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Preoperative therapy with pazopanib in high-risk soft tissue sarcoma: a phase II window-of-opportunity study of the German Interdisciplinary Sarcoma Group (GISG-04/NOPASS)

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ABSTRACT

Introduction: For resectable soft tissue sarcoma (STS), radical surgery, usually combined with radiotherapy, is the mainstay of treatment and the only potentially curative modality. Since surgery is often complicated by large tumor size and extensive tumor vasculature preoperative treatment strategies with the aim of devitalizing the tumor are being explored. One option is the treatment with antiangiogenic drugs. The multikinase inhibitor pazopanib, which possesses pronounced anti-angiogenic effects, has shown activity in metastatic and unresectable STS, but has so far not been tested in the preoperative setting.

Methods and analysis: This open-label, multicentre phase II window-of-opportunity trial assesses pazopanib as preoperative treatment of resectable STS. Participants receive a 21-day course of pazopanib 800 mg daily during wait time for surgery. Major eligibility criteria are resectable, high-risk adult STS of any location, or metachronous solitary STS metastasis for which resection is planned, and adequate organ function and performance status. The trial uses an exact single-stage design. The primary endpoint is metabolic response rate (MRR), i.e. the proportion of patients with >50% reduction of the SUVmean in post-treatment compared to pre-treatment FDG-PET-CT. The MRR below which the treatment is considered ineffective is 0.2. The MRR above which the treatment warrants further exploration is 0.4. With a type I error of 5% and a power of 80%, the sample size is 35 evaluable patients, with 12 or more responders as threshold. Main secondary endpoints are histopathological and MRI response, resectability, toxicity, recurrence-free and overall survival. In a translational sub-study, endothelial progenitor cells and vascular epithelial growth factor receptor are analyzed as potential prognostic and predictive markers.

Ethics and dissemination: Approval by the ethics committee II, University of Heidelberg, Germany (2012-019F-MA), German Federal Institute for Drugs and Medical Devices (61-3910-4038155) and German Federal Institute for Radiation Protection (Z5-22463/2-2012-007).

Registration details: Clinicaltrials.gov: NCT01543802, EudraCT: 2011-003745-18

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STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- One of the first trials to evaluate pazopanib, a multikinase inhibitor with pronounced antiangiogenic properties, in neoadjuvant treatment of soft tissue sarcoma
- As neoadjuvant antiangiogenic treatment of soft tissue sarcoma is not established, the trial uses a window-of-opportunity design to minimize potential risks for patients and yet provide valid information on efficacy and safety
- Multimodal response assessment: dynamic FDG-PET-CT (primary outcome), dedicated MRI protocol, specific protocol for histopathological assessment
- Translational sub-study exploring the role of circulating endothelial progenitor cells and soluble vascular endothelial growth factor receptor as potential biomarkers for antiangiogenic treatment of soft tissue sarcoma

Limitations

- Heterogeneous study population in terms of histological sarcoma sub-type
- Heterogeneous study population in terms of clinical setting (primary tumors and solitary recurrences / metastases)

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INTRODUCTION

Soft tissue sarcomas (STS) constitute a heterogeneous group of malignant mesenchymal tumors with varying histological differentiation. High grade, deeply located, large STS have a poor outcome. For both primary and recurrent tumors, radical surgery, usually combined with postoperative radiotherapy, is the mainstay of treatment and the only potentially curative modality.[1] Likewise, oligometastatic disease with one or few manifestations in one single organ is treated surgically in selected patients with the aim of prolonging survival and reducing symptom burden. Surgery is however often hampered by large tumor size with infiltration of adjacent structures, and extensive vasculature of the tumor.

For primary and recurrent tumors, various preoperative treatment strategies have been tested in clinical trials.[2] Preoperative doxorubicin/ifosfamide has not been shown to yield any benefit in overall or progression-free survival in a randomised trial when compared with surgery alone.[3] The addition of regional hyperthermia to preoperative chemotherapy has improved local progression-free and disease-free survival.[4] Regarding preoperative cytotoxic chemotherapy, additional concerns exist because the administration of drugs with a lifetime dose limit, such as anthracyclines, in a non-metastatic setting might narrow future treatment possibilities in case of recurrence. Preoperative radiation has been shown to have a slight survival benefit compared to postoperative radiation, but the latter is often preferred because of a lower rate of wound complications.[5] Isolated limb perfusion is an option for selected patients but requires a high logistic effort and is limited to referral centres.[2] In conclusion, there is still no consensus if and which preoperative treatment should be applied in patients with STS,[6,7] and further modalities are to be tested in clinical trials.

The ideal preoperative treatment for STS would be fast-acting and effective in terms of devitalisation of the tumor and disruption of its hypervasculature, thus facilitating resection. It would have a low incidence of side effects, thus not hampering surgery and post-surgery recovery. In addition, a valid possibility of early response assessment would be highly desirable, since this could spare non-responders from ineffective treatment and a potentially harmful delay of surgery.

The multikinase inhibitor pazopanib has been approved as treatment for metastatic or non-resectable STS based on the results a phase III trial which compared pazopanib treatment to placebo in patients with metastatic non-adipocytic STS who were angiogenesis inhibitor-naïve and had progressed on at least one prior chemotherapy regimen.[8] Patients in the pazopanib arm had a significantly longer progression free survival (median: 20 versus 7

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weeks; HR=0.31, 95% CI 0.24-0.40), which was the primary endpoint of the trial. There was no significant difference in overall survival (12.5 months with pazopanib versus 10.7 months with placebo; HR=0.86, 95% CI 0.67-1.11), but the trial was not powered for this secondary endpoint. In the heavily pretreated trial population, treatment was sufficiently well tolerated, with a median dose intensity of 96.3% and 14% of toxicity-related treatment interruptions in the pazopanib group. The most frequent adverse events were fatigue, hypertension, anorexia, and diarrhea. The trial was restricted to patients with non-adipocytic STS because of the previous phase II trial which showed no activity in the stratum of adipocytic STS.[9] Subsequent central pathology review, however, re-classified two patients with stable disease (internal communication by the trial sponsor). Based on this finding activity of pazopanib against liposarcoma is probable

Given this proof of efficacy, its fast and pronounced anti-angiogenic effects mediated by kinase inhibition,[10] and its favorable safety profile,[11] pazopanib might be an ideal candidate for neoadjuvant treatment in STS. Therefore, we decided to conduct a pertinent phase II trial as a “window of opportunity” study.[12,13] In this design, patients receive an investigational agent in a “window period” before commencing the established treatment. The risk that effective treatment is unduly delayed is minimised by keeping the duration of the investigational treatment short. The described window-of-opportunity design is feasible for pazopanib, as shown in a trial for non-small cell lung cancer.[14].

A crucial precondition for an effective use of preoperative treatment is good and early response monitoring. Dynamic F-18 labeled Fluorodeoxyglucose Positron Emission Tomography and Computed Tomography (dFDG-PET-CT) appears to be ideal for this purpose. It has been prospectively validated as a strong predictor of histopathological response and progression-free survival both for neoadjuvant and palliative chemotherapy in patients with STS.[15-18] Unlike histopathological response assessment, which can only be performed retrospectively after surgery, it allows for early selection of patients who benefit from preoperative therapy. Magnetic resonance imaging (MRI) is another viable option for early response assessment.[19,20] However, it has not yet been validated as a predictor of progression-free survival. Therefore, up to now, dFDG-PET-CT can be deemed the best available modality for early prospective response assessment of chemotherapy for STS.

