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Phase Ib/II Study of the Safety and Efficacy of Combination Therapy with Multikinase VEGF Inhibitor Pazopanib and MEK Inhibitor Trametinib In Advanced Soft Tissue Sarcoma.

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Phase Ib/II study of the safety and efficacy of combination therapy with multikinase vascular endothelial growth factor inhibitor Pazopanib and MEK inhibitor Trametinib in advanced soft tissue sarcoma

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Abstract

Purpose—Pazopanib, a multi-receptor tyrosine kinase inhibitor targeting primarily vascular endothelial growth factor receptors 1-3 (VEGFRs1-3), is approved for advanced soft tissue sarcoma and renal cell cancer. Downstream of VEGFR, trametinib is an FDA-approved MEK inhibitor used for melanoma. We hypothesized that vertical pathway inhibition using a trametinib would synergize with pazopanib in advanced soft tissue sarcoma (STS).

Experimental Design—In an open-label, multicenter, investigator-initiated NCCN-sponsored trial, patients with metastatic or advanced STS received pazopanib 800 mg and 2 mg of trametinib continuously for 28-day cycles. The primary endpoint was 4-month progression-free survival (PFS). Secondary endpoints were overall survival, response rate and disease control rate.

Results—Twenty-five patients were enrolled. The median age was 49 years (range 22–77 years) and 52% were male. Median PFS was 2.27 months (95% confidence interval [CI] 1.9–3.9), and the 4-month PFS rate was 21.1% (95% CI 9.7–45.9%), which was not an improvement over the hypothesized null 4-month PFS rate of 28.3% (p = 0.79). Median overall survival was 9.0 months (95% CI 5.7–17.7). A partial response occurred in 2 (8%) of the evaluable patients (95% CI 1.0–26.0%), one with PIK3CA E542K mutant embryonal rhabdomyosarcoma and another with spindle cell sarcoma. The disease control rate was 14/25 (56%; 95% CI 34.9–75.6%). The most common adverse events were diarrhea (84%), nausea (64%), and fatigue (56%).

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Conflict of interest: The authors declare no potential conflicts of interest.

Conclusion—The combination of pazopanib and trametinib was tolerable without indication of added activity of the combination in STS. Further study may be warranted in RAS/RAF aberrant sarcomas.

Keywords

Sarcoma; Pazopanib; MEK; Trametinib; VEGF

INTRODUCTION

Soft tissue sarcoma (STS) comprises a heterogenous group of mesenchymal neoplasms, including more than 50 subtypes. STS is usually treated using a multidisciplinary approach with surgery, radiation therapy, and chemotherapy (1, 2), but the prognosis of patients with relapsed or metastatic advanced STS remains poor. Cytotoxic chemotherapy has been the mainstay of first- and second-line treatment of these cancers, but patients invariably relapse or become refractory to therapy. Pazopanib is a multi-tyrosine kinase receptor inhibitor targeting vascular endothelial growth factor receptors 1-3 (VEGFR1-3), c-kit, and platelet-derived growth factor as its major targets. Though other agents (e.g. trabectedin and Eribulin) have recently been approved for select STS subtypes, pazopanib was the first targeted therapy to be approved for STS based upon the phase III trial of pazopanib in metastatic STS (PALETTE), which reported a median progression-free survival (PFS) duration of 4.6 months (3). These patients had received between one and four lines of previous therapy, with 50% of patients having received fewer than two lines of previous therapy. However, because patients develop resistance to pazopanib monotherapy, combination therapies with pazopanib are warranted.

The oncogenic RAS/RAF pathway has been implicated as a mechanism of resistance to antiangiogenic therapy (4). Previous work from other groups including ours has used a strategy of combination targeting of VEGF signaling with vertical signaling inhibition downstream of the VEGFR receptor to improve efficacy (5). Hence, a co-targeting strategy with a MAPK inhibitor may overcome resistance to single-agent VEGF inhibition. Treatment with sorafenib, an inhibitor of RAF and VEGFR1, dramatically inhibited growth of malignant peripheral nerve sheath tumors in vitro, inducing marked inhibition of phospho-MEK and phospho-ERK with downstream suppression of cyclin D1 (6). Additionally, an in vitro study of the MEK inhibitor U0126 showed inhibited cell proliferation and downstream phospho-ERK expression in bone sarcoma and STS cell lines in a dose- and time-dependent fashion (7). This has been shown in other malignancies as well (8). RAS pathway hyperactivation has been reported in sarcomas as well (9, 10).

