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A randomised phase II trial of selumetinib *vs* selumetinib plus temsirolimus for soft-tissue sarcomas

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Background: The MEK inhibitor, selumetinib, suppresses soft-tissue sarcoma (STS) cell proliferation *in vitro*. Mammalian target of rapamycin inhibitors possess modest activity against STS; however, resistance develops via MAPK pathway feedback activation. The combination of selumetinib and temsirolimus synergistically inhibits STS cell line growth. Therefore, a randomized phase II trial of selumetinib *vs* selumetinib plus temsirolimus was conducted.

Methods: Seventy-one adults with advanced STS who received $\leqslant 2$ prior chemotherapeutics were randomized to selumetinib 75 mg p.o. bid and allowed to crossover upon progression, or to selumetinib 50 mg p.o. bid plus temsirolimus 20 mg i.v. weekly, with primary endpoint of progression-free survival (PFS).

Results: There was no difference in PFS between the two arms for the overall cohort (median 1.9 *vs* 2.1 months); an improved median PFS was observed in the combination arm ($N = 11$) over single agent ($N = 10$) in the prespecified leiomyosarcoma stratum (median 3.7 *vs* 1.8 months; $P = 0.01$). Four-month PFS rate was 50% (95% confidence interval 0.19–0.81) with the combination *vs* 0% with selumetinib alone in the leiomyosarcoma cohort. Most common grade 3/4 adverse events with the combination were mucositis (29%), lymphopenia (26%), neutropenia and anaemia (20% each).

Conclusions: While single-agent selumetinib has no significant activity in STS, the combination may be active for leiomyosarcomas.

Soft-tissue sarcomas (STS) are a heterogeneous group of malignant, mesenchymal tumours that are comprised of over 50 different subtypes. Outcomes for recurrent STS are often poor because of their relative resistance to chemotherapy. Chemotherapy with doxorubicin alone or in combination with ifosfamide is often used in the first-line setting and the combination of gemcitabine and docetaxel is commonly used in the second-line setting. Pazopanib, a vascular endothelial growth factor receptor inhibitor, was the first targeted therapy agent to be approved in STS. The FDA approval is based upon a phase 3 trial in the second- or third-line setting and showed a median progression-free survival (PFS) of 4.6 *vs* 1.6 months with placebo.(van der Graaf *et al*, 2012) Cotargeting signalling pathways may be a promising strategy to overcome resistance pathways that rapidly develop during therapy for STS.

The mammalian target of rapamycin (mTOR) is a protein kinase that regulates protein translation, cell growth, autophagy, and apoptosis.(Sabatini, 2006) Intracellular signalling through mTOR and associated upstream signalling pathways are dysregulated in most sarcoma subtypes. (Wan and Helman, 2007)

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Mammalian target of rapamycin is regulated in part by AKT, a serine/threonine kinase. Mammalian target of rapamycin inhibitors (mTORi) may increase PI3K activity towards MAPK activation in a feedback activation loop, therefore, promoting both AKT and ERK phosphorylation.(Kinkade *et al*, 2008) (Figure 2A) The insulin-like growth factor 1 receptor (IGF-1R) pathway is a commonly activated pathway in a variety of sarcomas, and the MAPK pathway is also downstream of IGF-1R (Wan and Helman, 2007). In preclinical breast and prostate cancer murine models, inhibition of mTORC1, which is composed of regulatory-associated protein of mTOR (RAPTOR), mLST8 (also known as GβL), and AKT substrate of 40 kDa (PRAS40), with the mTORi rapamycin, led to MAPK pathway activation through a PI3K-dependent feedback loop. However, when rapamycin was combined with the MEK1/2 inhibitor, PD0325901, MAPK feedback activation was abrogated and resulted in an enhanced antitumoral effect (Carracedo *et al*, 2008; Kinkade *et al*, 2008).

We also evaluated the combination of temsirolimus, an inhibitor of mTOR complex 1, and selumetinib (AZD6244, ARRY-142886) for inhibition of STS proliferation *in vitro*. As detailed below, the combination synergistically inhibited growth of STS cell lines. Thus, cotargeting signalling pathways may be a potential strategy to overcome the mTORi-activated PI3K–Akt feedback pathway, and a clinical trial combining temsirolimus and selumetinib for STS was carried out.

PATIENTS AND METHODS

Preclinical studies. Human uterine leiomyosarcoma (SK-UT-1), fibrosarcoma (HT1080), and liposarcoma (SW872) cell lines (American Type Culture Collection, Manassas, VA, USA) were treated in triplicate with temsirolimus (1–100 nM) and selumetinib (10–1000 nM) alone or in combination for 6 days. Proliferation was evaluated on a fluorescence-based digital image microscopy system (DIMSCAN, Bioimaging Solutions Inc., San Diego, CA, USA). The potential for antagonistic, additive, or synergistic interaction of temsirolimus with selumetinib was quantified by the CalcuSyn automated software (Biosoft, Ferguson, MO, USA).