Another reason for conducting a neoadjuvant study in STS patients is that it can provide information on biomarkers for response prediction. Ultimately, such markers would allow distinguishing responders from non-responders, thus facilitating true “targeted therapy”. In the context of anti-angiogenic therapy, there are several circulating angiogenic factors which

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might serve this purpose. Endothelial progenitor cells (cEPCs) and soluble vascular epithelial growth factor (sVEGF) correlate with tumor burden in STS,[21] and might thus serve as early predictors of clinical and histological response.

Here, we present the trial protocol. The trial has been initiated in April 2013 and the first patient has been recruited in May 2013. At present, the trial is ongoing.

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METHODS AND ANALYSIS

Study objectives

The primary objective is to evaluate whether neoadjuvant treatment with pazopanib in patients with STS has therapeutic effects, measured as metabolic response. The secondary study objectives are

- To assess the safety of preoperative pazopanib treatment in patients undergoing resection of STS.
- To evaluate potential correlation between metabolic (dPET-CT), radiological (MRI) and histopathological assessment of tumor response to pazopanib treatment in STS.
- To provide exploratory data on blood levels of circulating endothelial progenitor cells (cEPCs) and soluble vascular epithelial growth factor (sVEGF) as potential predictive biomarkers during pazopanib treatment of STS.

Study outline

This is a multi-center (three recruiting centers and one center for PET-CT), single-arm, open-label phase II trial under the auspices of the German Interdisciplinary Sarcoma Group (GISG). Pazopanib is administered for 21 days followed by resection of the tumor after a 7-14 days break (figure 1).

Patient selection

The study population should consist of patients with STS for which efficacy of pazopanib is assumed and with adequate organ function and performance status to tolerate the treatment. This led to the in- and exclusion criteria listed in table 1.

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Table 1: Major in- and exclusion criteria of the trial

Inclusion criteria

- Non-metastatic primary tumor or locoregional recurrence of histologically confirmed high-risk (G2/3, diameter ≥5 cm) soft tissue sarcoma (STS) of any location; or metachronous solitary metastasis of STS for which surgical resection is planned according to the individual choice of the multidisciplinary treatment team (no grade or size restrictions apply for metastasis).
- Resectable and solitary tumor, as assessed by the investigator based on staging exams (CT scan of the chest, CT or MRI of the abdomen, MRI of the limb in case of extremity STS). Measurable disease according to RECIST 1.1
- STS except the following subtypes: embryonal rhabdomyosarcoma, chondrosarcoma, osteosarcoma, Ewing tumor / PNET, Gastrointestinal stromal tumors, dermofibromatosis sarcoma protuberans, inflammatory myofibroblastic sarcoma
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
- Adequate organ system function
- Effective contraception
- Written informed consent
- Age ≥ 18 years

Exclusion criteria

- Prior malignancy other than the STS under study with active disease within the last 5 years
- History or clinical evidence of central nervous system (CNS) metastases
- Prior or concurrent systemic chemotherapy or molecularly targeted therapy or radiotherapy for STS or other malignancies within five years before study entry
- Clinically significant gastrointestinal abnormalities that may increase the risk for gastrointestinal bleeding
- Clinically significant gastrointestinal abnormalities that may affect absorption of pazopanib
- Corrected QT interval (QTc) > 480 ms
- Presence of uncontrolled infection.
- History of severe cardiovascular conditions within the past 6 months:
- Cardiac angioplasty or stenting
- Poorly controlled hypertension [defined as systolic blood pressure (SBP) of ≥140 mmHg or diastolic blood pressure (DBP) of ≥ 90mmHg)
- Cerebrovascular accident including transient ischemic attack (TIA), pulmonary embolism or untreated deep venous thrombosis (DVT) within the past 6 months
- Major surgery or trauma within 28 days prior to first dose of pazopanib
- Evidence of active bleeding or bleeding diathesis
- Known endobronchial lesions and/or lesions infiltrating major pulmonary vessels that increase the risk of pulmonary hemorrhage)
- Any serious and/or unstable pre-existing medical, psychiatric, or other condition that could interfere with subject's safety, provision of informed consent, or compliance to study procedures
- Any ongoing toxicity from prior anti-cancer therapy that is > grade 1 and/or that is progressing in severity, except alopecia

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Treatment plan

All patients are routinely discussed in an institutional multidisciplinary sarcoma board, and a recommendation for the best available treatment and possible inclusion into the trial is jointly determined.

Pazopanib treatment

The treatment consists of the oral administration of 800 mg (two tablets of 400 mg or four tablets of 200 mg) pazopanib once daily. The treatment will be administered for 21 days. Pazopanib should be taken orally without food at least one hour before or two hours after a meal. The tablets should be swallowed whole and must not be crushed or broken. The time of day the tablets are taken should be relatively constant. If a dose is missed, the subject should take the dose as soon as possible, but only if there are 12 or more hours remaining before the next dose is due. If the next dose is due in less than 12 hours, the subject should skip the missed dose and take the next dose as scheduled. If vomiting occurs after taking pazopanib, the subject should not take a replacement dose on that day. The subject should resume taking pazopanib at the next scheduled dose on the following day. If vomiting persists, the subject should be instructed to notify the investigator. Pazopanib will be provided by the manufacturer. It should be stored at room temperature up to 25^{o}C. When stored at these temperatures and in unopened bottles, pazopanib tablets will remain stable until the expiration date indicated on the bottle label. For detailed information on the administration of pazopanib refer to the Investigator's Brochure for pazopanib.[22]

Given the short duration of 21 days of planned treatment, in case of drug toxicity only two dose reductions are permitted in a stepwise fashion: initially to 600 mg once daily, and subsequently to 400 mg once daily if necessary. Given the short treatment period, no dose re-escalation is foreseen. Recommendations for investigational product dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the online supplementary material.

In each individual patient, pazopanib treatment should be discontinued if one of the following events occurs:

- The patient withdraws consent to the study.
- The investigator is of the opinion that continuation of treatment would jeopardize the health status of the patient.
- A Serious Adverse Drug Reaction (SADR).

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- A specific AE requiring treatment discontinuation as defined in the online supplementary material.
- In female subjects: pregnancy.
- Severe non-compliance of the subject which jeopardizes validity of the data in a relevant way.

Surgery

Surgery should be performed 7-14 days after the end of the study treatment. It is not part of the study protocol and is performed according to the discretion of the treating surgeon. Although not mandatory, for reasons of quality assurance regarding pathological assessment, it is strongly recommended that surgery is performed at one of the study centres.

Postoperative treatment / radiotherapy

A recommendation for possible postoperative treatment is again jointly determined in an institutional multidisciplinary sarcoma board. If adjuvant radiotherapy is carried out, it is not part of the study protocol and can be performed according to the choice of the treating physician. However, for subjects participating in the translational study it should not start before blood samples to determine postoperative cEPC / sVEGF levels were taken (14 days after surgery).