On the basis of these findings, we hypothesized that we could increase the impact of pazopanib in advanced STS by employing vertical pathway inhibition using a MEK inhibitor, trametinib. We used the recommended phase II doses of pazopanib and trametinib that we established in a prior dose escalation trial of pazopanib and trametinib in advanced solid tumors; in that study, both agents were able to be escalated to their single agent phase II dose(11).

PATIENTS AND METHODS

Patient eligibility

This was an open-label, multicenter [Sidney Kimmel Comprehensive Cancer Center (SKCCC) at John Hopkins University (JHU) and The University of Texas MD Anderson Comprehensive Cancer Center (MDACC)] trial sponsored by the National Comprehensive Cancer Network (NCCN). Patients older than 18 years with advanced, inoperable STS that was refractory to standard of care treatment options (or patients who refused standard of care treatment options) were eligible. Other eligibility criteria included the presence of measurable disease, defined as at least one lesion accurately measured in at least one dimension (longest diameter for nonnodal lesions and short axis for nodal lesions) as ≥10 mm according to spiral computed tomography. In addition, objective evidence of tumor progression in the 6-month period preceding initiation of treatment in the trial, as assessed by unequivocal progression of objectively measured disease on successive computed tomography scans, was a specific requirement for enrollment. Other requirements included an Eastern Cooperative Oncology Group performance status ≤1 (or Karnofsky performance status ≥60%) and adequate organ function as defined by absolute neutrophil count ≥1,500 cells/μL, platelet count ≥100,000 cells/μL, international normalized ratio ≤1.2× upper limit of normal (unless stabilized with anticoagulation therapy and within the recommended range for the desired level of anticoagulation), total bilirubin ≤1.5× upper limit of normal (or, in patients with Gilbert syndrome, total bilirubin >1.5× as long as direct bilirubin is normal), and serum creatinine ≤1.5× upper limit of normal or creatinine clearance ≥45 mL/minute and urine protein to creatinine ratio <1 (or, if >1, 24-hour urine protein <1 g).

Evaluation and treatment

The study was approved by the institutional review boards of the participating sites. The study drugs (trametinib and pazopanib) were provided by GlaxoSmithKline (now Novartis). Eligible patients were enrolled centrally at the SKCCC at JHU.

Patients in the study received 800 mg of pazopanib and 2.0 mg of trametinib. Protocol allowed dose delays or reduction if patients experienced unacceptable side effects and adverse reactions. Patients were evaluated every cycle for trial therapy compliance and monitoring of adverse events. The National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 was implemented for adverse event monitoring [9]. Disease assessments (computed tomography or magnetic resonance imaging) were performed every other cycle. Response was evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) guidelines version 1.1 [10]. Upon progression of disease, patients were monitored for long-term adverse events, new primary tumors, and survival.

One patient in the trial, who had a partial response, had comprehensive genomic profiling done on 50 ng of genomic DNA in a Clinical Laboratory Improvement Amendments-certified laboratory. Another patient, who had Ewing sarcoma, had T200 research panel sequencing done as per MD Anderson protocol capturing and sequencing all the exons in 201 cancer-related genes(12).

Statistical methods

The primary outcome measure was 4-month PFS rate. Secondary outcome measures included overall survival (OS) duration and disease control rate (DCR). Proportions are reported with exact 95% binomial confidence intervals (CI). Event time distributions for OS and PFS were estimated using the Kaplan-Meier method [1] and CIs were calculated using the Brookmeyer-Crowley method. The median follow-up was calculated using the reverse Kaplan Meier method. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina) and R version 3.0.

The benchmark for improvement in the PALETTE study of pazopanib in advanced STS was to increase the 6-month PFS rate from 15% to 30%, HR=0.63, (or, assuming exponential survival, increasing 4-month PFS from 28% to 45%). The primary endpoint of the current study was 4-month PFS. This trial could not be powered to detect a hazard ratio of 0.63, however, our pre-specified benchmark for monitoring the study for futility was based on achieving a 4-month PFS rate of 45%. The study was designed to stop if we became 75% certain that the 4 month PFS was below 45%.

Analysis of sarcoma next generation sequencing data

We analyzed next generation sequencing data from the Cancer Genome Atlas (TCGA) and six other sarcoma studies, and figures were generated to show copy number alterations and mutations in selected genes in these sarcoma projects. All figure panels were created using the cBioPortal(13) for the seven sarcoma data sets available on the portal.