Patients. Adult patients with histologically confirmed STS with metastatic (*de novo* or recurrent) or locally advanced, unresectable disease were eligible. Patients must have had measurable disease, defined as at least one lesion that could be accurately measured in at least one dimension as $\geqslant 10$ mm with spiral CT scan. Patients may have received $\leqslant 2$ prior chemotherapeutic regimens (single agent or combination chemotherapies), with a life expectancy of at least 12 weeks. Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status of two or less, absolute neutrophil count $\geqslant 1000\ \mathrm{mm}^{-3}$, platelet count $> 100\,000\ \mathrm{mm}^{-3}$, haemoglobin $> 8\ \mathrm{g\,dl}^{-1}$, serum creatinine $< 1.5 \times$ upper limits of normal (ULN), or calculated creatinine clearance $\geqslant 45\ \mathrm{ml\,min}^{-1}$, total bilirubin $\leqslant 1.5 \times$ ULN, SGPT (ALT) $\leqslant 5 \times$ ULN for age, and serum albumin $\geqslant 2\ \mathrm{g\,dl}^{-1}$. Patient must have had no evidence of dyspnoea at rest, no exercise intolerance, and a pulse oximetry $> 94\%$ if measured.

Patients were excluded if they had known brain metastases, prior MEK inhibitor use, or received cancer treatments including radiation within three weeks (at least 6 weeks for mitomycin-C and nitrosureas). Patients with pediatric-type sarcomas (Ewing/Ewing-like or rhabdomyosarcoma) were also not eligible. Institutional review board approval was obtained for the study protocol, and all patients provided written informed consent before entering the study.

Study design and assessments. This was a multicentre randomised, open-label, phase 2 study with a goal to accrue 35 patients per arm. (Clinicaltrials.gov identifier: NCT01206140) Randomisation was conducted by the study biostatistician using a permuted block design (block-size of 4), stratified by prior therapy (0 *vs* 1 or 2) and sarcoma subtype (liposarcoma, leiomyosarcoma, synovial

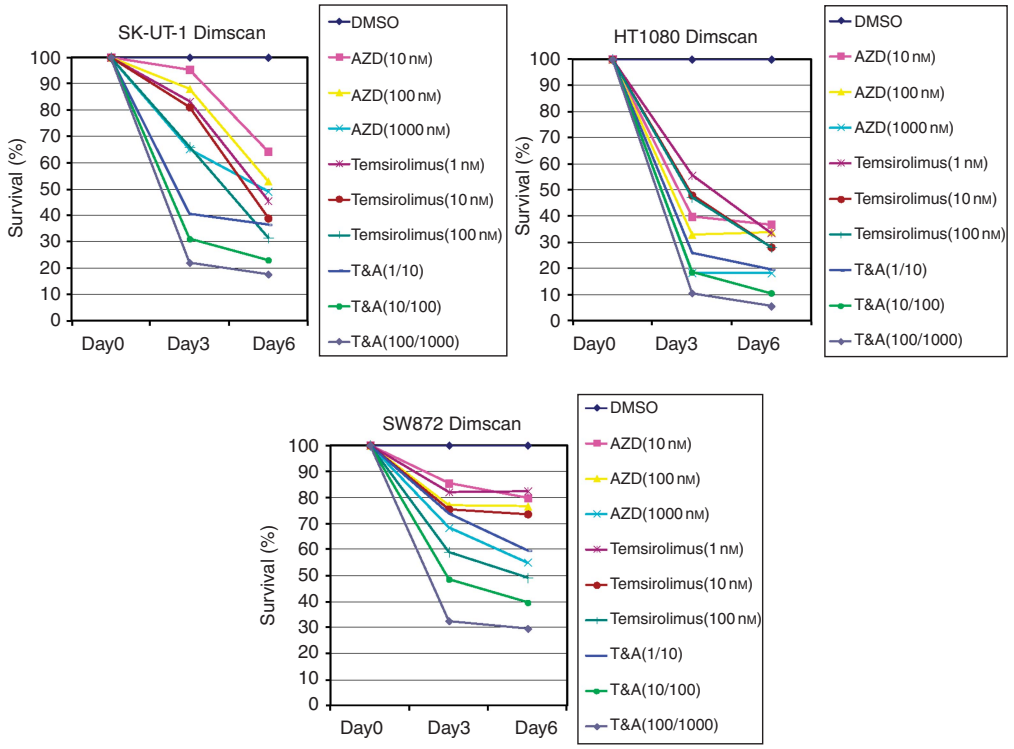

Figure 1. Inhibition of proliferation by temsirolimus, selumetinib (AZD), and the combination (T&A) in logarithmic concentrations in STS cell lines (SK-UT-1: leiomyosarcoma, HT1080: fibrosarcoma, SW872: liposarcoma) compared with control (dimethyl sulfoxide, DMSO).

sarcoma, pleomorphic undifferentiated sarcoma, other). Randomisation log was maintained by the central data coordinating centre, and slots were assigned after eligibility was confirmed. The primary endpoint was PFS by RECIST 1.1 criteria. Secondary endpoints were 4-month PFS rate, response rate, and toxicity. Inhibition of activated ERK1/2 in stimulated peripheral blood mononuclear cells (PBMCs), and activation status of mTOR–AKT pathway in tissue biopsies from normal skin were assessed (Ki67, p62, phospho-p70, BCL-2, phospho-AKT, cleaved caspase 3) within 7 days pre- and post-cycle 1 treatment.

