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A phase Ib/II translational study of sunitinib with neoadjuvant radiotherapy in soft-tissue sarcoma

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Background: Preoperative radiotherapy (RT) is commonly used to treat localised soft-tissue sarcomas (STS). Hypoxia is an important determinant of radioresistance. Whether antiangiogenic therapy can 'normalise' tumour vasculature, thereby improving oxygenation, remains unknown.

Methods: Two cohorts were prospectively enrolled. Cohort A evaluated the implications of hypoxia in STS, using the hypoxic tracer ^{18}F-azomycin arabinoside (FAZA-PET). In cohort B, sunitinib was added to preoperative RT in a dose-finding phase 1b/2 design.

Results: In cohort A, 13 out of 23 tumours were hypoxic (FAZA-PET), correlating with metabolic activity ($r^2 = 0.85$; $P < 0.001$). Two-year progression-free (PFS) and overall (OS) survival were 61% (95% CI: 0.44–0.84) and 87% (95% CI: 0.74–1.00), respectively. Hypoxia was associated with radioresistance ($P = 0.012$), higher local recurrence (Hazard ratio (HR): 10.2; $P = 0.02$), PFS (HR: 8.4; $P = 0.02$), and OS (HR: 41.4; $P < 0.04$). In Cohort B, seven patients received sunitinib at dose level (DL): 0 (50 mg per day for 2 weeks before RT; 25 mg per day during RT) and two patients received DL: −1 (37.5 mg per day for entire period). Dose-limiting toxicities were observed in 4 out of 7 patients at DL 0 and 2 out of 2 patients at DL −1, resulting in premature study closure. Although there was no difference in PFS or OS, patients receiving sunitinib had higher local failure (HR: 8.1; $P = 0.004$).

Conclusion: In STS, hypoxia is associated with adverse outcomes. The combination of sunitinib with preoperative RT resulted in unacceptable toxicities, and higher local relapse rates.

Soft-tissue sarcomas (STS) are diverse tumours arising from connective tissues, accounting for 1–3% of adult cancers and up to 15% of childhood cancers (Lewin *et al*, 2013). Regardless of histology, curative utilises a multimodal approach based on radical surgical resection. Limb-sparing surgery has replaced amputation and compartmental resection for STS (Ngan, 1997), often combined with radiotherapy (RT) which increases local control rates, facilitating limb preservation and improving outcomes (Ngan, 1997; Yang *et al*, 1998). Despite the use of RT, 10–45% of patients relapse at the primary site (Spiro *et al*, 1997). The reason for the relative radioresistance of STS is not well understood.

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Hypoxia in cancers is associated with poorer prognosis, malignant progression, increased metastasis, and resistance to radiation and chemotherapy (Harris, 2002; Vaupel and Harrison, 2004; Vaupel, 2004). Oxygen generates free oxygen radicals during RT that contribute to DNA damage and tumour cell death. Good preclinical and clinical evidence suggests that hypoxia contributes to radioresistance in head and neck (Brizel *et al*, 1997) and cervical cancers (Harrison and Blackwell, 2004) among other types. Since sarcomas are relatively hypoxic tumours (Brizel *et al*, 1994; Evans *et al*, 2001) we hypothesised that at least part of their radioresistance may be due to hypoxia rather than intrinsic factors. Most studies in sarcomas have assessed hypoxia using immunohistochemical or probe data. More recently, modern imaging technologies with a first-generation hypoxic tracer 18F-misonidazole (F-MISO) have suggested that 76% of sarcomas are hypoxic (Rajendran *et al*, 2003). Tumour hypoxia is a result of the imbalance between oxygen supply and consumption in some tumours. Factors causing hypoxia include the remodelling of microvessels supplying the tumour, leading to compromised blood flow and tumour perfusion, driven by the disordered growth of tumour cells and dysregulation of growth factors such as Vascular Endothelial Growth Factor-A (VEGF-A) (Ferrara, 1999). The VEGF family members are overexpressed in human cancers, and drive behaviour such as solid tumour growth and metastatic spread (Stacker *et al*, 2004, 2014), including in STS (Chao *et al*, 2001; Pakos *et al*, 2005). High plasma (Yoon *et al*, 2004) and serum (Linder *et al*, 1998; Graeven *et al*, 1999; Hayes *et al*, 2004) levels of VEGF-A have been documented in patients with STS, and correlate with tumour grade (Graeven *et al*, 1999; Yoon *et al*, 2004), potentially serving as a useful prognostic marker (Linder *et al*, 1998; Hayes *et al*, 2004; Yoon *et al*, 2004).