RESULTS

Twenty-five patients with advanced STS were enrolled in the trial. The median age was 49 years (range 22–77 years). The median number of prior chemotherapies was 3 (range 1–10). All 25 patients were evaluable for objective response, and the baseline characteristics and histologies are outlined in Table 1. Thirteen patients (52%) were male. Six patients (24%) had leiomyosarcoma and four patients (16%) each had liposarcoma [1 pleiomorphic liposarcoma, and 3 de-differentiated liposarcoma], Ewing sarcoma, and spindle cell sarcoma. Other subtypes included rhabdomyosarcoma, epithelioid fibrosarcoma, hemangiopericytoma, pleomorphic sarcoma, synovial sarcoma, dedifferentiated chondrosarcoma, and malignant peripheral nerve sheath tumor associated with NF1 syndrome (n = 1 each).

Clinical outcomes

Partial responses occurred in 2 (8%) of the evaluable patients (95% CI 1.0–26.0%). No patient achieved a complete response. The DCR was 14/25 (56%; 95% CI 34.9%–75.6%). Stable disease was the best response in 12 (48%) and progressive disease was the response in 11 patients (44 %).

Survival outcomes

Patients who withdrew early from the treatment protocol were censored for PFS at the time of withdrawal. All other patients were followed for OS for 3 years, or until death, regardless

time on study therapy. Follow-up was 95% complete through 16 months, with only one censored patient after month 20 (Figure 1). The median follow-up, calculated as the 50%-point of the censoring function, was 25.56 months (ranging from 1.8–25.6 months) The overall percentage censored for OS was 12% (3 patients).

PFS and OS are shown in Figure 2. The median PFS was 2.27 months (95% CI 1.9–3.9). The 4-month PFS rate was 21.1% (95% CI 9.7–45.9%), which was not an improvement over the hypothesized null 4-month PFS rate of 28.3% (p = 0.79). Median OS was 9.0 months (95% CI 5.7–17.7).

Adverse events

All relevant grade 1 and 2 toxic effects related to the protocol and all grade 3 and 4 toxic events and dose-limiting toxicities are summarized in the Table 2. The most common adverse events attributable to study drug were diarrhea (84%), nausea (64%), and fatigue (56%) and generally low-grade. Other toxicities that were attributable to drug and grade ¾ included anemia (8%), hypokalemia (12%), anemia (12%), and thrombocytopenia (16%). The left ventricular ejection fraction was transiently reduced in one patient who had previously received high cumulative exposures to doxorubicin and an IGF1R inhibitor, but the ejection fraction returned to normal after the pazopanib/trametinib combination was discontinued.

Response

Two patients (8%) achieved partial response and 12 patients had stable disease per RECIST. One of the patients who achieved partial response, who had a sinonasal embryonal rhabdomyosarcoma, had a 59% tumor size reduction (Figure 3E–H). Next-generation sequencing analysis of the patient revealed PIK3CA E542K aberration (14). The implications of this mutation are discussed below. The other patient had a 63% tumor size reduction (Figure 3A–D). This patient had undifferentiated sarcoma and did not have any molecular testing done owing to nonavailability of tissue.

Ewing sarcoma responses have been noted with single-agent pazopanib (15–17). However, we did not see any responses to the combination of pazopanib and trametinib (GSK1120212) in patients with Ewing sarcoma. Next-generation sequencing of the molecular profile of one of the patients with Ewing sarcoma was compared with a previous patient who had a response to pazopanib in our clinic, and we found that three genes (FGFR3, FGFR4, and FLT4) were amplified only in the responder (Table 3).

Dose Reductions

Eight patients/25 patients (32%) required dose reductions of the pazopanib or trametinib beyond the first cycle. The dose was reduced in 5 patients treated with pazopanib (from 800 mg to 600 mg) and 4 patients who received trametinib, decreasing 1.5 mg. Twelve of twenty-five were safely maintained at the recommended phase 2 dose of both the drugs. 100% dose intensity for pazopanib was 22,400 mg and trametinib is 56 mg.

Next generation sequencing data of sarcomas

Because the trial targeted the MAPK pathway and one of the exceptional responders harbored the PIK3CA E542K mutation, we analyzed sequencing data from the TCGA and six other sequencing projects available on cBioPortal(13) for aberrations in the following genes: BRAF, NRAS, KRAS, MAPK1, MAPK3, MAP2K1 and PIK3CA. Mutations and copy number alterations from seven sarcoma studies are depicted in Figure 4. Interestingly, most of the alterations in these genes were mutually exclusive, and PIK3CA alterations showed various frequencies in these studies, with 41% cases altered in uterine carcinosarcoma.

DISCUSSION

The FDA approval of pazopanib for the treatment of STS has opened up several possible combinations with this agent, given that nearly all patients experience disease progression with monotherapy. Combinations involving VEGF-based multikinase tyrosine kinase inhibitors are a challenge because many other targeted therapies have overlapping toxicities. There is a need to balance clinical benefit with clinical toxicity. In the current study, the combination of pazopanib and trametinib was reasonably well tolerated, although several patients had dose reductions. Clinical responses were observed, but did not meet the primary study endpoint of reduced 4-month PFS.