The starting dose of selumetinib arm was 75 mg p.o. bid (Arm A), and selumetinib 50 mg bid when combined with temsirolimus at 25 mg i.v. weekly (Arm B). This dosing was based on a phase 1 study where this dosing combination was used (Patel *et al*, 2013). Disease response was assessed by RECIST 1.1 criteria and Choi criteria (Choi *et al*, 2007). Patients initially randomized to Arm A were allowed to cross-over to Arm B after progression by RECIST criteria. Each cycle was 4 weeks, with imaging obtained every two cycles until unacceptable toxicity or progressive disease. Adverse events were graded according to National Cancer Institute Common Terminology Criteria of Adverse Events version 4.0.

Correlative studies. Peripheral blood mononuclear cells and normal skin biopsies were obtained pretreatment and after completion of cycle 1. Skin biopsy specimens were bisected in half. One-half were snap-frozen in liquid nitrogen, and the other half were formalin fixed and paraffin embedded. Immunohistochemisty (IHC) for Ki67, p62, phospho-p70, BCL-2, phospho-AKT, cleaved caspase 3 were performed on the formalin fixed and paraffin-embedded specimen, and the phosphorylation state of the signalling proteins was performed in snap-frozen samples.

For PBMC analysis, ERK phosphorylation levels were assessed as a surrogate marker for selumetinib activity. Eight mililiters of peripheral blood was collected in a preload sodium citrate-containing cell preparation tube with 12-O-tetradecanoylphorbol-13-acetate pretreatment and after completion of 1 cycle of selumetinib. To activate ERK, the whole blood was treated with 12-O-tetradecanoylphorbol-13-acetate for 10 min at 37 °C within 1 h of being drawn. Peripheral blood mononuclear cells were separated and collected though centrifugation. ERK phosphorylation was preserved by immediate fixation of the cells with 1.2% methanol-free formaldehyde and transferred to a cryovial tube frozen at −80 °C. The frozen cell pellets of PBMCs were subsequently thawed and stained with an anti-phospho-ERK antibody (Cell signaling Technology, Inc, Danvers, MA, USA), followed by a fluorescein isothiocyaanate-conjugated secondary antibody detection by fluorescence-activated cell sorting analysis.

Statistical analysis. The accrual goal of 35 patients to each arm would allow a 90% power at the 0.1 one-sided significance level to detect a hazard ratio (HR) of 2 in favour of the combination arm. Analysis was performed by an as-treated principle. Subgroup analysis was performed on the prespecified sarcoma subgroups, with no adjustment for multiple comparisons in the context of this randomised phase 2 study. Biological correlates were analysed in an exploratory manner.

Outcomes reported on patients who crossed over to the combination arm upon progression were summarised using data before the cross-over from single-agent selumetinib. Progression as per RECIST 1.1 was defined as at least a 20% increase in the sum of the diameters of target lesions in reference to the smallest sum on study, or the appearance of one or more new lesions, or death. Time to PFS was calculated from date of randomisation to the earliest qualifying event (last date of contact for censored patients with no events), and was summarised by the Kaplan–Meier method. Progression-free suvrvival rates were calculated using the survival distribution function, and 95% confidence limits were calculated using the log–log transformation. The log-rank test was used to assess differences in PFS by treatment arm overall, as well as by major histology group stratifications. Further, the Cox proportional hazards model was used to estimate hazard ratios between the two treatment arms.

RESULTS

Preclinical studies. The drug combinations of temsirolimus (1, 10, 100 nM) with selumetinib (10, 100, 1000 nM), respectively, led to inhibition of proliferation combination Index (CI) values at the lowest concentrations of 0.001 (SK-UT-1, leiomyosarcoma), 0.047 (HT1080, fibrosarcoma), and 0.152 (SW872, liposarcoma). (Figure 1) At the highest concentrations, the corresponding values were 0.024, 0.046, and 0.011. A CI value <0.1 indicates very strong synergism, and 0.1–0.3 indicates strong synergism as described by Chou and Talalay (Chou and and Talalay, 1984). These results were consistent with strong to very strong synergism in all STS cell lines tested at the lowest concentration of temsirolimus (1 nM) and selumetinib (10 nM). Further, the SK-UT-1 cell line appeared to be the most sensitive cell line by nearly 50-fold over HT1080 and over 150-fold over SW872 cells. These promising preclinical results led to the current clinical trial.

