A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study

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Purpose: After standard multimodal therapy, the prognosis of relapsed and unresectable high-grade osteosarcoma is dismal and unchanged over the last decades. Recently, mitogen-activated protein kinases were shown to be activated in osteosarcoma specimens, suggesting, therefore, they are suitable targets for the multikinase inhibitor sorafenib. Thus, we explored sorafenib activity in patients with relapsed and unresectable osteosarcoma.

Experimental design: Patients >14 years, progressing after standard treatment, were eligible to receive 400 mg of sorafenib twice daily until progression or unacceptable toxicity. The primary end point was progression-free survival (PFS) at 4 months. Secondary objectives were PFS, overall survival (OS), clinical benefit rate (CBR), defined as no

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progression at 6 months and safety. This nonrandomized phase II study used a Simon two-stage design. PFS and OS at 95% confidence intervals (95% CIs) were calculated by the Kaplan–Meier method. All tests were two sided.

Results: Thirty-five patients were enrolled. PFS at 4 months was 46% (95% CI 28% to 63%). Median PFS and OS were 4 (95% CI 2–5) and 7 (95% CI 7–8) months, respectively. The CBR was 29% (95% CI 13% to 44%). We observed 3 (8%) partial responses (PRs), 2 (6%) minor responses (<30% tumor shrinkage) and 12 (34%) stable diseases (SDs). For six patients (17%), PR/SD lasted ≥6 months. Noteworthy, tumor density reduction and [^{18}F]2-fluoro-2-deoxy-D-glucose–positron emission tomography responses were observed among SD patients. Sorafenib was reduced or briefly interrupted in 16 (46%) patients and permanently discontinued in one (3%) case due to toxicity.

Conclusions: Sorafenib demonstrated activity as a second- or third-line treatment in terms of PFS at 4 months with some unprecedented long-lasting responses. Sorafenib, the first targeted therapy showing activity in osteosarcoma patients, deserves further investigations.

Key words: bone neoplasms, MAPK, osteosarcoma, relapse, sorafenib, target therapy

introduction

High-grade osteosarcoma is the most common primary bone tumor [1] and mainly affects limbs in a young population; its incidence peaks in the second decade [2]. Despite optimal surgery, osteosarcoma prognosis was poor until the introduction of chemotherapy [3]. Presently, 60%–70% of high-grade osteosarcoma patients are cured via multidisciplinary treatment [4]. However, with the exception of the monocyte/macrophage immune-modulator muramyl tripeptide phosphatidyl-ethanolamine, recently shown to increase overall survival (OS) when combined with standard chemotherapy, results have not improved in the last 30 years [5, 6]. Two large series have studied the characteristics of patients relapsed after multimodal therapy [7, 8] and both concluded that the most important prognostic factor was a not surgically amenable disease at relapse. Prognosis was also influenced by chemotherapy response, time to relapse, number of pulmonary metastases and metastatic pleural disruption. Therefore, the impregnable core of high-grade osteosarcoma is still represented by unresectable either primary or relapsed disease which, as of 2010, is cured anecdotally through actual therapeutic strategies [7, 8]. Thus, new therapeutic tools are awaited.

Osteosarcoma has been extensively studied to identify oncogenes suitable to become targets of monoclonal antibodies and small inhibitors. Tyrosine kinase receptors as KIT, platelet-derived growth factor receptors (PDGFRs), vascular endothelial growth factor receptors (VEGFRs) [9–13] are expressed and activated, but their inhibition lacked antitumor activity. Also, the monoclonal antibodies anti-insulin-like growth factor receptor-I (IGF-IR) were promising preclinically, but their activity was not confirmed in the clinical setting [14]. Recently, several authors have focused on the signal transduction pathways of phosphatidylinositol 3′-kinase/mammalian target of rapamycin (PI3K/mTOR) [15] and mitogen-activated protein kinases (MAPK). In particular, MAPK activation was demonstrated in high-grade osteosarcoma specimens [16, 17] and their inhibition by sorafenib [16] proved highly effective in osteosarcoma preclinical models (tumor cell lines and xenograft).