In-depth analysis of one of the exceptional responders revealed a PIK3CA E542K aberration in a patient with sinonasal rhabdomyosarcoma. This patient had previously received treatment with a PI3K inhibitor, but the tumor had progressed. It is difficult to determine whether the tumor responded to pazopanib or trametinib in our protocol, but evidence from the literature suggests that the combination of agents could have contributed. RAS pathway overactivity has been shown in rhabdomyosarcoma (18), and the RAS/RAF pathway is one of the mechanisms of resistance to P13K pathway inhibition. In addition, PI3K activation occurs in more than 80% of rhabdomyosarcomas, and MAPK pathway co-activation occurs in 46% of embryonal rhabdomyosarcomas, suggesting that dual blockade could be an effective treatment strategy (19). The multi-kinase VEGF inhibitor could have potentiated the MEK inhibition in this patient, leading to the partial response. Interestingly, it has also been shown that pazopanib suppresses the PI3K pathway in rhabdomyosarcomas (10).

In-depth sequencing of Ewing sarcoma from a patient who responded to pazopanib in our clinic compared with the non-responder from the current study revealed divergence in the genotype of Ewing sarcoma in a three-gene signature consisting of FGFR3, FGFR4, and FLT4, which are all targets for pazopanib. These observations are strictly hypothesis-generating, but they may warrant further studies comparing pazopanib responders with non-responders. Ewing sarcoma is a translocation-positive sarcoma, and it would be helpful to identify mechanisms of response and resistance in these patients (20–22). In patients with Ewing sarcoma in the current study, the combination of the pazopanib and trametinib was tolerated but did not add any benefit to pazopanib as a single agent. However, we treated only 4 patients with Ewing’s sarcoma in this trial.

Toxic effects in our trial included diarrhea, which was grade 1/2 in 18 patients and grade 3 in three patients which was consistent with expected toxicity from other trials. This was managed by loperamide and atropine/diphenoxylate. The unexpected reduction of left ventricular ejection fraction in a patient with Ewing sarcoma was reviewed in detail. This patient had received heavy prior treatment with doxorubicin, a anthracycline known to increase the risk of cardiomyopathy, and an IGF1R inhibitor that is unlikely to have contributed. Though the toxic effect could have resulted from an interaction between MEK inhibition and chemotherapy-induced cardiomyocyte damage induced by prior therapies, our study cannot conclusively address this question. The adverse event was attributed as possibly related to study drugs, and the patient withdrew from the protocol.

The current study demonstrates the feasibility and efficiency of performing a phase II trial through the National Comprehensive Cancer Network. Two sites, major cancer referral centers, allowed very easy accrual of patients. However, one of the major limitations of the current study is that it included all diagnoses of STS and there was no selection for tumors with hyperactivity of the RAS/RAF pathway. A recently published trial noted that MEK inhibitors as monotherapy did not have any activity in STS (23). The median PFS reported in this study is 2.27 months compared to 4.6 months in the PALETTE study. Firstly, the number of prior therapies and the eligibility criteria for the trial varied. This trial was written as a phase 1 trial and this sarcoma cohort was the expansion part. The eligibility criteria for trial entry for the PALETTE Phase 3 trial were more rigorous than this Phase 1 trial. Secondly, this trial specifically required objective evidence of tumor progression in the 6-month period preceding initiation of treatment in the trial, as assessed by unequivocal progression of objectively measured disease on successive computed tomography scans. This could have probably selected for aggressive biology patients for this trial. Thirdly, PALETTE ineligible patients with histology such as embryonal rhabdomyosarcoma, liposarcoma and Ewing's sarcoma were included on the trial. In addition a recent study by Nakano et al notes that PALLETTE study ineligible patients might have worse prognosis on pazopanib than PALETTE-eligible patients and our study confirms this (24). The resistance mechanisms to VEGF inhibitors such as pazopanib are complex. Although pathways in sarcomas like VEGF have often been depicted as linear, clearly there is a complicated interaction among signaling elements (25, 26). A recent study showed the mTOR inhibitor could overcome pazopanib resistance(27). Several trials with pazopanib combinations are ongoing, including pazopanib + vorinostat (HDAC inhibitor; NCT01339871), pazopanib + everolimus (mTOR inhibitor; NCT01430572), pazopanib + lapatinib or trastuzumab (Her2 inhibitors; NCT01454804), pazopanib + gemcitabine (NCT01532687), pazopanib + pemetrexed or crizotinib (NCT01548144), and pazopanib + topotecan (NCT02357810). The results of these trials may be helpful in evaluating which combination is better than single-agent pazopanib in STS.

