Criteria, version 3.0.

Immunohistochemistry

Sections of paraffin-embedded tissue were deparaffinized in xylene, dehydrated in ethanol, and pretreated with citrate buffer (10 mmol/l), pH 6.1, for 20 min in a 95°C water bath for antigen retrieval. Endogenous peroxidases were blocked by methanol containing 3% H_2O_2 for 30 min. Immunohistochemical analysis was carried out using mouse monclonal antibody against PTEN (Cascade Biosciences, Winchester, Massachusetts, USA) and rabbit polyclonal antibody against phospho-S6 ribosomal protein (P-S6RP; Cell Signaling, Danvers, Massachusetts, USA). Incubation of the primary antibody lasted for 30 min at room temperature and was followed by incubation with secondary antibody and peroxidase-based revelation system (Dako REAL Detection Kit, K 5001, Dako, Hamburg, Germany) according to the manufacturer's protocol. The slides were counterstained with hematoxylin and were differentiated in lithium carbonate. Between each step, the slides were rinsed in a 1X phosphate buffer solution. PTEN expression was assessed in comparison with normal vascular endothelial cells present in each sample that were used as internal positive controls [14]. It was semiquantitatively evaluated from score 0 (no expression in tumoral cells, presence of positive endothelial cells), to 1 (decreased expression intensity in tumoral cells) and 2 (same intensity of expression in tumoral and in normal endothelial cells) [14]. Slides without signal even in endothelial cells or with 'zone effect' (i.e. alternation of entirely positive and entirely negative zones) were considered as 'nonassessable'.

P-S6RP protein expression was assessed using a semiquantitative scoring system described earlier [15] evaluating the percent of stained cells (0: none, 1: $< 10\%$, 2: 10–25%, 3: 25–50%, 4: $> 50\%$) and the intensity of staining scored from 1 to 3. We used the product of these scores to define the histological scoring from 0 to 12 (≤ 3: low expression, 4–6: intermediate expression, > 6: high expression).

Results

Patients and treatment

Six patients entered the study, and their characteristics are described in Table 1. The mean duration of treatment was 4.1 months. Overall, temsirolimus was well tolerated. The major nonhematologic toxicities include asthenia (patients 1 and 3: grade 1, patients 4 and 5: grade 2), mucitis (patients 1, 3, and 4: grade 1, patient 2: grade 2), anorexia (patient 4: grade 1), and hypertriglycidemia (patient 2: grade 1). The most common hematologic adverse events were: chronic anemia and neutropenia (patient 4: grade 2). No treatment interruption for toxicity was required. No dose reductions were required due to adverse events.

Response

All patients were evaluable for response (Table 2). According to the RECIST, three patients had stable disease (SD; patients 1, 2, and 4) and three patients had progressive disease. The three patients with RECIST SD had partial response (PR) according to the Choi criteria

Table 1 Patients' characteristics

Patient	Sex	Age	Performance status	Primary site	Grade	Sites of metastasis	Number of previous lines of chemotherapy	Presence of symptoms
1	Male	58	1	Popliteal vein	2	Liver, lung	4 (MAID, docetaxel–gemcitabine, clinical trial[a], metronomic cyclophosphamide)	No
2	Female	48	2	Mesentery	3	Liver	4 (doxorubicin, docetaxel–gemcitabine, clinical trial[a], metronomic cyclophoshamide)	Yes
3	Male	33	1	Bone (femur)	NA	Liver, lung, bone	3 (API, docetaxel–gemcitabine, trabectedin)	Yes
4	Female	63	1	Retroperitoneum	3	Liver, lung	3 (MAID, gemcitabine, trabectedin)	No
5	Female	56	2	Uterus	3	Liver	4 (API, docetaxel–gemcitabine, trabectedin, clinical trial[b])	Yes
6	Female	40	2	Bone (femur)	3	Liver, lung, and bone	4 (MAID, clinical trial[a], trabectedin, gemcitabine)	Yes