One potential action of antiangiogenic agents is to normalise tumour vasculature by inhibiting signalling for vessel growth and remodelling. The 'vascular normalisation hypothesis' proposed that blocking angiogenesis would improve delivery of therapeutics and oxygen to tumour cells, and therefore enhance the efficacy of chemotherapy and RT via the oxygen enhancement effect (Jain, 2005). Sunitinib (Sutent, Pfizer Inc., New York, NY, USA) is a small molecular inhibitor of transmembrane receptor tyrosine kinases (PDGFR, VEGFR, c-Kit, FLT-3, and RET) with antiangiogenic (Osusky *et al*, 2004) and radiosensitising effects (Cuneo *et al*, 2008), and has clinical activity in advanced STS (George *et al*, 2009). It is currently registered in Australia for treatment of renal cell carcinoma, second-line GIST and pancreatic neuroendocrine tumours. Given preclinical evidence that anti-VEGF-A or anti-VEGFR therapy is a radiosensitiser (Wachsberger *et al*, 2003), we also hypothesised that sunitinib may improve the efficacy of neoadjuvant RT in STS. In this two-stage prospective study of patients with STS undergoing preoperative RT, we analysed the incidence and clinical implications of hypoxia using the novel hypoxic tracer ^{18}F-azomycin arabinoside (FAZA), and then conducted a phase Ib/II trial of sunitinib in combination with RT to assess safety as well as biological and anti-tumour activity.

MATERIALS AND METHODS

Study design and eligibility. This open label phase Ib/II prospective study was conducted at the Peter MacCallum Cancer Centre and was approved by the Institutional Review Board (study identifiers: NCT00753727; ASSG 08/05 and 06/26). Patients with resectable STS were enrolled before neoadjuvant RT into two cohorts. Cohort A received neoadjuvant RT alone, whereas Cohort B received RT in conjunction with sunitinib. The overall study design of the two cohorts is shown in Figure 1. The drug and funding were provided by Pfizer Australia and sponsored by the Australasian Sarcoma Study Group.

Patient population. Inclusion criteria included histologically confirmed STS suitable for neoadjuvant RT and surgery (high-grade non-metastatic tumours, and low-grade tumours greater than 5 cm in diameter where tumour reduction might facilitate resection); age >16; ECOG 0–1; life expectancy >6 months with normal organ function. Exclusion criteria included evidence of distant metastasis.

Pretreatment and follow-up evaluations. Patients underwent baseline clinical staging with chest CT, an ^{18}F-fluoro-2D-deoxyglucose (FDG) PET/CT scan, and a dynamic contrast-enhanced MRI (DCE-MRI) scan. In addition, patients underwent FAZA-PET/CT imaging for hypoxia and had weekly blood samples for VEGF family members weekly. Patients in Cohort B underwent repeat DCE-MRI and FAZA-PET/CT after 2 weeks of sunitinib before the addition of RT. Preoperatively, patients underwent standard restaging including FDG-PET/CT, DCE-MRI, and FAZA-PET/CT. The patient's tumour was reviewed for intratumoral vascularity and vascular invasion, and, at resection, for

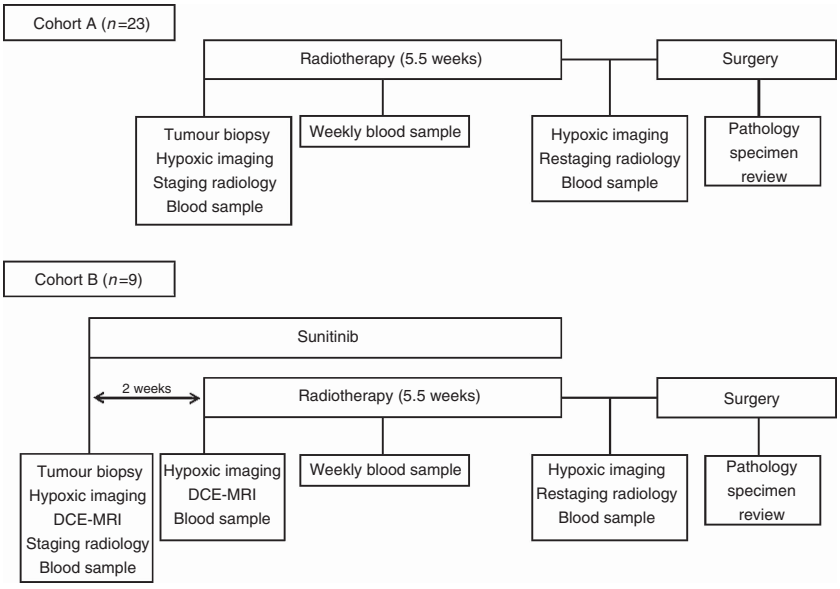

Figure 1. Design schema for Cohort A and Cohort B.

tumour necrosis, as well as for markers of hypoxia, proliferation, and VEGF family member expression.