Sorafenib is an orally active multikinase inhibitor that targets MAPK, VEGFRs, PDGFRs and KIT [18]. Previously, this drug was approved by regulatory authorities for the treatment of kidney and liver cancer [19, 20]. These positive results, along with our preclinical data, provided the background to design and conduct a phase II trial of sorafenib in advanced and unresectable high-grade osteosarcoma patients after standard therapy failure.

methods

patients

Eligible patients had the following characteristics: age >17 years; diagnosis confirmed histologically and reviewed centrally; prior treatment (completed >4 weeks before trial entry) consisted of standard high-grade osteosarcoma chemotherapy agents including doxorubicin, cisplatin, high-dose methotrexate, and ifosfamide; metastatic relapsed and unresectable progressive disease (PD); Eastern Cooperative Oncology Group performance status ≤2 with a life expectancy >3 months; adequate renal, hepatic, and hemopoietic function. Additionally, we required normal or controlled blood pressure, as well as surgery and/or radiotherapy completion at least 1 month before enrollment. Later, the protocol was amended to enroll patients >14 years and those treated for relapsed osteosarcoma with up to two lines of treatment (e.g. gemcitabine, Taxotere®, Sanofi-Aventis US, Bridgewater, NJ or monoclonal antibody against IGF-IR). All enrolled patients showed radiological evidence of disease progression before treatment start.

treatment

Patients were treated with a dose of sorafenib 400 mg twice daily. The dose was reduced or temporarily suspended according to predefined rules and after considering any observed toxicity [21], which was assessed according to the Common Terminology Criteria for Adverse Events version 3.0 [22]. Following adverse event resolution, sorafenib was restarted at the maximally tolerated dose and continued until progression, unacceptable toxicity or patient refusal. The study was approved by participating centers institutional review boards, and conducted according to the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines. Each patient provided written informed consent.

efficacy assessment

Before starting treatment, patients were staged with chest and abdomen computed tomography (CT) and magnetic resonance imaging (MRI) (whenever indicated by the clinical situation). Baseline assessment included also full blood count, serum chemistry, electrocardiogram and physical examination. In light of its potential role in osteosarcoma response assessment [23], [^{18}F]2-fluoro-2-deoxy-D-glucose–positron emission tomography (FDG-PET) was suggested but not mandated for patient enrollment, and its impact on tumor response assessment was purely exploratory. All tests were repeated after 2 months and, thereafter, at 2-

month intervals unless there were toxic effects or disease progression suspicion. Response was assessed by CT/MRI scan according to RECIST v1.0 [24]. Thus, both complete and partial remission needed confirmation within 4 weeks of when a response was first demonstrated. Stable disease (SD) was confirmed after a minimum of ≥8 weeks. We thoroughly probed for and recorded any sign(s) of treatment-induced improvement, be it minor response (MR) as tumor shrinkage <30%, and/or nondimensional tumor responses including Hounsfield unit measured tissue density changes or osteoid matrix calcification.

The primary end point progression-free survival (PFS) at 4 months was calculated from the date of treatment start until the time of disease progression or death, whichever came first. Patients alive and free from progression were censored. Secondary end points included the following: PFS; OS; overall response rate, defined as complete responses (CRs) + partial responses (PRs) + MRs; disease control rate (overall response rate + SDs); patterns of nondimensional response; clinical benefit rate (CBR) (PFS rate at 6 months) and duration of response. Duration of response was calculated from the day of first response assessment until either progression/death (event) or last day of follow-up (censored). Last, we evaluated any clinical improvement by means of the Pain Analgesic Score via the Brief Pain Inventory (BPI) score form that was filled in by patients themselves [25]. Analgesic medication use was recorded according to the analgesic score: 0 = none; 1 = minor analgesics; 2 = tranquillizers, antidepressants, muscle relaxants and steroids; 3 = mild narcotics; 4 = strong narcotics.