Patients. A total of 71 patients were enrolled from October 2010 to January 2013 across four sites, City of Hope, University of Pittsburgh, UC Davis, and University of Southern California. Thirty-six patients were randomised to selumetinib alone and 35 patients to the combination arm. (Figure 2B) One patient in the selumetinib arm was found to have bone sarcoma after enrollment and was deemed ineligible owing to incorrect diagnosis, and another patient never started treatment; therefore, 34 selumetinib patients were deemed eligible for analysis. Patient characteristics are reported in Table 1. Nineteen patients crossed over to the combination after documented progression and received a median of 2 cycles (range 1.7–16.4 months), with 5 patients receiving $\geqslant 6$ cycles after crossover. Patients were followed for a median of 4.5 months in selumetinib arm and 3.4 months with the combination regimen. Study completed full accrual as planned, and follow-up continued until all patients were off therapy.

Clinical outcomes. There was no difference in PFS between the single agent *vs* combination arm for the overall cohort (median 1.9 *vs* 2.1 months, $P=0.77$, HR: 0.92). (Figure 3A) However, compared with single agent ($N=10$), an improved median PFS was observed in the combination arm in the prespecified leiomyosarcoma stratum (median 1.8 *vs* 3.7 months; $P=0.01$, HR: 4.1). (Figure 3B) For the 19 patients who crossed over to combination arm, 12 of 19 patients eventually progressed; following their cross-over date, median PFS was 5.9 months (95% CI 1.8–8.5).

Three-month PFS rate was 34.6% (95% CI 16–51) with combination and 27.6% (13–44) for single-agent regimen in the overall cohort; it was 50% (95% CI 0.19–0.81) with the combination *vs* 15% (0–51) with selumetinib alone in the leiomyosarcoma cohort. Four-month PFS rate was 24% for both arms in the overall cohort; it was 50% (95% CI 0.19–0.81) with the combination *vs* 0% with selumetinib alone in the leiomyosarcoma cohort. In leiomyosarcoma patients, 6-month PFS rate was 37.5% (11–69) with the combination, *vs* 0% with selumetinib alone. (Table 2)

Overall response rate. Two patients with single-agent selumetinib had a partial response per RECIST criteria (confirmed PR in an undifferentiated pleomorphic sarcoma and an unconfirmed PR in a synovial sarcoma patient), and nine patients had stable disease with a median duration of 7.4 months (range: 1.8–20.9). (Figure 3C and D) With the combination regimen, although there were no