Immunohistochemistry and ELISA. Immunohistochemistry for VEGF-A was with polyclonal antibodies to human $VEGF_{165}$ (R&D Systems catalogue number AB-293-NA) according to the manufacturer's instructions; for VEGF-C was with affinity-purified polyclonal antibody (R&D Systems catalogue number AF752) according to the manufacturer; for VEGF-D was as described previously (Achen *et al*, 2001).

Peripheral blood samples were collected weekly. Plasma was isolated, aliquoted, and stored at $-80\,^{\circ}C$ until assayed. Measurement of VEGF-A, -C, and -D concentrations was performed using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA), specific for each VEGF isoform. All samples were run in duplicate for each assay.

Treatment plan. All patients received 50.4 Gy in 28 fractions of external beam radiation over 5½ weeks. Definitive surgery occurred 3–6 weeks after completion of RT. For Cohort B, sunitinib dose levels (DLs) were predefined as follows: DL 0: 50 mg per day for 2 weeks before RT, then 25 mg per day given during RT; DL 1: 50 mg per day for 2 weeks before RT, then 37.5 mg per day given during RT; DL −1: 37.5 mg per day for 2 weeks before RT, then 37.5 mg per day given during RT. Patients were monitored weekly during treatment for toxicity; premedication was not routinely prescribed. Dosing used a 6 + 6 phase 1 dose escalation design, with the first six patients accrued at DL 0, and subsequent DL's determined according to evaluation of defined dose-limiting toxicities (DLTs) (Supplementary Table 1; Supplementary Figure 1). Toxicity was evaluated weekly during treatment, at 1 month after completing RT, and 3 monthly for 2 years. Acute and available late toxicities were included in consideration of dose escalation. All toxicities were graded according to the Common Toxicity Criteria (CTCAE Version 3.0, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, USA). For cohort B, intrapatient dose modification occurred if grade $\geqslant 3$ toxicity developed, and was temporarily discontinued, then restarted at the lower DL when the toxicity resolved to grade 2 or lower. For patients with persistent DLT's despite dose reduction, sunitinib was discontinued and RT was continued at the discretion of the treating physician.

RT delivery. Preoperative RT consisted of external beam RT at a recommended dose of 50.4 Gy given in 28 fractions, 5 days a week, over 5 weeks and 3 days to the planning target volume (PTV). Variations to the dosing regimen consistent with a curative approach were allowed and were recorded on the CRFs. Gross tumour volume (GTV) was determined by MRI and CT. Clinical target volume (CTV) was defined as GTV plus 4 cm of grossly uninvolved tissue but at risk proximally and distally. The radial margin included the intact fascial boundary. Planning target volume was defined as CTV plus 1 cm. Customised immobilisation devices were utilised routinely. The CT simulation was performed and treatment area delineation was defined on planning CT. A longitudinal strip of skin and subcutaneous tissue of a limb was left untreated whenever possible. Shielding blocks or multileaf collimators were used for field shaping. Planning, dosimetry, and dose prescription followed International Commission on Radiation Units (ICRU) guidelines 50 and 62. All fields were treated 5 days per week and verification port film was performed weekly over the period of RT.

Response assessment and endpoints. Hypoxia on FAZA-PET was defined quantitatively by a tumour-to-background ratio (TBR) of greater than 1.4, and qualitatively by an experienced nuclear medicine physician. Equivocal hypoxia was defined as a TBR of 1.2–1.4. Response to RT was assessed by tumour necrosis on resected tumour specimens, and FDG-PET response where this was unavailable. Progressive disease was defined as an increase in FDG-PET activity after RT or clinical progression.

For Cohort A, the primary objective was to determine the incidence of hypoxia in STS and estimate its correlation this with radiologic and scintigraphic response, and percentage necrosis in the resected tumour following RT. Secondary objectives were to estimate correlation between hypoxia and circulating levels of VEGF family members and to correlate incidence of hypoxia with time to loco-regional failure (TTLF), progression-free survival (PFS) and overall survival (OS). For Cohort B, the primary objective was to determine the maximum dose of sunitinib at which its combination with preoperative RT was safe and tolerable. Secondary objectives were to determine measurable changes in radiologic hypoxia, and circulating VEGF family members, and to make preliminary comparisons with Cohort A.