In addition, although pazopanib as a targeted agent is approved for STS, the biomarkers of response or resistance are unknown, and the responses to such antiangiogenic agents are complex. As noted above, we identified a three-gene signature in a pazopanib responder that was not present in a non-responder, and these findings may warrant further study. As MEK inhibitors are active in RAF/RAS pathway-activated melanoma (28), a trial that includes STS patients harboring a RAF/RAS-overactive genotype may yield better results. Our

analysis of sequencing data from the TCGA and other projects shows that RAS/RAF is activated in a subset of sarcoma patients. Further evaluation of the combination of pazopanib and a MEK inhibitor specifically in RAS/RAF-activated tumors such as rhabdomyosarcomas, in a randomized design comparing pazopanib monotherapy with the combination, is one future goal.

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STATEMENT OF TRANSLATIONAL RELEVANCE

Pazopanib, US FDA approved for advanced soft tissue sarcoma is a multi-tyrosine kinase receptor inhibitor targeting vascular endothelial growth factor receptors 1-3 (VEGFR1-3), c-kit, and platelet-derived growth factor as its major targets. However, because patients develop resistance to pazopanib monotherapy, combination therapies with pazopanib are warranted. The oncogenic RAS/RAF pathway has been implicated as a mechanism of resistance to antiangiogenic therapy. Previous work from other groups including ours has used a strategy of combination targeting of VEGF signaling with vertical signaling inhibition downstream of the VEGFR receptor to improve efficacy. Hence, a co-targeting strategy with a MAPK inhibitor may overcome resistance to single-agent VEGF inhibition. We hypothesized that we could increase the impact of pazopanib in advanced soft tissue sarcoma by employing vertical pathway inhibition using a MEK inhibitor, trametinib and report the results of the Phase 1b/2 trial.

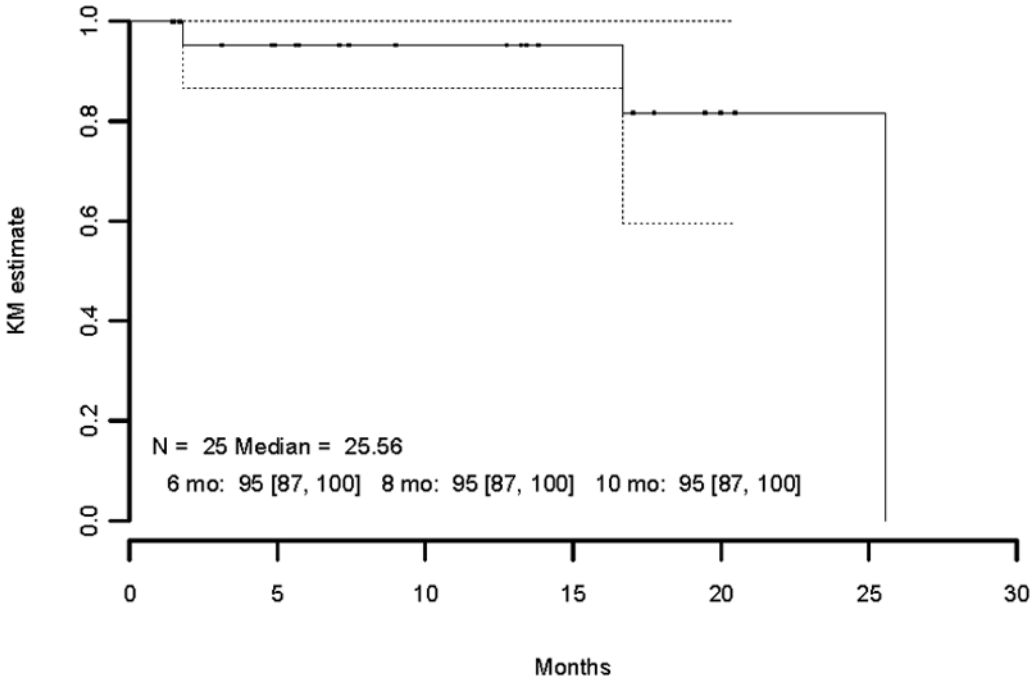


Figure 1.
Kaplan-Meier estimate of potential follow-up times.