Evaluation, laboratory tests, follow-up

A signed, written informed consent form must be obtained from the subject prior to any study-specific procedures or assessments. Procedures conducted as part of the subject's routine clinical management and obtained prior to signing of informed consent may be utilized for screening or baseline purposes provided these procedures are conducted as specified in the protocol. The study assessments schedules and visit windows are summarized in Table 2. A certified laboratory is to conduct all clinical laboratory assessments. Assessment at laboratories other than the site laboratory (e.g. at a laboratory collaborating with the patient's general practitioner) is possible. Laboratory assessments should be performed as indicated in table 3. All laboratory tests with values that become abnormal and clinically significant while the subject is participating in the study or within 28 days after the last dose of study drug should be repeated until the values return to normal or baseline.

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Table 2: Time and Events Table

Required measures	Screening (≤14 days before study entry)	d7	d14	d22-28	≥d29	3 monthly until end of follow-up (36 months/ recurrence/ death)
Informed Consent	♦					
History, ECOG PS, toxicity / compliance assessment	♦	♦	♦	♦		♦
Physical assessment incl. pulse, blood pressure, weight, cancer symptoms	♦	♦[a]	♦	♦		♦
Complete blood count, sodium, potassium, creatinine, bilirubin, alkaline phosphatase, AST, ALT, glucose, albumin, PT/INR, PTT, fT4, TSH	♦		♦[b]	♦		
UPC	♦					
12-lead ECG	♦					
Pregnancy test	♦					
Chest CT	♦					♦
MRI of tumor region (upon screening and on d22-28 according to specific protocol; during follow-up according to local clinical practice; see section **Error! Reference source not found.**)	♦[c]			♦[c]		♦
FDG-dPET-CT of tumor region (according to protocol; see section **Error! Reference source not found.**)	♦			♦		
Surgery (not part of study protocol)					♦	
cEPC / sVEGF levels	♦			♦	♦ (14 days after surgery)	

[a]Monitoring of BP: A measurement of BP should be taken at day 7+/- 3 days. BP can be assessed by any method (i.e., at home or by another physician) as long as the study physician is informed of the measurement, verifies any measurement that is not normal and takes appropriate action.
[b]Only LFTs
[c] optional according to local availability

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Table 3: Clinical Laboratory Assessments

Clinical Chemistry	
Renal function	Urea, Creatinine[a]
Liver function test (LFT) Panel	Albumin, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-GT and Bilirubin (total)[b]
Electrolytes and others	Calcium, Potassium, Sodium, Magnesium, Inorganic phosphate, Glucose, and Lactate Dehydrogenase (LDH)
Hematology	Hematocrit, Hemoglobin, White Blood Cell Count, Red Blood Cell Count, Neutrophils, and Platelets
Coagulation Tests	Activated partial thromboplastin (aPTT) and International Normalization Ratio (INR)[c]
Urinalysis for Proteinuria	UPC[d]
Thyroid Function Test	TSH[e]

a) Estimated creatinine clearance should be calculated using the Cockroft and Gault method (appendix D). Alternatively, creatinine clearance can be measured directly by 24-hour urine collection.
b) A direct bilirubin level should be obtained if the total bilirubin level is greater than 1.5 X upper limit of normal (ULN). See Section 5 for stopping criteria and dose modification guidelines for treatment-emergent liver function abnormality.
c) Coagulation tests may also be performed in response to an AE/SAE of bleeding and as clinically indicated.
d) UPC should be evaluated as described in appendix E or by 24-hour urine protein. If UPC ≥ 3 or if urine protein is ≥3g, then the dose modification table guidelines should be followed (Section 5).
e) Unscheduled thyroid function tests [TSH and thyroxine (free T_4)] should be performed if clinically indicated (e.g., if a subject develops signs and symptoms suggestive of hypothyroidism).

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Study evaluation

FDG-dPET-CT

At the time points day -14 to 0 and day 22-28, patients will undergo dPET-CT with FDG over the tumor area followed by a baseline whole-body PET-CT. All exams will be performed centrally at the same PET-CT-scanner. A topogram and a low dose CT are used for the positioning of the patient to include the primary volume of interest for the dPET-CT. Following the intravenous application of FDG, a dynamic data acquisition (4D mode) is performed for 60 minutes. Then a whole body PET-CT is acquired using 2 minutes per bed position. The iteratively reconstructed images are evaluated using dedicated software. Besides the SUV (standardized uptake values) further parameters of the tracer kinetics are assessed using compartment and non-compartment models. The SUV_{mean} 60 minutes after the FDG injection is used to assess the therapeutic effect. Exploratory analyses of dynamic PET-CT response will be based on a Volume of Interest (VOI) analysis. The following parameters of the FDG kinetics will be calculated: 1. SUV (mean, max) 55-60 min post injection; 2. Influx rates and transport rates based on a support vector machine algorithm and a two-tissue compartment analysis; and 3. Calculation of the fractal dimension of the FDG kinetics based on fractal analysis. The percentage change of all parameters of the FDG kinetics as well as discriminant analysis based on the absolute values of the FDG kinetics and their changes will be determined along with confidence intervals. In particular, the SUV_{mean} will be used primarily for the assessment of response. Metabolic response is assumed with a change of 50 % following pazopanib treatment (21 days). The dynamic parameters are used as secondary parameters for the assessment of treatment.

MRI

MRIs upon study entry and on day 22-28 are optional according to local availability and should be performed as follows: A 1.5T scanner with phased-array coil will be used. T1W (2D or 3D) pre- and post-contrast sequences with fat suppression will be undertaken. After localizer sequences (T2 weighted HASTE sequences), T1 weighted turbo Spin Echo (TSE) sequences with and T2 weighted turbo Spin Echo (TSE) without fat suppression pre-contrast, followed by an EPI DWI sequence with four b-values (0, 50, 400, 800) and quantification of ADC maps will be performed. Afterwards, T1 weighted fat-saturated gradient echo sequences in breathhold technique before and 30, 60, and 90 sec after contrast media application (0,1 mmol/kg bw Gadolinium chelates), covering at least 20 cm of the tumor size, will be done. The matrix size should be at least 256, and the slice thickness should be 3 mm or less.

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All quantitative analysis of dynamic and diffusion exams as well as evaluation according to the RECIST and modified Choi criteria will be performed by an experienced board certified radiologist at the study centre Mannheim. Data material will be provided for all included patients on CD or be electronic data export.

Evaluation of changes of tumor size during therapy will be performed in accordance with RECIST criteria v1.1 [23]). *Complete response (CR)* is defined as the disappearance of all lesions. *Partial response (PR)* is defined as a decrease of at least 30 % of the longest diameter of the tumor, taking as reference the baseline longest diameter; *progressive disease (PD)* is defined as an increase of at least 20 % in the longest diameter of the tumor, taking as reference the baseline longest diameter, or the appearance of one or more new lesions; *stable disease (SD)* is defined as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the baseline longest diameter. For evaluation during therapy, partial response, stable disease and progressive disease will also be defined according to Choi criteria modified for MRI.[19] Here, *CR* is defined as the disappearance of all lesions. *PR* is defined as ≥10% decrease in the greatest maximal diameter or a ≥15% decrease in contrast enhancement; *PD* is defined as a ≥10% increase in the greatest maximal diameter while the criteria for PR by using contrast enhancement are not met, or a ≥15% increase in contrast enhancement while criteria for partial response by using tumor size are not met, or the occurrence of one or more new lesions; *SD* is defined as all cases who do not meet criteria for CR, PR, or PD.