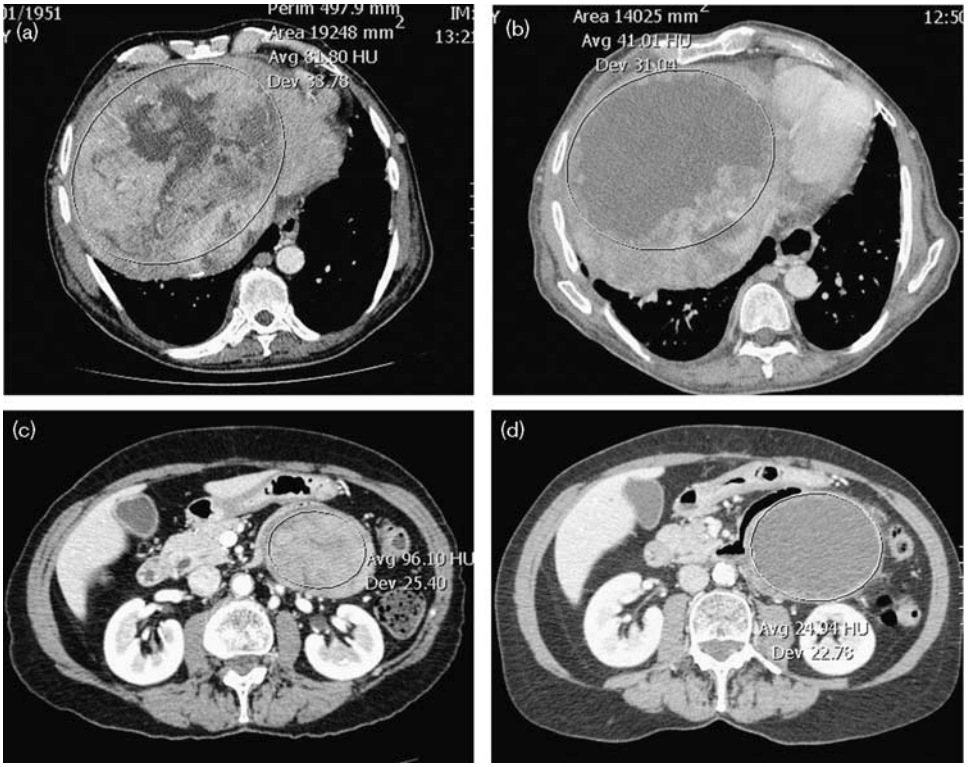
API, doxorubicin, cisplatin, ifosfamide; MAID, mesna, doxorubicin, ifosfamide, dacarbazine; NA, not available.
[a]*http://clinicaltrials.gov/ct2/show/NCT00699517.*
[b]*http://clinicaltrials.gov/ct2/show/NCT00413192.*

Table 2 Results

Patient	Progression free (months)	Toxicity grades 1–2	Toxicity grades 3–4	Symptom improvement	Best RECIST response	Best Choi response	P-S6RB score	PTEN score
1	7	Mucitis, asthenia	None	Not applicable	SD	PR	9 (high)	2
2	4	Mucitis, hypertriglycidemia	None	Yes	SD	PR	0	1
3	4	Mucitis, asthenia	None	No	PD	PD	9 (high)	2
4	7 (ongoing)	Mucitis, anorexia, neutropenia	None	Not applicable	SD	PR	12 (high)	NA
5	5	None	None	No	PD	PD	9 (high)	2
6	2	None	None	No	PD	PD	6 (intermediate)	2

NA, not available; PD, progressive disease; PR, partial response; P-S6RB, phospho-S6 ribosomal protein; RECIST, response evaluation criteria in solid tumor; SD, stable disease.

Fig. 1



Patterns of radiological response in patients with leiomyosarcoma treated with temsirolimus. (a and c): liver [81 Hounsfield units (HU)] (a) and peritoneal metastasis (96 HU; c) in patients 1 (a) and 4 (c) before temsirolimus treatment. (b and d) Partial response according to the Choi criteria in patients 1 (b) and 4 (d): computed tomography scan obtained 2 months after treatment shows that the lesions have become significantly hypodense (patient 1: 41 HU, patient 4: 24 HU). According to the response evaluation criteria in solid tumor, the response was a stable disease (−6.5% in variation size for patient 1 and +11% in variation size for patient 4).