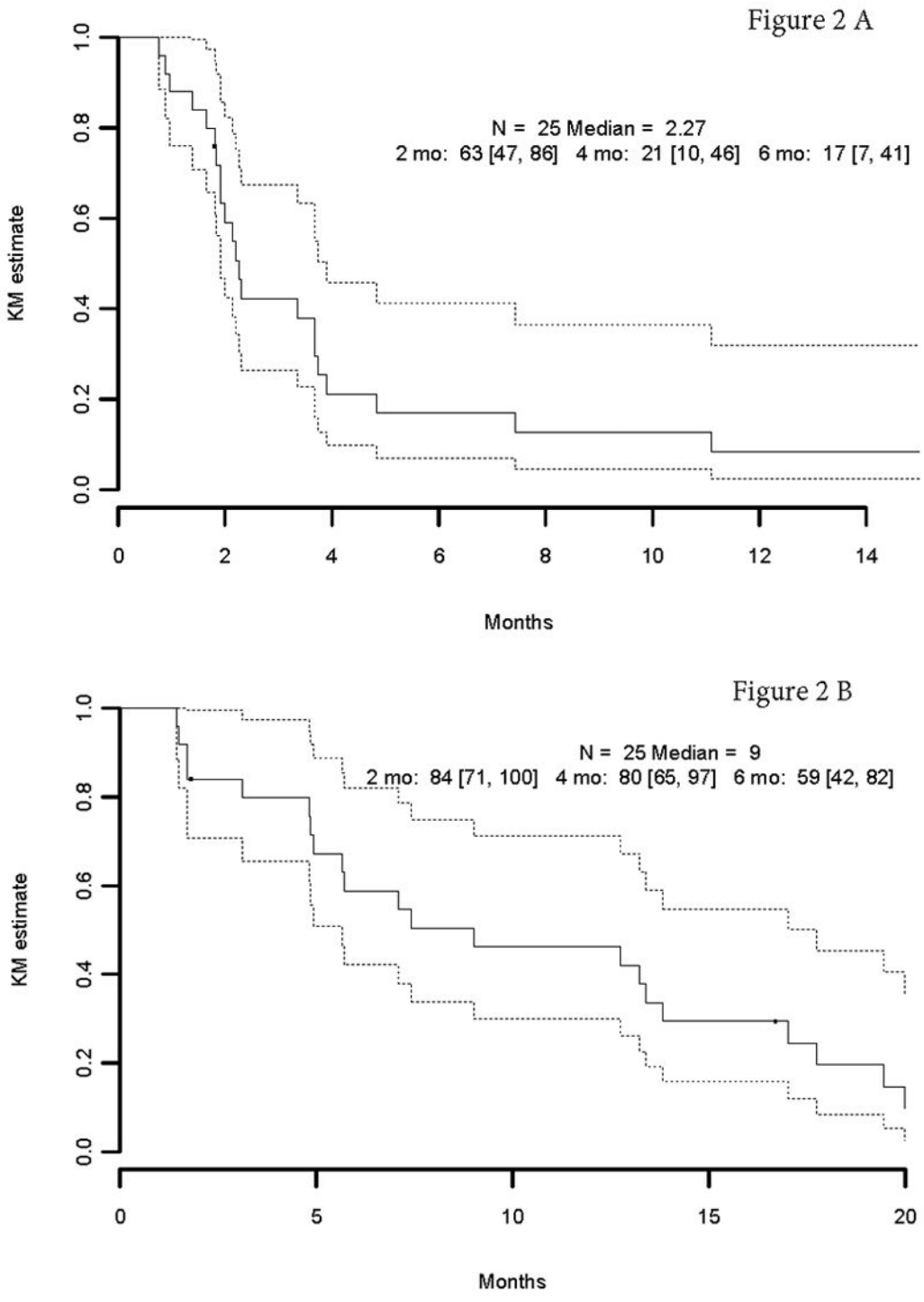


Figure 2.

A: Progression-free survival **(B)** overall survival curves Two-month, 4-month, and 6-month survival rates and 95% confidence intervals are shown.