If technically feasible at the local MRI unit, 2D and 3D measurements of the tumor will be performed. The apparent diffusion coefficient (ADC) values of two reproducible tumor localizations as well as the plasma flow (PF) and mean transit time (MTT) of the tumor at the same localizations evaluated by FDA approved Tissue 4D software or another adequate perfusion quantification tool will be documented and compared between measurements. MRI response will be quantified as the change in percent between pre- and post-therapeutic apparent diffusion coefficient (ADC).[20]

During the follow-up period, all MRIs can be performed according to local clinical practice without adhering to a specific protocol.

Chest CT

For screening, all patients require a multi-slice chest CT with a slice thickness ≤5mm (reconstruction interval) covering the whole area from the lung apices to the diaphragm. The

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use of intravenous contrast medium is recommended unless contraindicated. The images will be assessed by the responsible radiologist, according to local standards.

During the follow-up period (after surgery), all chest CTs can be performed according to local clinical practice without adhering to a specific protocol.

Histopathological procedures

The histopathological report should include the following information:

- Tumor size in three dimensions
- Resection status (free margins, margins microscopically infiltrated, margins macroscopically infiltrated)
- Smallest distance between resection margin and vital tumor tissue found in the specimen, together with the localisation where it was found
- Histological subtype
- Grading according to the FNCLCC system (G1-3) [24]
- Overall regression grading after slicing of the specimen (in percent, semi-quantitative value)
- Most prevalent type of regression: hyalinous necrosis, apoptosis, scar tissue, hemorrhagic necrosis

Evaluation of efficacy

Metabolic response and metabolic response rate (MRR)

Metabolic response is defined as the achievement of an at least 50% reduction of the mean standardized uptake value (SUV_{mean}) over the tumor area in the post-treatment compared to the pre-treatment FDG-dPET-CT. The *metabolic response rate (MRR)* is defined as the proportion of patients achieving a metabolic response.

Other outcome measures

dPET-CT response is defined as the change of FDG influx as well as of transport rates k1-k4 and distribution volume VB and fractal dimension. Absolute values of all parameters of FDG kinetics will also be used for discriminant analysis evaluation.

Resection status is determined by the assessing pathologist in the respective report.

Recurrence-free survival is defined as the time from resection to the date of diagnosis of recurrence or date of death or the last day when the patient was known to be disease-free or alive in the case of loss to follow-up (censoring). The date of diagnosis of recurrence is defined as the first day when the below mentioned criteria for recurrence are met. In case of

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a diagnosis by imaging, the day when the imaging procedure was performed is recorded as the date of recurrence. In case of a clinical diagnosis, the date of the visit is recorded.

Local recurrence-free survival is defined as survival free from local recurrence, which in this context is defined as a newly occurred lesion within a range of 10 cm of the margin of the original tumor. The assessment if a recurrence is considered local or distant should be made by the investigator at the study centre based on the physical examination and the patient's file. Patients with R2 resection are automatically assigned a local recurrence-free survival of zero.

Distant recurrence-free survival is defined as survival free from any recurrence not fulfilling the criteria of local recurrence.

For both local and distant recurrence, prior death will be counted as a competing interest and not included. The date of diagnosis of local / distant recurrence is defined as the first day when the criteria for recurrence are met. In case of a diagnosis by imaging, the day when the imaging procedure was performed is recorded as the date of recurrence. In case of a clinical diagnosis, the date of the visit is recorded

Overall survival is computed from the date of resection to the date of death (whatever the cause). Patients not known to be dead at the time of the analysis will be censored at the date of last follow-up.

Toxicity: All adverse events will be recorded according to CTCAE, version 4.0. (http://evs.nci.nih.gov/ftp1/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf).

Delay in planned time to resection: Any occurring delay between the actual date of resection and the planned date of resection will be quantified by the treating surgeon and categorized into "treatment-related" and "non treatment-related".

Study design, sample size and analysis plan

Study design and sample size

The trial is designed as a non-comparative single-arm phase II trial. Its primary end-point is the metabolic response rate (MRR), defined as the proportion of patients achieving a 50% reduction of the mean standardized uptake value (SUVmean) in the post-treatment compared to the pre-treatment FDG-dPET-CT. The trial uses an exact single-stage design based on the exact binomial distribution.[25] The MRR below which the treatment is considered ineffective is set at 0.2 (H0: MRR≤0.2). The MRR above which the treatment warrants further exploration in a subsequent phase III trial is set at 0.4 (H1: MRR≥0.4). This magnitude of response can be expected after preoperative therapy with a supposedly active agent for STS, as shown in several non-controlled trials.[15,26-28] With a predefined significance level of 5% and a power of 80%, the sample size is calculated to be 35

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evaluable patients with an actual significance level of 0.034 and an actual power of 0.805 with a critical value of 12 patients at the upper proportion limit (STPLAN, Version 4.5, 2010, The University of Texas, Houston, USA). If after treatment of 35 patients, the primary endpoint MRR cannot be assessed for one or more patients, e.g. for technical or medical reasons, up to 3 additional patients can be enrolled.

Analysis plan

The final analysis of the primary endpoint MRR will be carried out after all patients have received their post-treatment FDG-PET-CT. All patients registered in the study will be included (intention-to-treat analysis). The number of patients who are not evaluable, who died or who withdrew before treatment began or during treatment will be specified. According to the exact single-stage design based on the exact binomial distribution, H_0 will be rejected if 12 or more patients show metabolic response.[25] If at the end of treatment of all enrolled patients, the number of individuals who can be evaluated for metabolic response is below 35, the border for acceptance of H_1 will be modified accordingly with a type I error not exceeding 5%. The MRR will be presented as a percentage with a 95% confidence interval. In addition, metabolic response will be visualised as waterfall plot with the change in percent of SUV_{mean} on the y-axis.

At the time of analysis of the primary endpoint, the secondary endpoints MRI response, dynamic PET-CT response, histopathological response, R0-resectability and toxicity will also be analyzed. MRI response will be quantified as the change in percent between pre- and post-therapeutic apparent diffusion coefficient (ADC)[20] as well as the rates of complete response, partial response, stable disease, and progressive disease according to Choi criteria modified for MRI and RECIST 1.1, all with 95% confidence intervals. A potential correlation between MRI and metabolic response will be evaluated by calculating Pearson's correlation coefficient for SUV_{mean} change in percent between the pre- and post-therapeutic PET-CT and the ADC change in percent between the pre- and post-therapeutic MRI. For the categorical variables "response according to modified Choi and RECIST 1.1 criteria", parameters of diagnostic accuracy (sensitivity, specificity, PPV, NPV) for complete or partial response will be calculated relating to the primary outcome metabolic response as "gold standard".

Exploratory analyses of dPET-CT response will be based on a Volume of Interest (VOI) analysis. The following parameters of the FDG kinetics will be calculated: (1) SUV (mean, max) 55-60 min post infusion; (2) Influx rates and transport rates based on a support vector machine algorithm and a two-tissue compartment analysis, and (3) Calculation of the fractal dimension of the FDG kinetics based on fractal analysis. Percentage change of all described parameters of the FDG kinetics as well as discriminant analysis based on the absolute

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values of the FDG kinetics and their changes will be presented along with confidence intervals.