statistical methods

This is a phase II, nonrandomized, multicenter open-label trial (EudraCT 2007-004396-19). The study sample size was calculated according to Simon as a phase II, optimal two-stage study [26] with PFS at 4 months as the primary end point, i.e. patients alive after 4 months without signs of progression were regarded as successes. We set error $\alpha = 0.05$ and error $\beta = 0.1$. Then, we calculated the number of needed patients under a hypothesis of interest in which sorafenib PFS at 4 months was ≥30% (H1 = 30%) and a null hypothesis in which sorafenib reached a PFS at 4 months ≤10% (H0 = 10%). These conditions required that we observed at least three responses among the first 18 patients to proceed to the second stage for a total of 35 enrollees. Greater than seven successes were needed to warrant further study of sorafenib. All patients who received at least one pill were included in an intention-to-treat analysis. PFS at 4 months, PFS, OS and duration of response were estimated according to the Kaplan–Meier method with their respective 95% confidence intervals (CIs). The RECIST objective response was evaluated and we reported the overall response rate and the disease control rate with their CIs.

results

patient characteristics

Between January 2008 and December 2009, 35 patients affected by metastatic high-grade relapsed and unresectable osteosarcoma were enrolled at four Italian Sarcoma Group centers. Table 1 enumerates the patient demographics and risk factors.

Since all patients were treated according to protocol, 35 patients were assessable for both safety and efficacy. The analyses were carried out 6 months after the last patient started therapy.

efficacy

The median follow-up for efficacy was 3.6 months. At last follow-up, four patients (11%) remained on therapy at 14, 6, 6 and 6 months, respectively. Progression in 30 (86%) and

toxicity in 1 (3%) patient were reasons to interrupt the drug. Three (9%) patients received sorafenib for >1 year. Sixteen patients were free from progression after 4 months of therapy for an overall PFS at 4 months of 46% (95% CI 28% to 63%) (Figure 1). The median OS was 7 months (95% CI 7–8), with a 17% (95% CI 4–30) probability to be alive at 12 months from study entry (Figure 1). The median PFS and duration of response were 4 (95% CI 2–5) and 4 (95% CI 3–5) months, respectively. The CBR was 29% (95% CI 13% to 44%).

We observed no CRs, but three (9%) patients did meet the criteria for PRs (Figure 2A) and two (6%) achieved a MR (tumor shrinkage of 15%). Twelve (34%) patients qualified for SD. The overall response rate and disease control rate were 14% (95% CI 2% to 26%) and 49% (95% CI 31% to 67%), respectively (Table 2). Table 2 describes the site and type of response. No patient became eligible for surgery.

Among nonprogressing patients, there were six (17%) patients who continued to take sorafenib for as long as 14 months (14, 14, 12, 9, 9 and 7.5); 3 (9%) patients continued to be progression free after 6 months.

Finally, BPI was assessable in 31 (89%) patients. We demonstrated a significant improvement in patients who reached an SD with a reduction in the 'best-response' BPI score to a mean score of 4/10 ($P = 0.009$) (Figure 3).

pattern of response

Consideration of the mounting evidence that targeted therapies may cause nondimensional tumor responses coupled with acknowledgment of how difficult it may be to detect chemotherapy activity in osteosarcoma led us to report all observed modifications other than tumor shrinkage. In two (6%) patients, we detected progressive calcification of the tumor mass without dimensional increase (Figure 2B). A biopsy of the mass showed a calcified lesion without tumoral residual cells (data not shown). In an exploratory analysis, FDG-PET was carried out in 12 patients. In two (6%) patients, FDG–PET predicted the progression of the disease earlier than CT. In seven (20%) patients, CT and FDG-PET response evaluations were consistent. In one patient with MR and two patients with SDs, FDG-PET showed a PR according to PET Response Criteria In Solid Tumors [27].

In Figure 4, we present a 73% reduction in the maximum standard uptake value in a stable calcified metastasis. A different pattern of response was noticed in a large pelvic metastasis, in which a moderate mass shrinkage was accompanied by a mean density reduction from 64 to 29 Hounsfield units (Figure 2C). This patient refused a fine needle biopsy. Finally, the only patient who stopped the study drug for toxicity did so due to a pneumothorax resulting from necrosis of a pleural metastasis that shrank >30% in its widest diameter. Nevertheless, this patient was considered a failure for study purposes.

toxicity

The median duration of therapy was 4.4 months. The overall incidence of sorafenib-related adverse events was 78%. In general, drug-related adverse events were limited to grade 1 or 2. We noted the following grade 3 and 4 toxic effects: anemia 2