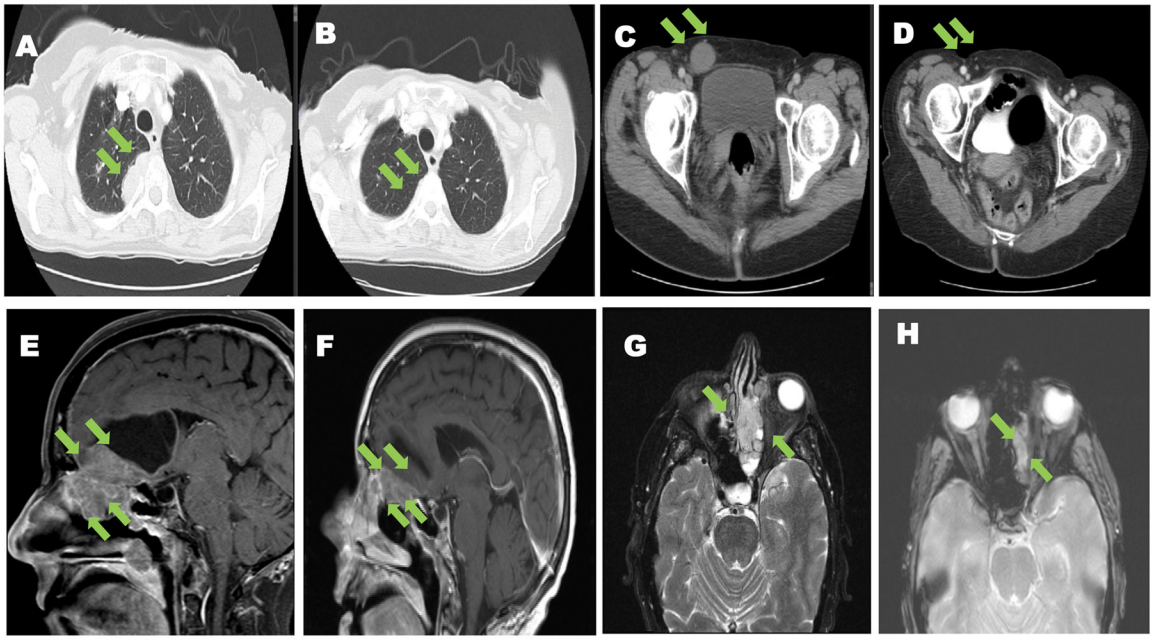
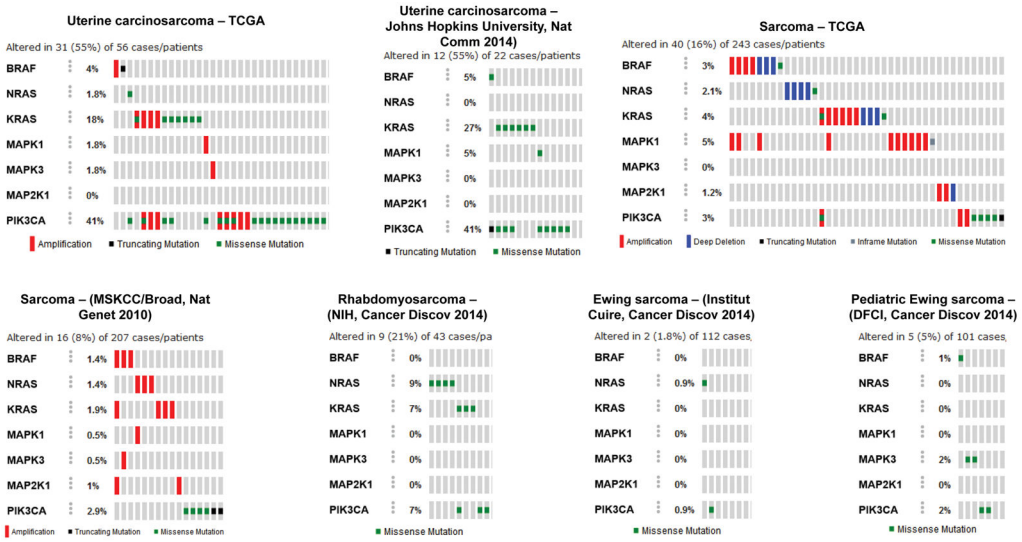


Figure 3.

Images showing response of sarcomas to pazopanib and GSK1120212 in two patients. (A, C) Baseline imaging in a patient with spindle cell sarcoma. (B, D) Re-staging scans during the trial showing a reduction in disease. (E, G) Baseline imaging in a patient with a sinonasal embryonal rhabdomyosarcoma. (F, H) Re-staging scans during the trial showing a reduction in disease.



Mutations and copy number alterations in seven sarcoma studies (screenshots are from the cBioPortal, patients with alterations are shown).

Figure 4.

Analyses of the SARCOMA TCGA data for aberrations in the following genes BRAF, NRAS, KRAS, MAPK1, MAPK3, MAP2K1 and P1K3CA. Mutations and copy number alterations from seven sarcoma studies are depicted.