Statistical methods. Descriptive summaries of variables were provided as means, standard deviations, medians, ranges, and interquartile ranges for continuous variables, and as counts, percentages, and their associated 95% binomial confidence intervals for categorical variables. Associations between ordinal variables and continuous variables (e.g., effect of TBR on radiological response) were assessed using ordinal logistic regression and the Jonckheere–Terpstra test. Associations between one continuous variable and one variable restricted between 0 and 1 (e.g., the association between TBR and proportion necrosis) were assessed using beta regression. Correlations between two continuous variables were assessed using the Spearman rank correlation. Comparisons in continuous variables between two groups were made using the Wilcoxon rank sum test. Time to loco-regional failure, PFS, and OS were analysed using standard survival analysis techniques based on Kaplan–Meier estimates. Time to loco-regional failure was defined as the period between the start of RT and the detection of a local recurrence. Both distant recurrence and death were censoring events. Progression-free survival was defined as the period between the start of RT and the occurrence of a local or distant recurrence or death from any cause, whichever occurred first. Overall survival was defined as the period between the start of RT and death from any cause. Median follow-up was estimated using the reverse Kaplan–Meier method. Cox proportional hazard models identified predictors of TTLR, PFS, and OS with *P*-values based on log-rank or Wald tests when comparing categorical or continuous variables, respectively. All analyses were performed in R (Version 3.0.1).

RESULTS

Study populations. In total, 23 patients in Cohort A and 9 patients in Cohort B were accrued ($n = 32$) between 2005 and 2013. Accrual in cohort B was terminated due to DLTs. Baseline demographics are shown in Table 1. The median tumour necrosis (%) in cohort A and B was 40 (range; 5–100) and 75 (1–95). The median follow-up in Cohort A and B was 5.3 and 3.7 years, respectively.

Hypoxic imaging and biomarkers. In all, 17 out of 23 patients in Cohort A and 7 out of 9 patients in Cohort B underwent baseline FAZA-PET imaging for hypoxia. At baseline across the cohorts, 5 patients demonstrated quantitative evidence of hypoxia, 8 patients had equivocal evidence of hypoxia, and 11 patients appeared normoxic. Although not correlated with tumour size, a correlation was found between hypoxia (documented by FAZA SUVmax) and metabolic activity (FDG SUVmax; correlation coefficient 0.85 (95% CI = 0.67–0.93; $P < 0.001$); Figure 2A. Figure 2B shows a representative image of a tumour with high FDG SUVmax and FAZA SUVmax. Baseline high TBR values were associated with a less favourable radiologic response to preoperative RT ($P = 0.012$),

Table 1. Baseline demographics

Baseline demographics	Cohort A	Cohort B
Number of patients	23	9
Age in years (average, range)	58 (36–81)	56 (33–77)
Sarcoma type (number, %)		
Undifferentiated pleomorphic sarcoma	11 (48)	4 (44)
Liposarcoma, myxoid	4 (17)	1 (11)
Liposarcoma, pleomorphic	2 (9)	2 (22)
Liposarcoma, dedifferentiated	1 (4)	
Fibrosarcoma	1 (4)	
Synovial sarcoma	1 (4)	1 (11)
Ossifying fibromyxoid tumour	1 (4)	
Leiomyosarcoma	1 (4)	
Hemangiopericytoma	1 (4)	
Clear cell		1 (11)
Size in mm (average, range)	87 (19–200)	100 (37–185)
Site (number, percentage)		
Lower limb	22 (96)	6 (67)
Upper limb	1 (4)	1 (11)
Other		2 (22)

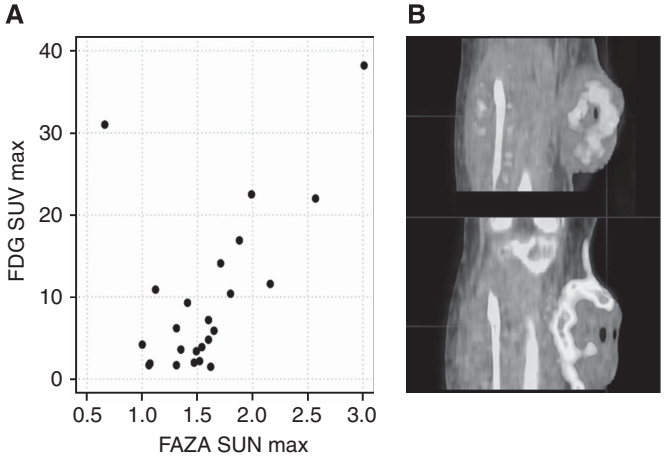

Figure 2. (**A**) Scatterplot of hypoxia against metabolic activity demonstrating a correlation coefficient of 0.85 (95% CI = 0.67–0.93; $P<0.001$); (**B**) Images of FAZA-PET (top panel) and FDG-PET (bottom panel) in a patient with a metabolically active hypoxic tumour.