R0-resectability will be presented as proportion with corresponding 95% confidence interval. Histopathological response will be displayed as the rate of responders with corresponding 95% confidence interval and range as well as a waterfall plot with the percentage of viable tumor tissue on the y-axis. Parameters of diagnostic accuracy for histopathological response will be calculated relating to the primary outcome metabolic response as “gold standard”. The incidence of adverse events (AEs) will be presented for the safety population, in a descriptive analysis. Delay in planned time to resection due to the treatment will be reported in a descriptive way with appropriate summary measures. The analysis of the secondary endpoints overall survival, recurrence-free survival, local and distant recurrence-free survival will be performed when data are mature. Survival curves will be estimated with the Kaplan-Meier method and displayed graphically.

A first exploratory analysis of biomarker data (see below), i.e. cEPC and sVEGF blood levels, will be carried out at the time of analysis of the primary endpoint. This will assess if the mentioned biomarkers have a predictive value for metabolic response. For this purpose, pre-treatment levels of cEPC and sVEGF will be plotted against the change in percent of SUV_{mean} in order to display a possible predictive relationship. If possible, linear regression will be performed. Likewise, a potential predictive value of cEPC and sVEGF level changes during treatment and metabolic response will be explored by plotting the change in percent of post-treatment levels (d22-28) compared to pre-treatment levels against the change in percent of SUV_{mean}. If possible, linear regression will be performed. cEPC and sVEGF levels measured at d14 post-surgery will be compared to levels measured after completion of pazopanib treatment (d22-28). The difference will be presented in a descriptive fashion (mean, median, standard deviation), related to resection status in an explorative way, and, if meaningful and feasible, formally compared with appropriate parametric or non-parametric tests between patients who received complete and incomplete resection. At the time of analysis of recurrence-free survival, prognostic properties of cEPC and sVEGF levels will be explored. Biomarker levels will be treated as continuous variables. If possible in a meaningful way, recurrence-free survival of patients in the different quartiles of pre-treatment and postoperative cEPC and sVEGF levels will be compared by means of Kaplan-Meier curves. In addition, pre-treatment and postoperative levels of recurred and recurrence-free patients will be compared with an appropriate parametric test.

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Translational research

There is an increasing amount of evidence suggesting that cEPCs, in conjunction with soluble cytokines such as sVEGF, are involved in neoangiogenic processes of solid tumors.[29] Studies suggest that the recruitment of cEPC, which is probably mediated through cytokines, is an expression of tumor-induced neoangiogenesis in situations of tumor growth. cEPCs have been shown to contribute to vasculogenesis and neoangiogenesis in several tumour entities.[30,31] On the other hand side, tumor response to chemotherapy or kinase inhibition seems also to be related with a rise in cEPC and sVEGF levels.[29,32]

Based on these preliminary findings, it seems probable that blood levels of cEPCs and sVEGF have a certain prognostic and/or predictive value for the course of disease and treatment of STS. We were able to show a positive association between clinical tumor load and cEPCs level in the blood of patients with lung cancer[33] and STS (unpublished data). Consequently, pre-treatment cEPC levels might reflect tumor load and metabolism and thus constitute a prognostic marker. The same might hold true for postoperative cEPC levels, which could be an indicator of residual microscopic tumor load and thus of recurrence risk. On the other hand side, high cEPC pre-treatment levels, indicating an extensive tumor vasculature and thus a high susceptibility to an anti-angiogenic treatment, might be a predictive marker for response. The same might be true for a drop in cEPC level during treatment. Similar associations have been observed between sVEGF levels and treatment response.

In order to explore these hypotheses, the study foresees measurement of cEPC and cVEGF levels at various time points. Patients will be eligible for the translational research project if they are eligible for the clinical trial and have given written informed consent to participate in this project. Patients will have the possibility to accept or refuse participation in the translational research project, or to accept participation in only a part of the project, without affecting their participation in the clinical study.

During the specified visits, 20 ml of full blood will be drawn by insertion of a 20-gauge cannula in a peripheral vein and collected in tubes containing sodium citrate (0.105 M) as anticoagulant. In addition, 5 ml of serum will be collected through the same cannula and stored in an appropriate tube. Full blood samples will be processed within 1 hour after collection.

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PBMCs will be prepared by gradient centrifugation using Ficoll-Hypaque (Amersham Biosciences, Freiburg, Germany). The expression of cell-surface antigens will be determined by four-color immunofluorescence staining. Of each sample, 100µl of PBMC (containing 1 x 106 cells) will be incubated with 10 µl of FcR-blocking reagent (Miltenyi Biotec, Bergisch-Gladbach, Germany) for 10 minutes to inhibit nonspecific bindings. Hereafter the cells will be incubated at 4°C for 30 min with 10µl PE-conjugated anti-human CD133 mAb (Miltenyi Biotec, Bergisch-Gladbach, Germany), 10 µl PerCP-conjugated anti-human CD34 mAb (BD Biosciences, Heidelberg, Germany), 10µl APC-conjugated VEGF R2 mAb (R&D Systems, Wiesbaden-Nordenstadt, Germany) and 10µl FITC-conjugated Annexin V mAb (BD Biosciences, Heidelberg, Germany). PE-, PerCP, APC and FITC conjugated isotype-matched immunoglobulin (Ig)-G1 and IgG2a antibodies (DakoCytomation, Hamburg Germany) will be used for each patient and measurement as negative controls. The cells will be washed three times to remove unbound antibodies and finally re-suspended in 400 µl of FACS solution (BD Biosciences, Heidelberg, Germany). FACS-analysis will be performed on a FACSCalibur flowcytometer (BD Biosciences, Heidelberg, Germany) and the data will be analysed using WinMDI 2.8 software. A minimum of 500,000 events is to be collected. FACS analysis of each probe will be performed in triplicate. The frequency of cEPCs in peripheral blood is determined by a two-dimensional side-scatter / fluorescence dot-plot analysis of the samples, after exclusion of Annexin V-positive cells and appropriate gating. The exclusion of Annexin V-positive cells is performed to rule out contamination with apoptotic cells. cEPC counts will be expressed as percentage of total PBMC in each patient.

sVEGF serum concentrations will be assessed using an enzyme-linked immunosorbent assay kit (R&D Systems, Wiesbaden-Nordenstadt, Germany) in triplicate samples obtained from 5 ml serum. ELISA will be performed according to the manufacturer's instructions. The cVEGF concentration will be measured in pg/ml. Further details will be described in a laboratory manual.

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ETHICS AND DISSEMINATION

After due consideration, all participating investigators are convinced that the trial has a positive risk-benefit ratio. Based on results from a phase III trial, which showed efficacy for pazopanib treatment in STS,[8] patients who are treated according to this protocol can be expected to have a higher chance of complete tumor resection and thus a lower risk of tumor recurrence, which should positively affect progression-free and overall survival. Moreover, the preoperative treatment has the potential to result in a less radical, tissue-sparing resection with fewer side effects. In contrast, the expected magnitude of potential risks and harmful side effects of the study treatment is small. Pazopanib is a registered drug. Overall, it is well tolerated and most side effects are manageable and quickly subside once the dosage is reduced or the therapy discontinued. Therefore, the risk that surgery is delayed or perioperative morbidity is increased due to pazopanib side effects is expected to be small. Given the short preoperative treatment period in the window-of-opportunity design and the demonstrated efficacy of pazopanib in STS, the risk of relevant tumor progression during treatment is judged to be very low.