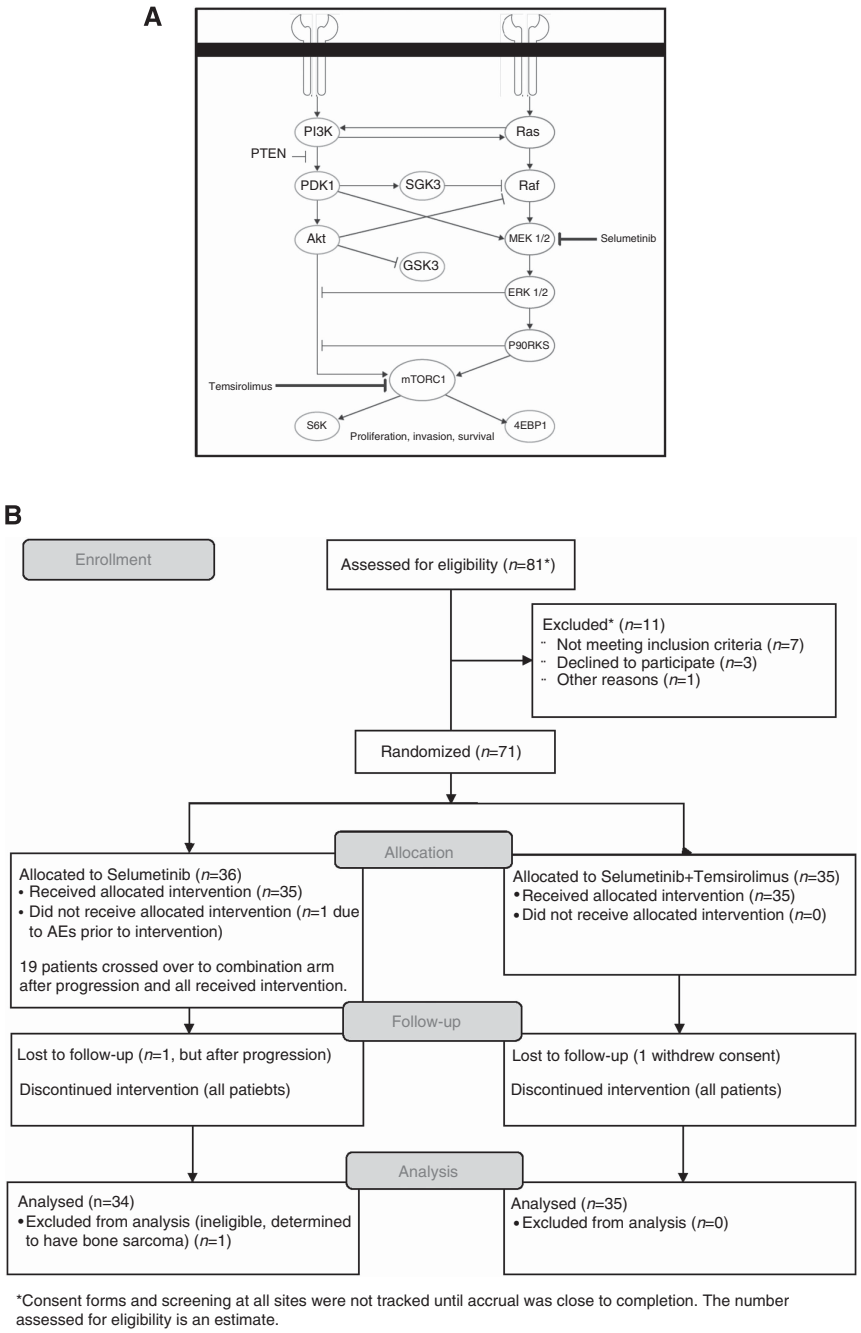

Figure 2. (**A**) Ras-PI3K-mTORC1 pathway; (**B**) CONSORT diagram.

objective responses seen per RECIST, 12 patients had stable disease, with a median duration of 2.1 months (range: 1.7–22.2). Five *vs* two patients in the leiomyosarcoma cohort had stable disease with the combination arm *vs* selumetinib alone, with no objective responses seen in either arm. Nineteen of 34 selumetinib patients crossed over to the combination; 2 patients had a partial response per RECIST, whereas 6 (32%) patients had stable disease, and 11 (58%) had progressive disease. With Choi response criteria analysis, five patients with selumetinib alone and four in the combination arm had PR; there were two PRs in the combination arm of the leiomysarcoma cohort and zero with selumetinib alone.

Toxicity and dose modifications. On the basis of an early interim review of toxicities, grade 3 mucositis/stomatitis was noted in four of 11 patients randomised to the combination arm or crossed over from single-agent selumetinib. Therefore, the study was amended to reduce the temsirolimus dose from 25 mg to 20 mg i.v. weekly. Of the 35 patients randomised to the combination regimen, 16 patients received the 25-mg dose, whereas 19 received 20 mg. Table 3 shows grade 3 and 4 adverse events in all patients before cross-over.

Oral mucositis was the most frequent grade 3 adverse event; it was observed in 29% of all patients in the combination arm. Lymphopenia (26%), neutropenia, and anaemia (20% each) were the next most common grade 3 events in this arm. For the selumetinib alone arm, hypertension (12%) was the most common grade 3 event. Most frequent grade 1/2 toxicities in the selumetinib alone and combination arms included acneiform rash (59% *vs* 43%, respectively), fatigue (53% *vs* 43%), anaemia (24% *vs* 48%) diarrhoea (37% *vs* 47%), and nausea (47% *vs* 31%).

All patients are off study, with the majority owing to progression of disease (61.8% in the single-agent and 74.3% in

Table 1. Patient characteristics

Patient characteristics	Selumetinib alone ($n=34$)	Selumetinib + temsirolimus ($n=35$)
Age at randomisation, median (range)	56.2 (34.4–84.3)	56.9 (20.2–83.7)
Gender, *N* (%) male	17 (50.0%)	10 (28.6%)
Race/ethnicity, *N* (%) Caucasian	17 (50.0%)	24 (68.6%)
Performance status, *N* (%)		
0	8 (23.5%)	14 (40.0%)
1	21 (61.8%)	19 (54.3%)
2	5 (14.7%)	2 (5.7%)
Tumor grade, *N* (%)		
Low grade	5 (14.7%)	5 (14.3%)
Intermediate grade	3 (8.8%)	2 (5.7%)
High grade	24 (70.6%)	24 (68.6%)
Unknown	2 (5.9%)	4 (11.4%)
Invasion depth, *N* (%)		
Superficial	4 (11.8%)	5 (14.3%)
Deep	26 (76.5%)	23 (65.7%)
Unknown	4 (11.8%)	7 (20.0%)
Stratum, *N* (%)		
Liposarcoma, prior therapy 0	3 (8.8%)	2 (5.7%)
Liposarcoma, prior therapy 1 or 2	2 (5.9%)	2 (5.7%)
Pleomorphic undifferentiated sarcoma, prior therapy 1 or 2	2 (5.9%)	2 (5.7%)
Synovial sarcoma, prior therapy 1 or 2	2 (5.9%)	3 (8.6%)
Leiomyosarcoma, prior therapy 1 or 2	10 (29.4%)	11 (31.4%)
Other, prior therapy 0	2 (5.9%)	2 (5.7%)
Other, prior therapy 1 or 2	13 (38.2%)	13 (37.1%)
Prior therapy, *N* (%)		
No prior chemotherapy	7 (20.6%)	2 (5.7%)
Noncytotoxic drugs only	1 (2.9%)	2 (5.7%)
Cytotoxic drugs		
Anthracycline based only	5 (14.7%)	1 (2.9%)
Nonanthracycline based only	8 (23.5%)	7 (20.0%)
Both	13 (38.2%)	23 (65.7%)
Number of prior lines of therapy, median	1	1
Total cycles precross-over, median (range)	2 (1–24)	2 (1–24)
Total cycles including postcross-over, median (range)	4 (1–24)	2 (1–24)