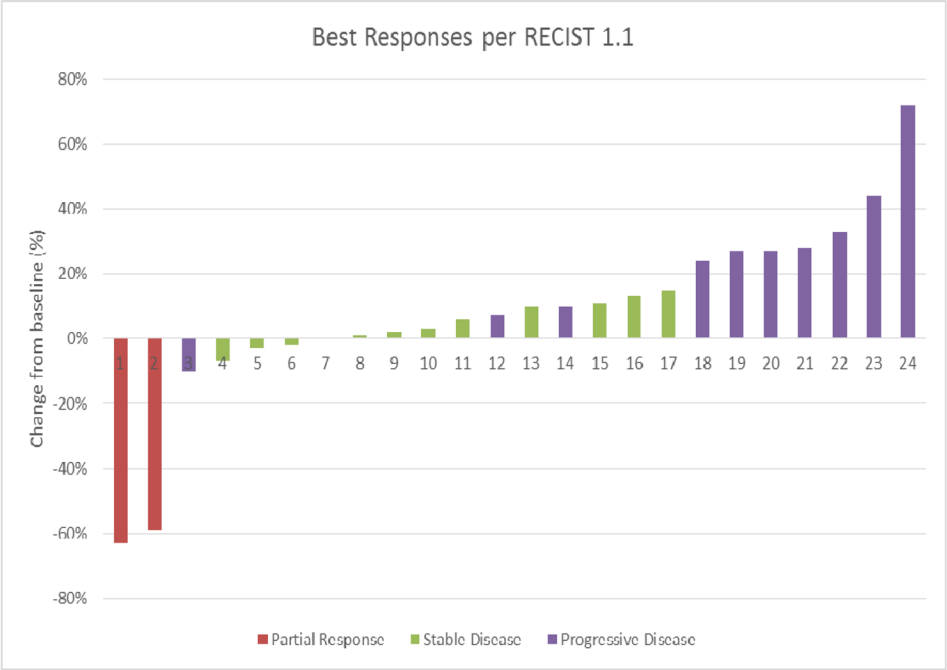


Figure 5.

Responses per RECIST 1.1 Two partial responses were seen. Patients 3, 12, and 14 had new lesions and hence progressive disease.

Table 1

Baseline characteristics and best responses of patients enrolled in the trial (n = 25).

Characteristic	No. of patients (%)
Median age (range)	49 years (22–77 years)
Sex	
Male	13 (52)
Female	12 (48)
Median no. of prior lines of chemotherapy (range)	3 lines (1–10 lines)
Median no. of prior radiation treatments (range)	1 treatment (0–2 treatments)
Sarcoma subtype	
Leiomyosarcoma	6 (24)
Liposarcoma[a]	4 (16)
Ewing sarcoma	4 (16)
Spindle cell sarcoma	4 (16)
Other[b]	7 (28)
Best response	
Partial response	2 (8)
Stable disease	12 (48)
Progressive disease	11 (44)

[a]Three De-differentiated liposarcoma and one pleiomorphic liposarcoma. One De-differentiated liposarcoma had focal rhabdoid features.

[b]Sinonasal embryonal rhabdomyosarcoma, epithelioid fibrosarcoma, hemangiopericytoma, pleomorphic sarcoma, synovial sarcoma, dedifferentiated chondrosarcoma, and neurofibrosarcoma (n = 1 each).

Table 2

Relevant grade 1 and 2 and all grade 3 and above toxic effects related to treatment with pazopanib and GSK1120212 in patients in our study (n = 25).

	No. of patients				
Adverse event	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total**
Alanine aminotransferase increase	2		1		3 (12%)
Anemia			2	1	3(12%)
Aspartate aminotransferase increase			1		1(4%)
Blood pressure increase	1	2	1		4(16%)
Blurred vision	2	1			3(12%)
Congestive heart failure			1		1(4%)
Constipation	2	1			3(12%)
Decreased appetite	5				5(20%)
Diarrhea	8	10	3		21 (84%)
Fatigue	8	6			14 (56%)
Headache	4	2	1		7(28%)
Hoarseness	1				1(4%)
Hypokalemia			3		3(12%)
Left ventricular ejection decrease			1		1(4%)
Maculopapular rash	3	1			4(16%)
Mucositis	1				1(4%)
Nausea	9	7			16 (64%)
Neutropenia	1	2	2		5(20%)
Periorbital edema			1		1(4%)
Thrombocytopenia	2		3	1	6(24%)
Presyncopal episode			1		1(4%)

Table 3

Amplification of three genes in patient with Ewing sarcoma from a previous study who responded to treatment with pazopanib, compared with a patient with Ewing sarcoma in the current study who did not respond to combination treatment.

	Amplification	
Gene	**Ewing sarcoma responder**	**Ewing Sarcoma nonresponder**
FGFR3	8.7	2.6
FGFR4	5.2	2.8
FLT4	4.8	2.6