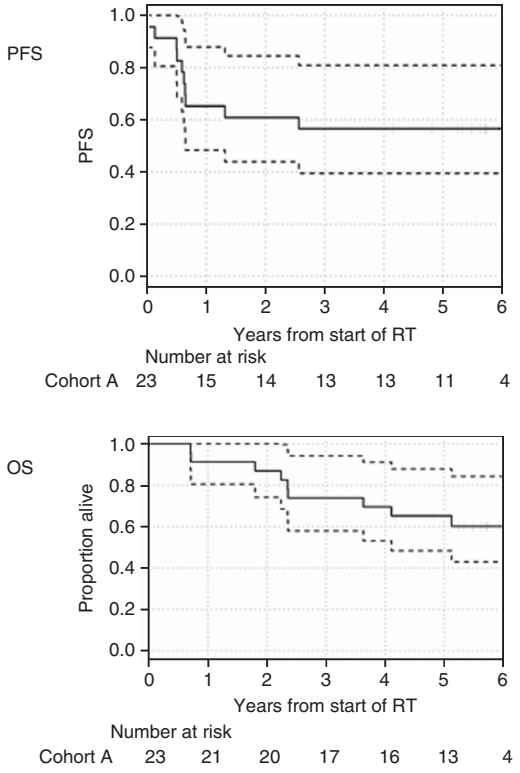

Figure 3. PFS and OS for Cohort A demonstrating a 2-year PFS and OS of 61% (95% CI = 0.44–0.84) and 87% (95% CI = 0.74–1.00), respectively.

Table 2. Predictors for relapse (local or distant) based on patients in Cohort A

Variable	Hazard ratio (per unit increase)	*P*-value (Wald test)
Baseline TBR	8.40 (95% CI: 1.33–53.11)	0.02
Baseline FAZA SUVmax	14.18 (95% CI: 2.1–96.03)	0.007
Baseline FDG SUVmax	1.07 (95% CI: 1.01–1.13)	0.03
Baseline tumour size	1.01 (95% CI: 1.00–1.02)	0.16
Pathological necrosis (%)	1.00 (95% CI: 0.98–1.02)	0.66

Abbreviations: CI = confidence interval; FDG = ^{18}F-fluoro-2D-deoxyglucose; FAZA = ^{18}F-azomycin arabinoside; SUVmax = maximum standardized uptake value; TBR = tumour-to-background ratio.

a trend to association with metabolic response ($P=0.066$), but there was no association with tumour necrosis following RT ($P=0.29$).

We studied the effect of hypoxia on clinical outcomes in Cohort A. Ten patients of 23 have relapsed. The 2-year PFS and OS were 61% (95% CI = 0.44–0.84) and 87% (95% CI = 0.74–1.00), respectively (Figure 3). Relapse was associated with increased baseline TBR, FAZA SUVmax, and FDG SUVmax, but not with initial tumour size or pathological necrosis (Table 2). Baseline quantitative hypoxia was associated with markedly higher risk of local recurrence (Hazard ratio (HR):10.15; 95% CI = 1.34–77; $P=0.02$), and reduced PFS (HR: 8.37; 95% CI = 1.32–53; $P=0.02$), and OS (HR: 41.42; 95% CI = 1.15–1488; $P<0.04$).

Baseline circulating VEGF-C levels (531 ± 82 pg ml^{-1}, mean ± s.e.m.) were higher than either VEGF-A (35 ± 8 pg ml^{-1}) or VEGF-D (131 ± 17 pg ml^{-1}). Quantitative hypoxia correlated with lower baseline levels of VEGF-C and a trend toward lower VEGF-A, whereas VEGF-D levels did not distinguish between patients with hypoxic and non-hypoxic tumours. During RT, VEGF-A levels in patients with hypoxic tumours increased over baseline levels ($P<0.001$). Although not statistically significant, similar results were observed in circulating levels of VEGF-D, but not VEGF-C. Changes in VEGF were not associated with statistically significant differences in TTLF, PFS, or OS.

Combining sunitinib and preoperative RT (Cohort B). Patients ($n=7$) were treated at DL 0 and two patients were treated at DL −1. Dose-limiting toxicities were seen in four patients at level 0 (G4 liver failure, G4 hyponatraemia, G3 hyperglycaemia, G3 rash and hyponatraemia) leading to dose reduction. Despite this, two DLTs were seen at DL −1 (G3 ALT, G3 neutropaenia).