the combination arm.) Four patients withdrew consent, and 11 patients were removed from the study owing to toxicity (eight in selumetinib alone and three in combination arm.)

Correlative studies. Twenty-eight patients had PBMC samples analysed both before and at end of cycle 1. In every patient except for three in selumetinib alone and two in combination arm, decrease in ERK phosphorylation was observed posttreatment. (Figure 4) Thus, selumetinib inhibited ERK phosphorylation at both the 50 and 75 mg twice daily schedule. Pre- and post-treatment skin biopsies were obtained simultaneously with the PBMCs to evaluate as a surrogate for tumor mTOR–Akt pathway pharmacodynamic markers including: phospho-p70, phospho-AKT, Ki67 (proliferation), p62 (autophagic flux), BCL-2 (anti-apoptosis), and cleaved caspase 3 (apoptosis). However, IHC demonstrated marked variable detection of these proteins in both the pre- and post-treatment specimens. The antibodies used did not reliably detect the desired antigen in the pretreatment skin biopsies. Further, in several samples there was inadequate dermis within the punch skin biopsy specimens for review. Accordingly, IHC of skin biopsies as a surrogate marker for tumour tissue was uninformative for the proteins evaluated in this study.

DISCUSSION

There was no difference in PFS observed between selumetinib alone and selumetinib with temsirolimus in 69 advanced STS patients who had up to two lines of prior therapy in this multicentre randomised phase 2 clinical trial. The MEK inhibitors have not been previously tested in STS to our knowledge, but do not appear to show significant activity in this patient population. Mammalian target of rapamycin inhibitors have been tested in other trials of advanced STS with varying results. In a first-line phase 2 study of 25 mg weekly temsirolimus in 41 advanced STS patients, median time to progression was only 2 months, with 6-month PFS rate of 13% seen (Okuno *et al*, 2011). However, in a phase 2 trial of another mTORi, ridaforolimus, in mostly pretreated STS patients, a median PFS 15.3 weeks, and 6-month PFS rate of 23.4% was observed (Chawla *et al*, 2012).

Whereas the initial dose of temsirolimus in this trial was 25 mg i.v. weekly, owing to the frequency of grade 3 mucositis observed, the starting dose was reduced to 20 mg i.v. weekly. Thus, 46% of patients received 20-mg dosing of temsirolimus in the combination arm. The FDA approved dosing of temsirolimus for advanced renal cell cancer is 25 mg i.v. weekly; however, it is not clear whether the amended lower dose of temsirolimus in this study can inhibit mTOR signalling as completely. There was a lower incidence of grade 3 mucositis after the dose was lowered; the side effects of the combination appeared to be tolerable at this dosing.

There were no significant difference in response rates between the two arms regardless of response criteria used. Choi criteria were originally developed and validated in assessing GIST response. It has been suggested that Choi criteria may be more accurate in predicting outcomes in STS than RECIST (Stacchiotti *et al*, 2012), although its use is not currently validated in STS.