Table 1. Patients demographics

Characteristics	N	%
Age at study entry (years)		
Median (range)	21 (15–62)	
<18	7	20
≥18	28	80
Gender		
Male	21	60
Female	14	40
ECOG performance status		
0	9	26
1	17	48
2	9	26
Location of primary tumor		
Limbs	19	54
Nonextremities	16	46
Osteosarcoma histotype		
Osteoblastic	23	66
Chondroblastic	4	11
Fibroblastic	5	14
Other	3	9
Metastatic at diagnosis		
No	21	60
Yes	14	40
Adjuvant chemotherapy	35	100
MTX-ADM-CDDP ± IFO	33	94
Other (ADM-CDDP based)	2	6
First-line chemotherapy	33	94
HD-IFO based	16	46
CE	7	20
GEM-TXT	6	17
Other	4	11
Second-line chemotherapy	13	37
HD-IFO based	7	20
ETO based (+ IFO or CTX)	6	17
Time to first relapse (months)		
Median (95% CI)	17 (95% CI 14–20)	
Site(s) of disease[a]		
Local relapse	8	23
Distant lung	13	37
Bone and lung	17	49
Extra	5	14

[a]There were patients progressing at both sites.
ECOG, Eastern Cooperative Oncology Group; MTX, methotrexate; ADM, adriamycin; CDDP, cisplatin; IFO, ifosfamide; HD-IFO, high-dose ifosfamide; CE, cyclophosphamide plus etoposide; GEM, gemcitabine; TXT, docetaxel; ETO, etoposide; CTX, cyclophosphamide; CI, confidence interval.

(6%), thrombocytopenia 2 (6%), nausea 1 (3%), asymptomatic lipase G4 increase 1 (3%), abdominal cramps 1 (3%), oral mucositis 1 (3%), hand–foot skin reaction 3 (9%) and 1 (3%) skin metastasis bleeding (after a minor trauma). Table 3 summarizes the main side-effects and all grades 3 and 4 adverse events. All reported toxic effects led to brief study drug interruptions and contributed to a discontinuation rate of 46% (16 patients). Whenever clinically needed, sorafenib was reduced by 25% (600 mg daily). However, this relatively young population was often able to resume the study drug at full dose. The mean administered dose was 0.85 of the full expected dose.

We recorded no drug-related death. As mentioned previously, we observed 1 (3%) pneumothorax in a patient due to the necrosis of a lung metastasis creating a bronchopleural fistula. Recovery was uneventful, but immediately upon sorafenib restarted, the pneumothorax relapsed leading to permanent treatment discontinuation.

discussion

This multicenter phase II trial was carried out to test the activity of the multikinase inhibitor sorafenib as second- or third-line therapy for patients with unresectable and relapsed high-grade osteosarcoma. In the last decades, only marginally effective therapies have been developed for such patients [28, 29] and most of them become candidates for either experimental therapies or supportive care [30]. Our preclinical study on osteosarcoma specimens demonstrated that sorafenib targets were expressed and effectively inhibited by this drug in xenografts, providing a rationale to explore sorafenib in progressing osteosarcoma patients.

Within this context, sorafenib produced two notable results. First, it achieved a far greater PFS at 4 months than the hypothesized threshold of interest and second, it delivered a CBR (PFS ≥6 months) to nearly one of three patients with PD at enrollment. Sorafenib also demonstrated objective responses: three RECIST PRs and two MRs. Moreover, at least two patients showed a nondimensional pattern of response that included a complete tumor calcification—a well-known pattern of response to chemotherapy in osteosarcoma—and a sharp density reduction with a minor shrinkage (<30% in its widest diameter) of a pelvic metastasis in a second patient. While density reduction is a relatively new type of radiological finding in osteosarcoma, it has gained recognition as a sign of response to several targeted therapies [31–33]. From this point of view, such objective findings (dimensional and nondimensional responses) represent the proof of principle standing for an antitumor activity of sorafenib in high-grade osteosarcoma. Also, the FDG-PET might be a helpful and complementary tool to assess response. Figure 4 strengthens our interpretation of the observed calcification as a response to the study drug despite its dimensional stability. Besides, this is fully consistent with what Hawkins recently demonstrated regarding FDG-PET use in osteosarcoma [23].