Study-related diagnostic procedures performed in addition to standard of care include two dPET-CTs. These expose the patient to radiation. However, the overall radiation dose of these two procedures does not exceed the annual dose threshold stipulated by relevant German laws for individuals who are professionally exposed to radiation. Thus, the risk of radiation-induced secondary malignancy is judged to be very low and outweighed by the scientific benefit of the study as well as the individual clinical benefit of each participating subject. dPET-CT, although not yet part of standard clinical algorithms for primary STS, does yield relevant additional information. It can detect occult metastases leading to a change in treatment strategy. Moreover, the planning of resection is facilitated for the surgeon, as PET-CT provides additional information on tumor extension and depicts the metabolism in different tumor areas, which potentially changes the surgical approach.

Before the start of the study, patients are informed in writing and verbally about the nature and implications of the proposed study, and especially about the possible benefits for their health and any risks. Patients document their consent by signing the informed consent form. Medical confidentiality and the provisions of the German Federal Data Protection Act are complied with. Moreover, the German Medicines Act (AMG) and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP) regulations are complied with. The principal investigator can decide to discontinue the entire study if he

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concludes that continuation would pose a relevant threat to the health or wellbeing of the individual subjects, or if he concludes that the risk-benefit-ratio of the study is unfavorable, i.e. if the risks clearly outweigh the potential benefits.

The trial was approved by the ethics committee II of the University of Heidelberg, Germany (Reference number 2012-019F-MA). Furthermore, it was approved by the German Federal Institute for Drugs and Medical Devices (Reference number 61-3910-4038155) and the German Federal Institute for Radiation Protection (Z5-22463/2-2012-007). Prior to initiation, the trial has been registered at clinicaltrials.gov (NCT01543802) and the European Clinical Trials database (EudraCT: 2011-003745-18). The University of Heidelberg is the legal sponsor of this trial.

The originals of all central study documents are archived at the principal study site for at least 10 years after preparation of the final report. The Principal Investigator retains the generated administrative documents (correspondence with Ethics Committee, supervisory authority etc.), patient identification list, signed informed consent forms and copies of the general study documentation (protocol, amendments) for the time period stated above. Original data of study patients (medical source records) are to be retained for the applicable archiving period of the study centre but for not less than 15 years, starting from study completion.

It is aimed to publish results from this study in the form of one or several manuscripts in international medical journals. The Principal Investigator will review all manuscripts to prevent forfeiture of patent rights to data not in the public domain. Publication of the first manuscript reporting study results is planned to take place as soon as possible after analysis of the primary endpoint.

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DISCUSSION

We present the protocol of a trial which uses pazopanib for preoperative treatment of STS in a window-of-opportunity study. While pazopanib has shown efficacy in metastatic and irresectable STS[8] and therefore been approved for this indication, the drug has not yet been extensively evaluated in the preoperative setting in resectable STS. Recently, results of a study on preoperative pazopanib combined with radiation therapy were published.[34] It assessed the safety of this combination but was a phase I trial and thus not designed to provide sufficient information on efficacy. An ongoing pilot trial (NCT01446809) evaluates pazopanib induction therapy prior to preoperative chemotherapy. Results are awaited in 2016. Another ongoing phase II/III trial (NCT02180867) compares the combination of pazopanib and chemoradiotherapy or radiotherapy with chemoradiotherapy or radiotherapy without pazopanib. Results are awaited in 2018.

Our trial is unique in testing preoperative pazopanib monotherapy in a window-of-opportunity design. The relatively short duration of pazopanib therapy was chosen to minimize the risk of tumor progression and consequent irresectability in non-responders. Notwithstanding, given the drug's mechanism of action, 21 days of treatment are considered sufficient to detect efficacy by assessing dPET-CT response, which is the trial's primary outcome.[35] The wide range of secondary outcomes allows validating other potential parameters for response assessment. In particular, the trial offers the unique possibility to prospectively compare different modalities of response assessment, i.e. MRI and histopathological features with dFDG-PET-CT, which is considered gold standard.

Another important element of the trial is its translational sub-study. The trial design offers the opportunity to measure two potential biomarkers, cEPCs and sVEGF, during antiangiogenic treatment and in the perioperative setting, and to correlate them with several parameters of treatment response.

In summary, this trial will provide initial evidence regarding preoperative pazopanib treatment of STS, and, if efficacy can be demonstrated, lead to a pertinent phase III trial. Moreover, cEPCs and sVEGF are further explored regarding their use as biomarkers for antiangiogenic treatment of STS.

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AUTHORS' CONTRIBUTIONS

UR and PH conceived of and planned the trial.

UR drafted the trial protocol and edited its final version.

ADS wrote the parts of the protocol relating to dPET-CT.

JJ, MS, and PH provided advice on the parts of the protocol relating to surgical treatment.

BK, GE, SF, and HGD provided advice on the parts of the protocol relating to medical treatment.

KN wrote the parts of the protocol relating to the translational study.

LRP did the final trial design, devised the analysis plan and wrote the corresponding parts of the trial protocol.

UA provided advice on the parts of the protocol relating to MRI diagnostics.

TG provided advice on the parts of the protocol relating to pathology.

All authors read and approved the final version of the protocol and this manuscript.

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COMPETING INTERESTS STATEMENT

UR, BK, JJ, and PH have received travel reimbursements from GlaxoSmithKline Oncology / Novartis.

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Figure 1: Study flowchart. d: day. PET-CT: Positron Emission Tomography and Computed Tomography. MRI: magnetic resonance imaging.

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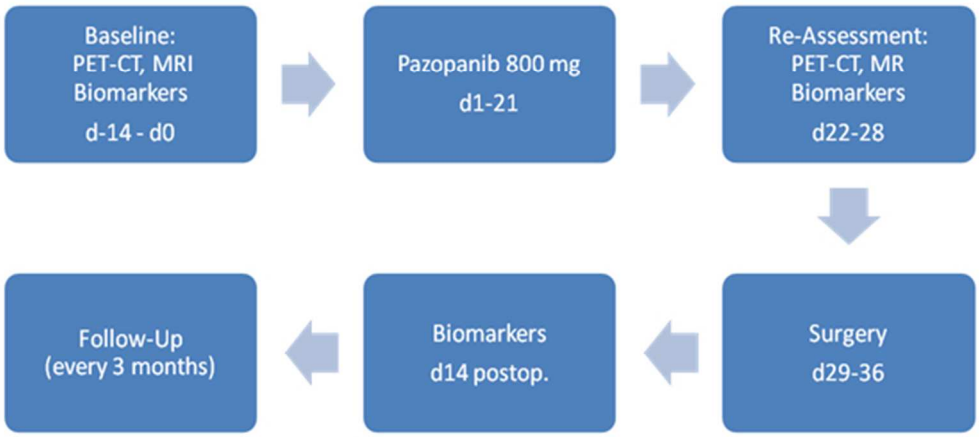

Study flowchart. d: day. PET-CT: Positron Emission Tomography and Computed Tomography. MRI: magnetic resonance imaging.
169x79mm (96 x 96 DPI)

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Online supplementary material: Recommendations for investigational product dose interruptions/modifications