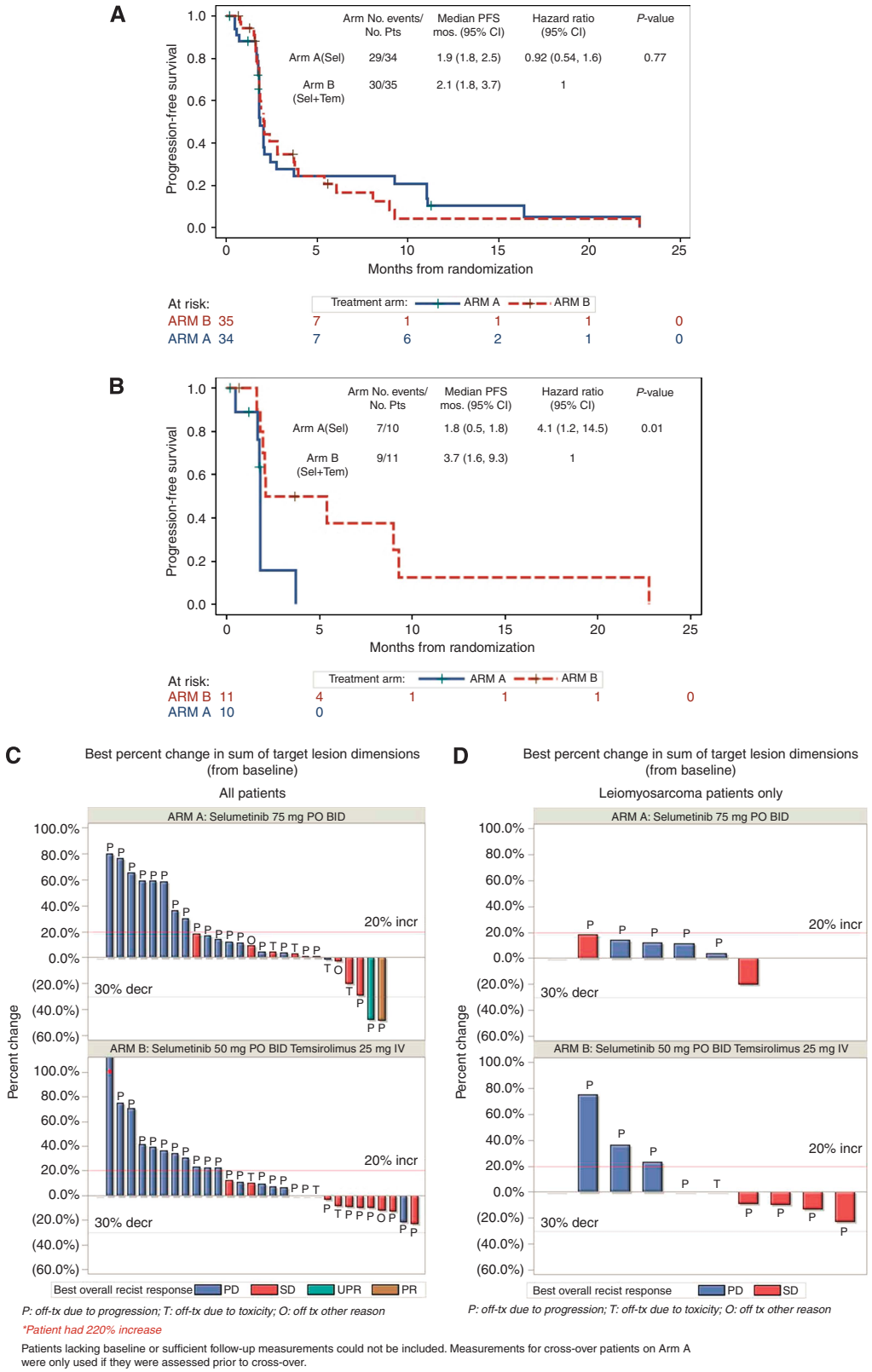

Figure 3. Progression-free survival (**A**) All patients (**B**) leiomyosarcoma cohort waterfall plots of response (by RECIST) (**C**) All patients (**D**) Leiomyosarcoma cohort.

A larger number of partial responses were observed with the Choi *vs* RECIST criteria (9 *vs* 2).

There was an improvement in PFS and PFS rates observed with the combination arm in the 21-patient leiomyosarcoma cohort; all patients in this cohort had 1 or 2 prior treatments. In a European Organisation for Research and Treatment of Cancer analysis of phase 2 trials for advanced sarcomas, the 3- and 6- month PFS rates for pretreated STS patients receiving an active agent were 39% and 14%, respectively. (Van Glabbeke *et al*, 2002) For second-line therapy, a 3-month PFS of $\geqslant$40% was suggested as a benchmark

Table 2. Progression-free survival rates

Selumetinib arm	PFS rate	95% CI	Sel + Tem arm	PFS rate	95% CI
All patients					
3 months	27.60%	13–44	3 months	34.60%	16–51
4 months	24.00%	9–40	4 months	24.00%	9–39
6 months	24.20%	11–14	6 months	20.80%	9–36
Leiomyosarcoma cohort					
3 months	15.00%	0–51	3 months	50.00%	19–81
4 months	0.00%	0	4 months	50.00%	19–81
6 months	0.00%	0	6 months	37.50%	11–69

Abbreviations: CI = confidence interval; PFS = progression-free survival; Sel = Selumetinib; Tem = temsirolimus.

Table 3. Grade 3/4 adverse events

	Treatment arm			
	Selumetinib ($n = 34$)		Sel + Tem ($n = 35$)[a]	
Grade 3 adverse events in >1 patient and grade 4 events	Grade 3	Grade 4	Grade 3	Grade 4
Anaemia	2 (6%)		7 (20%)	
Mucositis oral			10(29%)	
Nausea	2 (6%)		2 (6%)	
Vomiting	1 (3%)		3 (9%)	
Fatigue	2 (6%)		1 (3%)	
Lymphopenia	1 (3%)	1 (3%)	9 (26%)	
Neutropenia	1 (3%)		7 (20%)	
Thrombocytopenia			2 (6%)	
Leukopenia	1 (3%)		3 (9%)	
Dehydration	1 (3%)		2 (6%)	
Hypokalemia	2 (6%)	1 (3%)		
Syncope	2 (6%)		1 (3%)	
Rash acneiform	1 (3%)		2 (6%)	
Hypertension	4 (12%)		1 (3%)	
Thromboembolic event				1 (3%)

Abbreviations: Sel = selumetinib; Tem = temsirolimus.
[a]Adverse events for patients on selumetinib arm are reported prior to cross-over only.