We did observe some long-lasting disease stability. Whereas in a nonrandomized study, such behavior could be attributed to tumor growth variability rather than study drug activity, physicians involved in the care of high-grade osteosarcoma patients would estimate the likelihood of 'spontaneous' stability to be extremely low. Furthermore, all patients were progressing at baseline after receiving standard therapy plus one or two additional lines. Finally, in targeted therapies, tumor shrinkage may be delayed [34] or may not even occur [19], yet the drug may still be deemed 'efficacious'. Therefore, we believe SD should be regarded as the mark of success in this specific population [35–38] whose median OS was only 7 months.

Since stability and clinical benefit alone are somewhat slippery as evidence of drug activity, we designed our study to capture any clinical improvement in symptoms by means of the BPI scale. To this end, we found that 9 of 12 (75%) patients
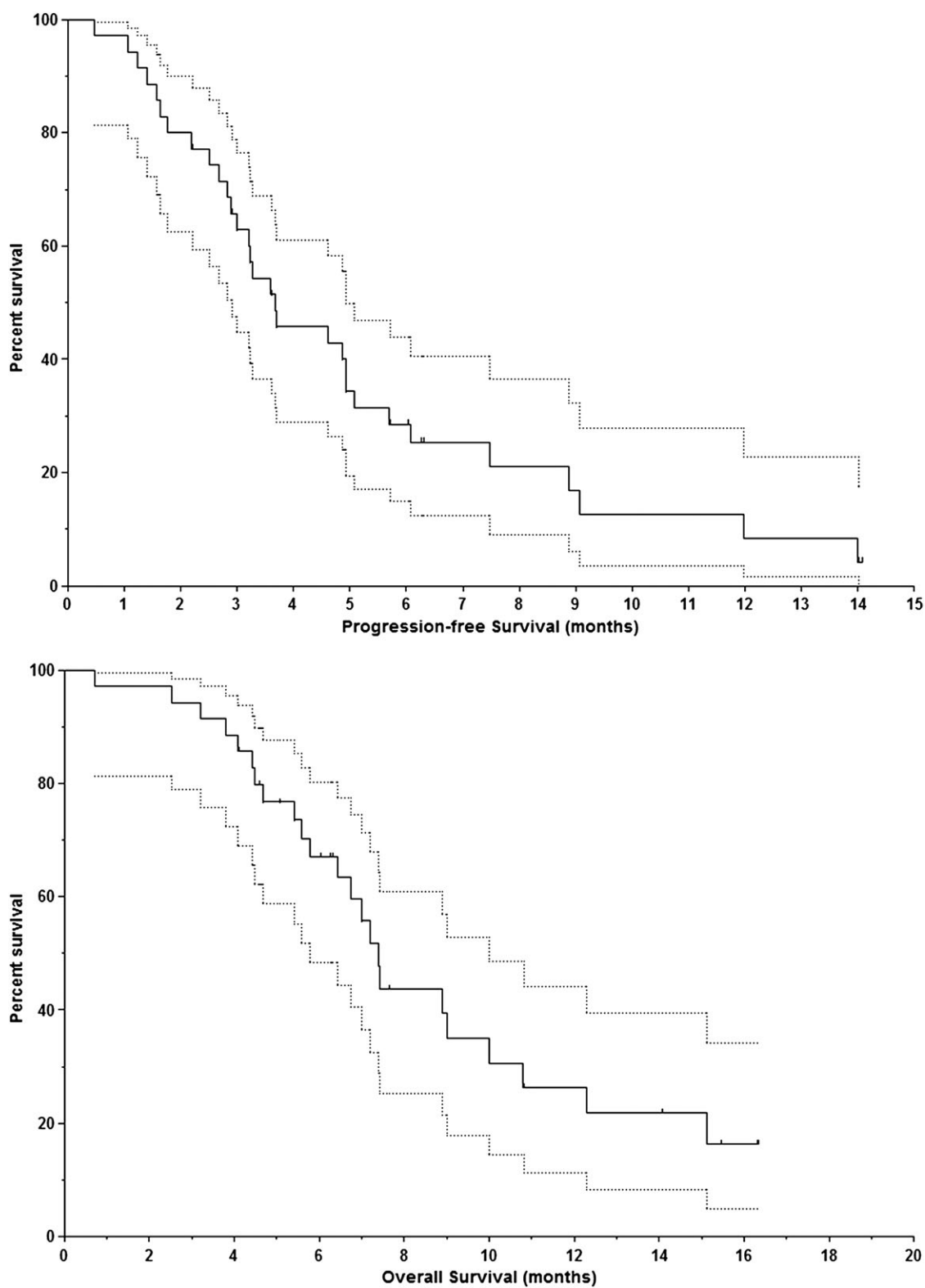
Figure 1. Kaplan–Meier plots for progression-free survival and overall survival.