(Fig. 1). The three patients who achieved PR according to Choi criteria received a mean of six cycles (median, 6; range, 4–7). One patient with PR remained on treatment after 7 months; the other two patients with PR stopped taking the drug after 7 months and 4 months because of progression. PR according to the Choi criteria was associated with clinical and biological signs of temsirolimus antitumor activity. One patient (patient 2) with severe pain due to advanced liver metastases presented symptomatic improvement and stopped morphine after one cycle of temsirolimus. In this patient, alkaline phosphatase and γ-glutamyl transpeptidase decreased from 455 and 713 μ/l to 198 and 333 μ/l, respectively, over 6 weeks of treatment with temsirolimus. In patient 3, lactate dehydrogenase decreased from 983 to 296 μ/l over 16 weeks of treatment with temsirolimus.

Correlative studies

PTEN expression was assessable in five of six cases. Four cases retained normal PTEN expression whereas only one showed partial loss of expression. Four tumors showed high level of P-S6RP expression, one tumor showed

intermediate level, and one tumor showed no expression of P-S6RP. Among the five patients with high or intermediate level of P-S6RP expression, two had PR according to the Choi criteria (SD according to RECIST) and three had progressive disease. Patient 2 with no expression of P-S6RP had PR according to the Choi criteria (SD according to RECIST). It should be noted that this patient showed partial loss of expression of PTEN.

Discussion

Activation of the mTOR pathway, leading to cell survival and persistent growth independent of nutritional factors, is one of the most important oncogenic pathways in human cancer [16], and seems to be of particular importance in at least a subset of sarcomas. Indeed, several reports indicated the frequent deletion of *PTEN* in human pleiomorphic sarcomas including leiomyosarcomas [3–8]. The functional importance of this genomic event has been suggested by a recent report showing that *PTEN* conditional knockout mice developed leiomyosarcomas with high penetrance and rapid onset as a result of a sustained activation of the insulin-like growth factor (IGF)/Akt/mTOR pathway [9]. These results lead us to evaluate temsirolimus, an inhibitor of mTOR, in a cohort of patients with advanced leiomyosarcomas for whom the sole alternative was symptomatic and palliative care. Temsirolimus controlled tumor growth in three of six patients.

There are three inhibitors of mTOR in various stages of clinical development: temsirolimus (CCI-779; Pfizer, New York, USA) and everolimus (RAD001; Novartis Pharmaceuticals, Basel, Switzerland), which have been proved by the Food and Drug Administration for the treatment of renal cell carcinoma, and ridaforolimus [AP23573; Ariad Pharmaceuticals (Cambridge, Massachusetts, USA), formerly known as deforolimus]. Whether these drugs are substantially different from each other clinically has yet to be elucidated but is unlikely. The clinical activity of ridaforolimus in sarcoma has been investigated in a phase II trial, which enrolled 216 patients and categorized them by sarcoma histology [17]. There were no restrictions on earlier therapy. The results of this trial are preliminary and only available in an abstract form. A total of 212 patients were treated for the following: bone sarcoma ($n = 54$), leiomyosarcoma ($n = 57$), liposarcoma ($n = 44$), and other soft tissue sarcomas ($n = 57$). Responses were identified according to the classical volumetric RECIST criteria. The clinical benefit rate (first end point of the study) was 29% (complete response, PR and SD > 4 months) consisting mainly of long-term disease stability (SD > 4 months). It should be noted that no significant difference was identified in terms of outcome among the four subgroups [17]. A phase II study investigating the efficacy of everolimus in patients with advanced sarcoma is currently under way (NCT00767819). A multicenter phase II study of temsirolimus enrolled patients with advanced soft tissue sarcoma in the first-line setting [18]. As in this study, patients received 25 mg intravenous temsirolimus weekly. The results of this study are preliminary and only available in an abstract form. This trial included 41 eligible patients and did not meet its primary endpoint based on objective response rate. Indeed, only one objective response according to the RECIST was observed and disease stabilization was not an end point. All these trials explored the efficacy of mTOR inhibitors in patients with a heterogeneous mix of advanced sarcomas histological subtypes. Moreover, there are no reported data about the status of mTOR activation of these sarcomas.