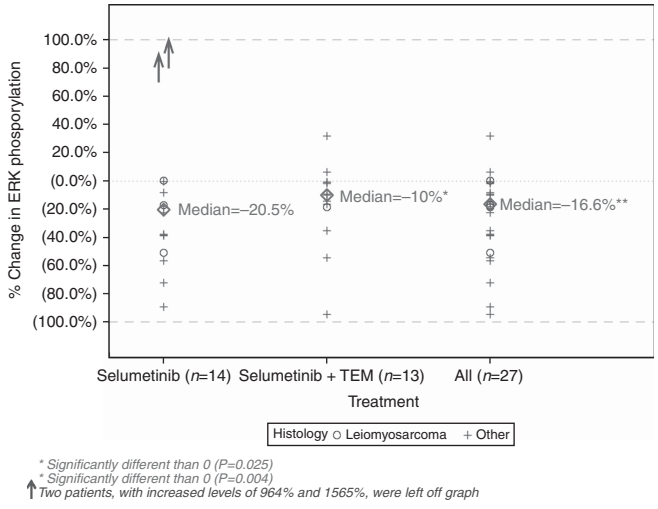

Figure 4. Change in ERK phosphorylation in PBMCs pre to posttreatment.

for drug activity. In the leiomyosarcoma cohort of the current study, a 3-month PFS of 50% (95% CI 19–81) was observed, which suggests clinically meaningful activity in this group. In the European Organisation for Research and Treatment of Cancer phase 2 study of pazopanib, the 3-month PFS rate was 44% for the 41 patients in the leiomyosarcoma cohort, for which 76% received prior chemotherapy for advanced disease. (Sleijfer *et al*, 2009) Although our results compare favourably with these outcomes, this is only based on a 21-patient subgroup analysis.

In leiomyosarcomas, the Akt/mTOR pathway has been shown to be activated and associated with worse prognosis (Setsu *et al*, 2012). Setsu *et al* (2012) reported over 70% of primary leiomyosarcoma tumours demonstrated phosphorylated forms of Akt, mTOR, ribosomal protein S6 kinase (S6), and eukaryotic translation initiation factor 4E-binding protein (4E-BP1). Interestingly, *PTEN* expression was lost in only 20% of the tumours, and mutational analysis failed to reveal any *PIK3CA* or *AKT1* mutations. Also consistent with those findings, absence of genetic alterations in the *AKT1*, *PI3K*, *PTEN*, and *EGFR* genes in leiomyosarcoma stem-like cells was reported, whereas proteins downstream of the PI3/AKT and MAPK/ERK pathways were strongly activated (Sette *et al*, 2012). The *PTEN* gene is located on chromosome 10q, and partial loss of 10q is also a frequent event in leiomyosarcomas. (Hu *et al*, 2005; Meza-Zepeda *et al*, 2006) Mice with smooth muscle lineage-specific knockout of *PTEN* also develop widespread smooth muscle cell hyperplasia and abdominal leiomyosarcomas, significantly implicating *PTEN* downregulation in leiomyosarcomagenesis. (Hernando *et al*, 2007) *KRAS* mutations can also activate the Akt/mTOR pathway, and *KRAS* mutations have been reported in a subset (7–33%) of leiomyosarcoma patients (Hill *et al*, 1997; Yoo and Robinson, 1999); MEK inhibitors act downstream of RAS in the MAPK pathway. These results may explain the relative sensitivity of leiomyosarcoma to the combination of temsirolimus and selumetinib as compared with selumetinib alone in this study.

Our results are also consistent with a clinical report evaluating temsirolimus in a small cohort of subjects with advanced

leiomyosarcomas. (Italiano *et al*, 2011) Although no objective responses were observed by RECIST criteria, stable disease was achieved in three of six subjects. These three subjects had partial response according to the Choi criteria. Given the lack of a temsirolimus alone comparator arm in this study, it is difficult to determine what benefit is gained by adding selumetinib to temsirolimus, and it is possible a similar outcome may have been observed with single-agent temsirolimus in leiomyosarcoma patients in our study as well.

In summary, although no improvement in PFS was seen in advanced STS patients with selumetinib alone or with addition of temsirolimus, the combination may have clinically meaningful activity in leiomyosarcoma patients, with improved median PFS, and an acceptable side effect profile. On the basis of these findings, we feel that testing of the combination regimen *vs* single-agent temsirolimus would be warranted in a randomised, phase 2 trial in leiomyosarcoma patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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