who achieved SD, patient records confirmed a sharp reduction or even elimination of opioids. Since such an improvement can only be attributed to sorafenib, this finding bolsters our assertion of stable disease SD as 'success'.

We acknowledge our study has some limitations due to its lack of a control group. However, we conceived this study to explore a completely different strategy in a rare sarcoma for which there are no other therapeutic options. The young age and the
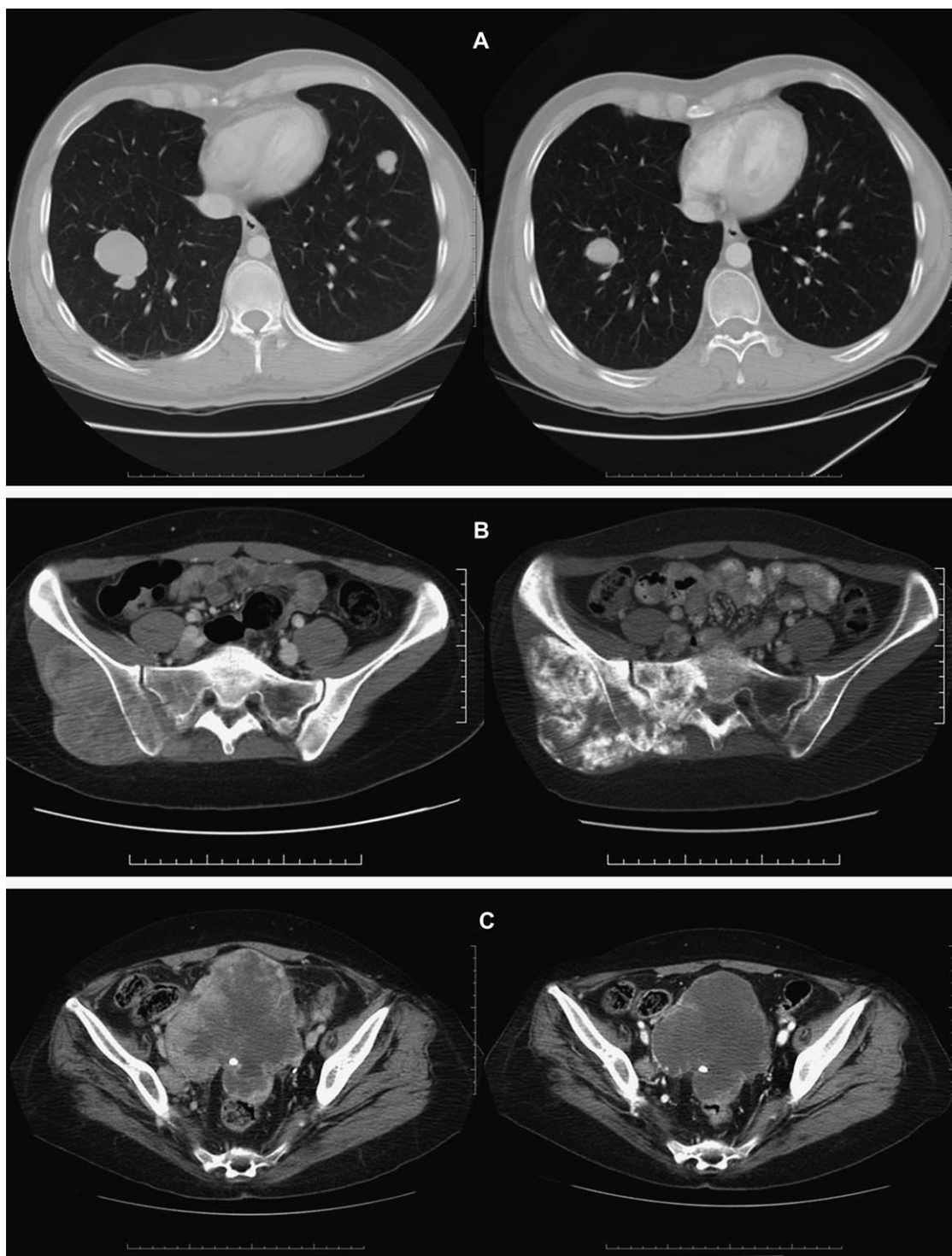
Figure 2. Computed tomography (CT): different patterns of response. (A) Dimensional response; (B) nondimensional response (calcification of the lesion); (C) nondimensional response (>50% mean lesion density reduction in Hounsfield units). On the left: CT scan carried out at baseline; on the right: CT scan carried out after 45 days of treatment.