Grade 3/4 toxicities occurred in seven patients (78%). Six G3/4 toxicities (26%) met prespecified criteria for DLT. Of the 24 G3/4 toxicities, 19 were considered attributable to sunitinib (including elevated GGT ($n=4$), AST ($n=1$), ALT ($n=2$), lipase ($n=2$), lymphopaenia ($n=1$), neutropenia ($n=2$), hyponatraemia ($n=1$), and hyperglycaemia ($n=1$)), 2 were potentially attributable to RT and sunitinib (rash, pain), and 2 were considered unrelated (post-operative pain and infection) (Table 3). Late toxicity of any grade

Table 3. Summary of the toxicities observed per patient reported during treatment and post-treatment assessments per DL

	Sunitinib and RT					
	Dose level 0 ($n=7$)			Dose level −1 ($n=2$)		
Toxicity	Total, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)	Total, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)
Alkaline phosphatase	4 (57)	1 (14)	0	0	0	0
ALT	5 (71)	1 (14)	0	2 (100)	1 (50)	0
AST	4 (57)	0	0	2 (100)	1 (50)	0
GGT	5 (71)	3 (43)	1 (14)	2 (100)	1 (50)	0
Bilirubin	4 (57)	1 (14)	0	0	0	0
Hyperglycaemia	1 (14)	1 (14)	0	0	0	0
Haemoglobin	1 (14)	0	0	1 (50)	0	0
Leukocytes	7 (100)	0	0	2 (100)	1 (50)	0
Neutropaenia	5 (71)	1 (14)	0	1 (50)	1 (50)	0
Platelets	4 (57)	0	0	2 (100)	0	0
Lipase	3 (43)	2 (29)	0	0	0	0
Mucositis	4 (57)	0	0	0	0	0
Diarrhoea	2 (29)	0	0	0	0	0
Nausea	5 (71)	0	0	2 (100)	0	0
Vomiting	4 (57)	0	0	0	0	0
Anorexia	2 (29)	0	0	0	0	0
Fatigue	6 (86)	0	0	2 (100)	0	0
Pain (limb extremity)	2 (29)	0	0	1 (50)	1 (50)	0
Skin rash associated with RT	4 (57)	1 (14)	0	0	0	0
Hyponatraemia	1 (14)	0	1 (14)	0	0	0
Hypertension	1 (14)	0	0	1 (50)	0	0

Abbreviations: AE=adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; DL = dose level; GGT = gamma-glutamyl transferase; RT = radiotherapy. Both treatment-related and non-treatment related AEs were included.

occurred in seven patients (78%), predominantly lymphoedema and skin fibrosis. Only two G3 late toxicities were seen (skin infection, fibrosis). Of most concern was the unexpected liver toxicity; following statistical review at both DLs by the Independent Data and Safety Monitoring Committee the study was closed prematurely in June 2013.

Of the nine patients treated with sunitinib, eight showed RECIST stable disease with one partial response. With a median follow-up of 3.7 years, 6 out of 9 patients have relapsed, compared with 10 out of 23 in Cohort A. The 2-year PFS and OS for Cohort B was 44% (95% CI 0.21–0.92) and 56% (95% CI 0.31–1.00). There were no statistically significant differences in PFS (HR 1.92; 95% CI = 0.70–5.30; $P=0.20$) or OS (HR 1.71; 95% CI = 0.51–5.73; $P=0.38$) between the cohorts. However, the time to local failure differed with patients receiving sunitinib being eight times more likely to relapse locally (HR 8.06; 95% CI = 1.54–42.2; $P=0.004$).

Biological effects of preoperative sunitinib. After 2 weeks of exposure to sunitinib, and before starting RT, both VEGF-A levels and VEGF-D levels increased ($P=0.06$ and $P=0.004$, respectively). While on average there was no change in VEGF-C levels after 2 weeks of sunitinib, levels increased in five patients. The peak induction of VEGF-C during RT at week 2 was lower for Cohort B than for Cohort A, while VEGF-D levels at 2 weeks of RT were higher for Cohort B than for Cohort A. The FAZA-PET and the DCE-MRI were assessed at baseline and after 2 weeks of sunitinib exposure. Although not statistically significant, after 2 weeks of sunitinib, 3 out of 5 assessable patients showed an increase in FAZA TBR, suggesting an increase in imageable tumoral hypoxia. Additionally, DCE-MRI showed that after 2 weeks of sunitinib, 5 out of 9 patients showed reductions in K_{trans} and 6 out of 9 showed reductions in iAUC, consistent with decreased tumour perfusion (Table 4).