AE Terms & Descriptions	Dose Modification Algorithms
Hypertension	
(A). Asymptomatic and persistent SBP of ≥140 and <170 mmHg, or DBP ≥90 and <110 mmHg, or a clinically significant increase in DBP of 20 mmHg (but still below 110 mmHg).	Step 1. Continue pazopanib at the current dose. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled[a] blood pressure (BP). If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B).
(B). Asymptomatic SBP ≥170 mmHg, or DBP ≥110 mmHg, or failure to achieve well-controlled BP within 2 weeks in scenario (A).	Step 1.Consider reducing or interrupting pazopanib, as clinically indicated. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled BP. Step 4. Once BP is well-controlled, restart pazopanib dose-reduced by 200 mg if pazopanib was interrupted.
(C). Symptomatic hypertension or recurring SBP ≥170 mmHg, or DBP ≥110 mmHg, despite modification of antihypertensive medication(s)	Step 1. Interrupt pazopanib. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled BP. Referral to a specialist for further evaluation and follow-up is also recommended. Step 4. Once BP is well-controlled, restart pazopanib dose-reduced by 200 mg.
(D). Refractory hypertension unresponsive to above interventions.	Discontinue pazopanib and continue follow-up per protocol.
Prolongation of QTc Interval: If the QTc is prolonged, the ECG should be manually read to ensure accuracy of the reading. The values below refer to manually-read ECGs (see section **Error! Reference source not found.**).	
QTc ≥ 480 < 500 msec	Continue pazopanib; monitor as clinically indicated.
QTc ≥500 msec	Discontinue pazopanib and continue follow-up per protocol.
Proteinuria	
UPC <3	Continue pazopanib at the current dose; monitor as clinically indicated.
UPC ≥3 or 24-h urine protein ≥3g	Step 1. Interrupt pazopanib. Step 2. Weekly UPC or 24-hr urine protein monitoring until UPC is <3 or 24-hr urine protein is <3 grams. Then restart pazopanib dose-reduced by 200 mg. Step 3. If UPC ≥3 or 24-h urine protein ≥3g recurs, repeat steps 1 and 2. Step 4. If UPC ≥3 or 24-hr urine protein ≥3 recurs and the pazopanib dose can no longer be reduced, discontinue pazopanib and continue follow-up per protocol.
Hemorrhage /Bleeding: Investigate and document underlying etiology of the bleeding	

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AE Terms & Descriptions	Dose Modification Algorithms
Grade 1	For hemoptysis, interrupt pazopanib and contact the GSK Study Physician to discuss whether further treatment with pazopanib is appropriate. For other Grade I hemorrhage/bleeding events, continue pazopanib at the current dose; monitor as clinically indicated.
Grade 2	Step 1. If pulmonary or GI bleed (other than hemorrhoidal bleeding), discontinue pazopanib and continue follow-up per protocol. Otherwise, interrupt pazopanib until the AE resolved to ≤ Grade 1. Step 2. Restart pazopanib; consider reducing dose and monitor as clinically indicated.
Grade 3 or 4, or Recurrent ≥ Grade 2 event after dose interruption/reduction.	Discontinue pazopanib and continue with follow-up per protocol.
Venous Thrombosis (DVT, PE)	
Grade 2	Continue pazopanib at the current dose; monitor as clinically indicated
Grade 3	Step 1. Interrupt pazopanib. Step 2. Initiate and monitor anticoagulation as clinically indicated. Step 3. Resume pazopanib at same dose only if all of the following criteria are met: • The subject must have been treated with anticoagulant at the desired level of anticoagulation for at least one week. • No Grade 3 or 4 or clinically significant Grade 2, hemorrhagic events have occurred while on anticoagulation treatment. Subject should be monitored as clinically indicated during anticoagulation treatment and after resuming study treatment. When treating with warfarin, international normalized ratio (INR) should be monitored within three to five days after any change in pazopanib dosing (eg, re-initiating, escalating/de-escalating, or discontinuing pazopanib), and then at least weekly until the INR is stable. The dose of warfarin (or its derivatives) may need to be adjusted to maintain the desired level of anticoagulation
Grade 4 and/or PE	Discontinue pazopanib and continue follow-up per protocol.
Arterial Thrombosis/Ischemia	
Any Grade	Discontinue pazopanib and continue follow-up per protocol.
Thrombocytopenia: Investigate and document underlying cause	
Grade 1 or 2	Continue pazopanib with current dose; monitor as clinically indicated.
Grade 3 or 4	Step 1. Interrupt pazopanib until toxicity resolves to ≤ Grade 2. Step 2. Restart pazopanib dose-reduced by 200 mg and monitor as clinically indicated. If no recovery to ≤ Grade 2 or recurrent Grade 3 or 4 thrombocytopenia, discontinue pazopanib and follow-up per protocol.
Anemia: No specific dose reduction rules are indicated for anemia unless due to hemorrhage or bleeding as noted above.	

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AE Terms & Descriptions	Dose Modification Algorithms
Palmar-plantar Erythrodysesthesia Syndrome	
Grade 1 Minimal skin changes or dermatitis without pain (erythema, oedema, hyperkeratosis)	1. Continue pazopanib at present dose
Grade 2 Skin changes with pain; limiting instrumental activities of daily living (ADLs) (peeling, blisters, oedema, bleed, hyperkeratosis)	1. Hold pazopanib 2. Treat as clinically appropriate 3. Upon resolution to Level 1 or better restart pazopanib with a dose reduction to 400 mg 4. If recurrent consider a further dose reduction to 200mg or discontinuation
Grade 3 Severe skin changes with pain and limiting self care ADLs	1. Discontinue pazopanib
Other Clinically Significant Adverse Events[b]	
Grade 1	Continue pazopanib; monitor as clinically indicated.
Grade 2 or 3, if clinically significant	Step 1. Interrupt pazopanib until toxicity resolves to ≤ Grade 1. Step 2. Restart pazopanib dose-reduced by 200 mg and monitor as clinically indicated.
Grade 4	Discontinue pazopanib and continue follow-up per protocol.

a. Well-controlled BP defined as SBP <140 mmHg and mean DBP <90 mmHg.
b. AEs are graded according to NCI Common Terminology Criteria for Adverse Events v4.0 (NCI CTCAE v4)
Abbreviations: BP, blood pressure..