good performance status of high-grade relapsed osteosarcoma make the 'no treatment option' difficult to propose. Moreover, there is no 'standard' second- or third-line of therapy by which to compare alternative treatments, and 94% of our patients (Table 1) had already received those 'salvage' regimens most commonly used in this context [28, 29]. Finally, the non-randomized design allowed us to complete our study quickly.

Regarding our primary end point, we do recognize that the lack of a comparator makes the meaningfulness of the absolute data of PFS at 4 months and at 6 months *per se* difficult to assess.

Table 2. Patients responses

Response	n	%
Total assessable patient	35	100
CR	0	0
PR (>30%)	3	9
Lung	1	3
Lung + bone + soft tissue	2	6
MR	2	6
Lung	1	3
Lung + bone	1	3
SD	12	34
Lung	4	11
Lung + bone	7	20
Lung + bone + soft tissue	1	3
PD	18	51
Lung	7	20
Lung + bone	9	26
Lung + bone + soft tissue	2	6
Nondimensional responses	2	6
Lung + bone	1	3
Lung + bone + soft tissue	1	3
Overall response rate (PR + MR)	5	14
Clinical benefit (PR + MR + SD)	10	29
≥6 months		
Analgesic score reduction	13	37

CR, complete response; PR, partial response; MR, minor response; SD, stable disease; PD, progressive disease.
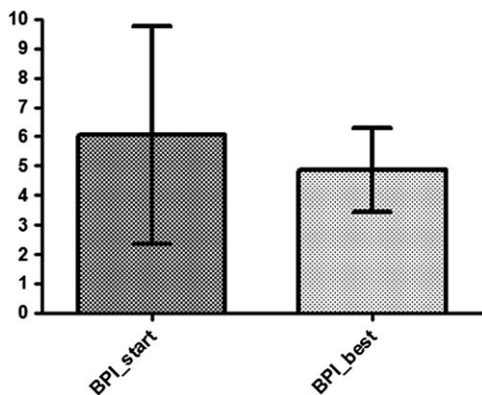

Figure 3. Brief Pain Inventory (BPI) score evaluation. The bar on the left refers to the BPI score at the start of the study [mean 6/10; 95% confidence interval (CI) 4.7–7.3]; the one on the right refers to the best score obtained during drug administration (mean 4.0/10; 95% CI 3.4–6.3).

Figure 4. [^{18}F]2-fluoro-2-deoxy-D-glucose–positron emission tomography. Maximum standard uptake value at the beginning (above) and after 3 months of therapy (below). SUV, Standard Uptake Value.

Table 3. Drug-related adverse events

Adverse event	Grade							
	1 or 2		3		4		Total	
	n	%	n	%	n	%	n	%
Anemia	16	46	2	6	0	0	18	51
Leucopenia	17	49	1	3	0	0	18	51
Hand and foot syndrome	10	29	3	9	0	0	13	37
Skin rash	11	31	1	3	0	0	12	34
Mucositis/stomatitis	9	26	1	3	0	0	10	29
Diarrhea	9	26	0	0	0	0	9	26
Thrombocytopenia	6	17	2	6	0	0	8	23
Nausea	5	14	1	3	0	0	6	17
Fatigue	5	14	1	3	0	0	6	17
Pruritus	5	14	0	0	0	0	5	14
Weight loss	4	11	0	0	0	0	4	11
Hypertension	3	9	0	0	0	0	3	9
Lipase elevation	1	3	1	3	1	3	3	9
Abdominal cramps	3	9	0	0	0	0	3	9
CK elevation	0	0	0	0	2	6	2	6
Alopecia	1	3	0	0	0	0	1	3
Pneumothorax	0	0	0	0	1	3	1	3
Bleeding	0	0	1	3	0	0	1	3

CK, creatine kinase.