DISCUSSION

Sarcomas have marginal blood supplies, and frequently demonstrate central necrosis. Hypoxia is reported using Eppendorf needles (Siemann *et al*, 1998) and more recently PET-based hypoxic imaging using ^{18}F-MISO (Rajendran *et al*, 2003). We measured intratumoral hypoxia using the novel hypoxic tracer ^{18}F-AZA. Iodine-123-iodoazomycinarabinoside (^{123}I-IAZA) has been studied in cancer patients (Groshar *et al*, 1993) with good correlation with historical measurements of oxygenation. ^{18}F-AZA has potential advantages over ^{123}I-IAZA because of higher resolution, higher contrast, and better radiation dosimetry, with higher TBR ratios than ^{18}F-MISO (Sorger *et al*, 2003). Higher clearance of ^{18}F-AZA compared with ^{18}F-MISO decreased specific background activity and thereby provide improved lesion contrast for PET (Piert *et al*, 2005). We observed equivocal or clear evidence of hypoxia using FAZA-PET in 54% of tumours, comparable to rates reported previously using ^{18}F-MISO (Rajendran *et al*, 2003).

We found that imageable hypoxia in STS confers worse outcomes, adding to evidence in other malignancies (Brizel *et al*, 1997; Harrison and Blackwell, 2004; Overgaard *et al*, 2005). In STS, expression of hypoxia-inducible factor (HIF)-1α predicts survival (Shintani *et al*, 2006), while an hypoxic microarray signature correlated with metastasis (Francis *et al*, 2007). Studies have linked angiogenesis to the growth and spread of STS (Rajendra *et al*, 2013) and elevated VEGF-A increased metastasis and worsened survival (Rutkowski *et al*, 2002). Hypoxia-inducible factor-1α is a central regulator of cellular responses to hypoxia, and regulates expression of GLUT1 and VEGF-A (Forsythe *et al*, 1996). We also observed a strong relationship between baseline hypoxia and FDG-PET activity, consistent with the known relationship between

Table 4. Changes in TBR, and DCE-MRI after 2 weeks of sunitinib in Cohort B

	TBR baseline	TBR at 2 weeks	Change in TBR	Change in K_{trans} by >20% from baseline to 2 weeks	Change in iAUC by >20% from baseline to 2 weeks
Patient 1	1.3	1.3	Stable	Decrease	Decrease
Patient 2	1.31	1.3	Stable	Decrease	Decrease
Patient 3				Stable	Stable
Patient 4	1.38	9.9	Increase	Increase	Increase
Patient 5				Stable	Decrease
Patient 6	4.91	1	Decrease	Decrease	Decrease
Patient 7	1	1	Stable	Stable	Stable
Patient 8	1.86	2.4	Increase	Decrease	Decrease
Patient 9	1.35	1.5	Increase	Decrease	Decrease

Abbreviations: DCE-MRI = dynamic contrast-enhanced magnetic resonance imaging; iAUC = initial area under the uptake curve; TBR = tumour-to-background ratio.

GLUT1, glycolytic activity, and hypoxia. The predominant circulating VEGF family members observed were VEGF-C and VEGF-D, with lower levels of VEGF-A. Tumoral hypoxia was associated with lower baseline circulating levels of VEGF-C, with a trend towards lower VEGF-A levels. While hypoxia induces VEGF-A expression under many conditions (Ikeda *et al*, 1995), the lower levels of VEGF-A seen here may be due to sequestration of VEGF-A isoforms by cell surface receptors or the extracellular matrix. During RT, circulating levels of VEGF-C and VEGF-D, and particularly VEGF-A, were induced in patients with hypoxic tumours and remained elevated, although these changes were not associated with differences in outcome. Both VEGF-C and VEGF-D mediate lymphangiogenesis and angiogenesis, and are linked to nodal metastasis in many cancers (Wang *et al*, 2011; Stacker *et al*, 2014), while VEGF-A is thought to mediate angiogenesis (Carmeliet, 2005). Notably, STS typically metastasise by the haematogenous route, raising questions as to the role of VEGF-C or VEGF-D-mediated lymphangiogenesis in STS. Both VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3, whereas VEGF-A binds to VEGFR-1 and VEGFR-2 (Ferrara *et al*, 2003; Stacker *et al*, 2004, 2014). Given the limited selectivity of current small molecule tyrosine kinase inhibitors, studies of more selective antagonists are underway.