Although preliminary, our results suggest a clinical benefit of temsirolimus in leiomyosarcomas. The patterns of radiological response observed in our study suggest that RECIST response is not an optimal end point to assess the efficacy of mTOR inhibitors in sarcoma. Indeed, in our series, the best response according to RECIST was SD in three of six patients. However, we also observed that in all patients with SD at first tumor evaluation, there was a significant change in tumor density and/or contrast enhancement. This change was still present at 4 months (patients 1, 4) and 6 months (patient 4) and correlated with clinical and/or biological signs of antitumor activity. For this reason, we decided to apply the Choi criteria as defined for gastrointestinal stromal tumor (GIST) [13]. Indeed, Choi criteria have been reported to correlate with outcome much better than RECIST in GIST, and in soft tissue sarcoma and other tumors such as renal cell carcinoma as well [13,19,20]. In this series, the Choi criteria resulted better than RECIST in identifying tumor response and tumor progression (two of three patients with RECIST SD at 2 months but with Choi's PR were still on treatment at 6 months). In clinical studies, the alternative is to use progression-free survival as the first end point. However, in an exploratory study, as this one, tumor response is the sole reliable marker.

PTEN expression was assessable in five of six cases. Four cases retained normal PTEN expression whereas only one showed partial loss of expression. This result contrasts with several previous data indicating that loss of PTEN play a crucial role in leiomyosarcomas tumorigenesis [3–9]. We believe that these results simply reflect the fact that immunohistochemistry is not a reliable technique to assess PTEN status in SGC. Indeed, earlier results showed that leiomyosarcomas and other solid tumors show a strong immunoreactivity for PTEN despite array-comparative genomic hybridization and expression data showing *PTEN* loss [14,21]. This may be related to a nonspecific crossreactivity of this antibody in these tumors, maybe with smooth muscle antigens. Five of six tumors showed high or intermediate levels of PS6RP expression suggesting an activation of PTEN/Akt/mTOR pathway in the majority of cases. Altogether, our data suggest that PTEN and PS6RP statuses assessed by IHC

are not reliable predictive markers of temsirolimus activity in leiomyosarcomas. Further comprehensive studies are needed in SGC to confirm these results and to determine which subgroups would benefit more from mTOR inhibitors.

We have recently reported the effectiveness of temsirolimus in metastatic PEComa, a rare form of sarcoma for which no effective therapy has been described previously [22]. PEComas are characterized by mutations in the *TSC1* or *TSC2* tumor suppressor genes. Normally, the cytoplasmic TSC1 and TSC2 proteins interact and inhibit mTOR activity. In the absence of a normally functioning TSC1–TSC2 complex, mTOR activity increases, leading to the development of tumors in various organ systems including the kidney, lung, brain, and skin. Our results also suggest that temsirolimus may be associated with clinical benefit in leiomyosarcomas, which is characterized by a different mechanism of activation of the PTEN/Akt/mTOR pathway. Given the limited size and the design of our study, these results must be confirmed by a clinical trial including a translational research program. Other sarcomas may also represent an ideal target for mTOR inhibition. For instance, a subset of myxoid liposarcoma has been recently shown to be characterized by phosphatidyl inositol 3-kinase catalytic subunit mutation [23].

Recent studies have shown that mTOR inhibition induces feedback activation of Akt signaling through a type 1 IGF receptor-dependent mechanism [24]. For this reason, there is currently a great deal of interest in using a combination of mTOR and type 1 IGF receptor inhibitors for the treatment of several solid tumors such as breast, colon, and prostate cancer. It is likely that more well-defined patient selection criteria according to specific molecular aberrations in the phosphoinositide 3-kinases/PTEN/Akt/mTOR pathway will improve our understanding of the role of mTOR inhibitors, either alone or in combination, in patients with advanced sarcoma.

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