However, and acknowledging the differences, PFS at 4 months has been put forth as a meaningful clinical end point by Van Glabbeke et al. [39], who examined European Organisation for Research and Treatment of Cancer (EORTC) phase II clinical trials of soft tissue sarcoma patients. The EORTC data demonstrated that further study was warranted for any new drug tested in a phase II study showing a progression-free ratio of 40% and 20% at 3 and 6 months, respectively. While no established parameters exist for osteosarcoma, we found
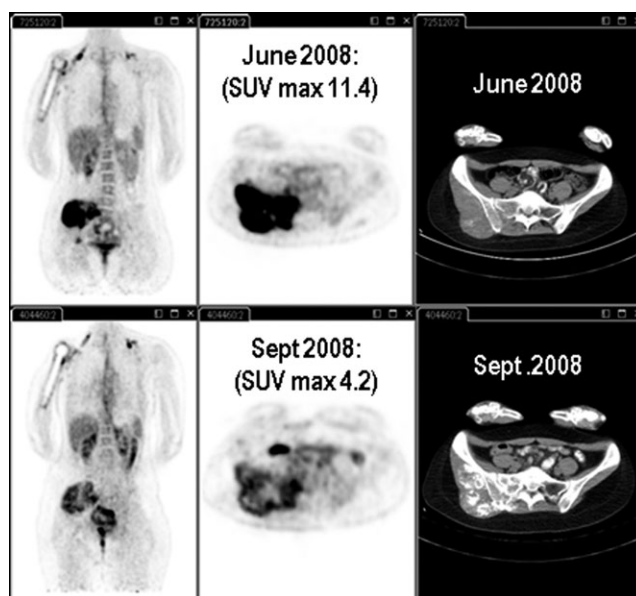
sorafenib yielded progression-free ratios at levels above those suggested in the literature for soft tissue sarcomas. Within the above-mentioned limits, these results compare very favorably not only with proposed cut-offs but also with historical controls. Indeed, in the postrelapse inoperable osteosarcoma setting, the results of three cooperative groups provide a useful point of reference. The Cooperative Osteosarcoma Study Group unresectable patient median survival time was 0.49–0.55 year

[8]; the Italian 2-year postrelapse survival was <2% for like patients [7]. Last, Leary et al. [40] demonstrated a median time to progression of 1.8 months with a 6% event-free survival at 6 months in their inoperable series.

In general, sorafenib was fairly well tolerated and its side-effects were manageable. We observed several of the expected adverse events previously reported and mostly grade 1 or 2. Grades 3 and 4 occurred relatively infrequently. Chronic use of the study drug might require short sorafenib holidays or dose reductions, even for milder toxic effects. The mean administered dose was 0.85 of the full expected dose, which is relatively high compared with another sorafenib phase II study composed of older sarcoma patients in which 61% of the population required at least one dose reduction [41]. We found no different toxic effects in younger (<18) patients. Regardless of age, careful identification and prompt treatment of adverse events are necessary to improve patient compliance.

Two observed shortcomings deserve emphasis. First, there was a high percentage of relatively short-lasting responses in patients who initially benefited from the study drug; second, almost half of the patients were refractory to this inhibitor. Several causes could lie behind these failures. The fact that osteosarcoma is a genetically complex disease [42, 43] makes it unlikely that a single small molecule, even if it is targeting many kinases at the same time, might shut off all activated pathways. Rather, we suggest that other transduction pathways are involved based on preclinical data of both others and ourselves [15, 16]. In particular, both PI3K [44] and the mTOR [45] seem likely to be involved in the escape mechanism, which also make them promising targets for future therapies.

In conclusion, sorafenib displayed clinical activity and an acceptable toxicity profile in what is the worst-case scenario of high-grade osteosarcoma. We suggest these encouraging results deserve further studies focusing not only on mechanisms of activity and resistance but also on combination therapies.

disclosure

The authors declare no conflict of interest.

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