The addition of sunitinib to neoadjuvant RT was based on the 'vascular normalisation hypothesis': that antiangiogenic agents can 'normalise' tumour vasculature leading to more efficient delivery of oxygen to tumour cells. This could enhance the efficacy of RT and chemotherapy (Jain, 2005). Overexpression of PDGFR in some STS (Yoon *et al*, 2006) further supported the biological rationale for the antitumour activity of sunitinib. In the metastatic STS setting, the PALETTE trial showed that the antiangiogenic agent pazopanib improved PFS (van der Graaf *et al*, 2012), whereas bevacizumab, sorafenib, and sunitinib have shown modest activity in STS. In the neoadjuvant setting, bevacizumab in combination with RT was well tolerated, with no reported wound healing problems, nor evidence of enhanced acute radiotoxicity, and a suggestion of increased effectiveness (Yoon *et al*, 2011). Although there is preclinical data suggesting sunitinib can radiosensitise the cytotoxic effects of RT (Cuneo *et al*, 2008), to our knowledge, only one published trial has examined the combination of sunitinib and RT (Kao *et al*, 2014) using a 4 + 2 schedule of sunitinib at 37.5 mg per day in combination with stereotactic RT for oligometastatic cancers, none of which included STS (Kao *et al*, 2014). The authors found the combination to be acceptable in the advanced disease setting, with 24 grade 3+ adverse events in 46 patients, including only 3 grade 3+ episodes of liver abnormalities (Kao *et al*, 2014). By contrast, in our study of curative disease, 78% of patients experienced G3 toxicities, including 4 of 9 patients with grade 3+ liver abnormalities (mainly raised gamma-glutamyl transferase; one case of acute liver failure from acalculous cholecystitis). Hepatotoxicity is a class effect with TKIs, although a meta-analysis of several thousand patients treated with sunitinib identified grade 3+ hepatotoxicity in fewer than 5% of patients (Shah *et al*, 2013). The increased frequency of liver dysfunction may represent an interaction with concomitant RT. It is not clear why such a high rate of adverse events was observed, but this may relate to the dosing and scheduling differences in the two studies, or to differences in RT administration. On the basis of IDMC review and prespecified endpoints, Cohort B was closed prematurely.

Of concern, time to local treatment failure decreased in sunitinib recipients, albeit without difference in PFS and OS in this small study. In a subset of patients, sunitinib appeared to decrease perfusion and increase hypoxia, contrary to the 'vascular normalisation hypothesis'. Sunitinib increased circulating VEGF-A and VEGF-D, in keeping with previous translational studies (Motzer *et al*, 2007). Data from Cohort A show that tumoral hypoxia is associated with increased relapse and poorer outcomes. While some studies have shown that anti-angiogenic agents improve tumour oxygenation and enhance blood flow, others have shown enhanced hypoxia (Gaustad *et al*, 2012). Since the inception of this study, preclinical studies reported that anti-angiogenic therapies, including sunitinib, may increase metastatic potential (Ebos *et al*, 2009; Paez-Ribes *et al*, 2009). In the limited adjuvant or neoadjuvant anti-angiogenic studies to date, while no increased rate of metastasis has been observed, neither has there been evidence of a survival benefit. Given the small numbers in this study, these findings are cautionary rather than conclusive as the study was not designed to make comparisons between the cohorts and other factors (e.g., patient selection, variation in histological subclass, degree of pathologic necrosis, and resection margins) may have accounted for the differences in local treatment failure seen.

In summary, this study confirms that hypoxia in STS is common, measurable, and clinically important. The addition of sunitinib to preoperative radiation was poorly tolerated, with more frequent locoregional relapse. Whether targeting the angiogenic pathway in STS is clinically useful in the neoadjuvant setting remains unclear, and will require further prospective clinical trials.

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CONFLICT OF INTEREST

JL received travel, accommodations, and expenses from Bayer; Relationship: You. RJH received honoraria from Novartis, Relationship: You; played a consulting or advisory role in Pfizer, Novartis, and Bayer, Relationship: Your Institution; received research funding from Pfizer, Novartis, Roche/Genentech, Amgen, and Bristol-Myers Squibb, Relationship: Your Institution; GCT played a consulting or advisory role in Ipsen, Bayerm, and Pfizer, Relationship: Your Institution; received research funding from Bayer, Bristol-Myers Squibb, Medivation from Company, Relationship: Your Institution. JD played a consulting or advisory role in Merck Serono, Novartis, Bayer, BiPar/sanofi-aventis, Bionomics, Vegenics, and GlaxoSmithKline, Relationship: You; received Research Funding from Novartis, GlaxoSmithKline, and Roche. Relationship: You; PFMC played a consulting or advisory role, in Johnson & Johnson, Relationship: You; received travel, accommodations, expenses from Johnson & Johnson, Relationship: You; SAS had stock or other ownership in Circadian Technologies, Ark Therapeutics, and Sonic healthcare, Relationship: You; played a consulting or advisory role in Circadian Technologies, Relationship: You; received patents, royalties, and other intellectual property from Circadian Technologies and Ark Therapeutics. MGA had stock or other ownership in Circadian Technologies and Ark Therapeutics, Relationship: You; played a consulting or advisory role in Circadian Technologies, Relationship: You; received patents, royalties, and other intellectual property from Circadian Technologies and Ark Therapeutics; DT received research funding from Pfizer, Relationship: Your Institution.

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