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Event	Dose Modification Algorithms
(A). ALT of ≤ 3.0 x ULN	Continue pazopanib at current dose with full panel LFTs[c] monitored as per protocol.
(B). ALT >3.0 x ULN to ≤8.0 x ULN **without** bilirubin elevation (defined as total bilirubin[d] <2.0 x ULN or direct bilirubin ≤35%) and **without** hypersensitivity symptoms (e.g., fever, rash)	**Liver Event Monitoring Criteria:** (1) Continue pazopanib at current dose levels. (2) Monitor subject closely for clinical signs and symptoms; perform full panel LFTs[a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1.
(C). ALT >8.0 x ULN **without** bilirubin elevation (defined as total bilirubin[b] <2.0 x ULN or direct bilirubin ≤35%) and **without** hypersensitivity symptoms (e.g., fever, rash)	**1st occurrence – Liver Event Interruption Criteria[e]:** (1) Interrupt pazopanib until toxicity resolves to ≤Grade 1 or baseline. Report the event to GSK as an SAE **within 24 hours** of learning of its occurrence and complete the eCRF liver event forms. Make every reasonable attempt to have subjects return to the clinic within 24 to 72 hours for repeat liver chemistries and liver event follow up assessments. (2) Liver imaging and other laboratory investigations should be considered as clinically appropriate. (3) Monitor subject closely for clinical signs and symptoms; perform full panel LFTs [a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1. (4) If the subject is benefiting from the study treatment, contact GSK Study Physician for possible re-challenge. Re-treatment may be considered if ALL following criteria are met: - ALT/AST reduced to Grade 1 - Total bilirubin <1.5 x ULN or direct bilirubin ≤35% - No hypersensitivity signs or symptoms - Subject is benefiting from therapy. If approval for re-treatment is granted, the subject must be re-consented (with a separate informed consent specific to hepatotoxicity). **Recurrence – Liver Event Stopping Criteria[e]:** Discontinue pazopanib permanently and monitor subject closely for clinical signs and symptoms; perform full panel LFTs [a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1. At the time of the recurrence, complete the eCRF liver event forms.
(D). ALT >3.0 x ULN **with** concomitant elevation in bilirubin[d] (defined as total bilirubin ≥2.0 x ULN; with direct bilirubin >35%) or **with** hypersensitivity symptoms (e.g., fever, rash).	**Liver Event Stopping Criteria[e]:** (1) Discontinue pazopanib immediately, report the event to GSK as an SAE **within 24 hours** of learning of its occurrence , and complete the eCRF liver event forms. Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries and liver event follow up assessments. (2) Consult a gastroenterologist / hepatologist and perform the following assessments to identify potential co-factors: - Eosinophil count - Viral serology for hepatitis A, B, C and E, cytomegalovirus, Epstein-Barr virus (IgM antibody, heterophile antibody, or monospot testing) - Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies. - Serum creatinine phosphokinase for possible muscle injury caused LFT elevation - Liver imaging -Consider toxicological blood screen for possible contributing chemical/medical entities (3) Monitor subject closely for clinical signs and symptoms; record the appearance or worsening of clinical symptoms of hepatitis, or hypersensitivity, such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever rash or eosinophilia as relevant on the AE report form. Perform full panel LFTs [a] weekly or more frequently if clinically indicated until LFTs are reduced to Grade 1.

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Event	Dose Modification Algorithms
For isolated total bilirubind elevation without concurrent ALT increases (defined as ALT <3 X ULN).	(1) Isolated hyperbilirubinemia (i.e., in the absence of elevated ALT or other signs/symptoms of liver injury) does not require dose modification. Pazopanib inhibits UGT1A1 and OATP1B1, which can cause elevation of indirect (unconjugated) bilirubin in the absence of liver injury.. (2) If bilirubin is >1.5 x ULN in the absence of ALT elevation, fractionation of bilirubin elevation should be performed. If bilirubin is >35% direct (conjugated), further evaluation for underlying cause of cholestasis should be performed.

c. Full panel LFTs include: AST, ALT, alkaline phosphatase, GGT, and total bilirubin. Coagulation tests should be performed as clinically indicated.

d. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >1.5 x ULN, then the event should be promptly reported as an SAE.

e. When a liver chemistry event meets the Liver Event Interruption Criteria, or Liver Event Stopping Criteria, blood samples should be obtained for PK and for clinical laboratory testing by the central laboratory (Liver Event Kits will be provided for this purpose).

Abbreviations: ALT alanine aminotransferase; AST aspartate aminotransferase; eCRF electronic case report form; IP investigational product; LFT liver function tests; SAE serious adverse event; ULN upper limit of normal

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Preoperative therapy with pazopanib in high-risk soft tissue sarcoma: a phase II window-of-opportunity study of the German Interdisciplinary Sarcoma Group (GISG-04/NOPASS)

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Primary Subject Heading:	Oncology
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Keywords:	Sarcoma < ONCOLOGY, Clinical trials < THERAPEUTICS, NUCLEAR MEDICINE, Magnetic resonance imaging < RADIOLOGY & IMAGING, VASCULAR MEDICINE, Surgical pathology < PATHOLOGY

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Preoperative therapy with pazopanib in high-risk soft tissue sarcoma: a phase II window-of-opportunity study of the German Interdisciplinary Sarcoma Group (GISG-04/NOPASS)

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soft tissue sarcoma; neoadjuvant treatment; pazopanib; antiangiogenic treatment; endothelial progenitor cells

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ABSTRACT

Introduction: For resectable soft tissue sarcoma (STS), radical surgery, usually combined with radiotherapy, is the mainstay of treatment and the only potentially curative modality. Since surgery is often complicated by large tumor size and extensive tumor vasculature preoperative treatment strategies with the aim of devitalizing the tumor are being explored. One option is the treatment with antiangiogenic drugs. The multikinase inhibitor pazopanib, which possesses pronounced anti-angiogenic effects, has shown activity in metastatic and unresectable STS, but has so far not been tested in the preoperative setting.

Methods and analysis: This open-label, multicentre phase II window-of-opportunity trial assesses pazopanib as preoperative treatment of resectable STS. Participants receive a 21-day course of pazopanib 800 mg daily during wait time for surgery. Major eligibility criteria are resectable, high-risk adult STS of any location, or metachronous solitary STS metastasis for which resection is planned, and adequate organ function and performance status. The trial uses an exact single-stage design. The primary endpoint is metabolic response rate (MRR), i.e. the proportion of patients with >50% reduction of the SUVmean in post-treatment compared to pre-treatment FDG-PET-CT. The MRR below which the treatment is considered ineffective is 0.2. The MRR above which the treatment warrants further exploration is 0.4. With a type I error of 5% and a power of 80%, the sample size is 35 evaluable patients, with 12 or more responders as threshold. Main secondary endpoints are histopathological and MRI response, resectability, toxicity, recurrence-free and overall survival. In a translational sub-study, endothelial progenitor cells and vascular epithelial growth factor receptor are analyzed as potential prognostic and predictive markers.

Ethics and dissemination: Approval by the ethics committee II, University of Heidelberg, Germany (2012-019F-MA), German Federal Institute for Drugs and Medical Devices (61-3910-4038155) and German Federal Institute for Radiation Protection (Z5-22463/2-2012-007).

Registration details: Clinicaltrials.gov: NCT01543802, EudraCT: 2011-003745-18

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STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths

- One of the first trials to evaluate pazopanib, a multikinase inhibitor with pronounced antiangiogenic properties, in neoadjuvant treatment of soft tissue sarcoma
- As neoadjuvant antiangiogenic treatment of soft tissue sarcoma is not established, the trial uses a window-of-opportunity design to minimize potential risks for patients and yet provide valid information on efficacy and safety
- Multimodal response assessment: dynamic FDG-PET-CT (primary outcome), dedicated MRI protocol, specific protocol for histopathological assessment
- Translational sub-study exploring the role of circulating endothelial progenitor cells and soluble vascular endothelial growth factor receptor as potential biomarkers for antiangiogenic treatment of soft tissue sarcoma

Limitations

- Heterogeneous study population in terms of histological sarcoma sub-type
- Heterogeneous study population in terms of clinical setting (primary tumors and solitary recurrences / metastases)
- Timing of metabolic response assessment several days after discontinuation of therapy, “rebound growth” is therefore possible; however, metabolic response is assessed close to the time of surgery, which is the clinically most relevant time point; surgery takes place at least seven days after treatment discontinuation to minimize risk of surgical complications

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INTRODUCTION

Soft tissue sarcomas (STS) constitute a heterogeneous group of malignant mesenchymal tumors with varying histological differentiation. High grade, deeply located, large STS have a poor outcome. For both primary and recurrent tumors, radical surgery, usually combined with postoperative radiotherapy, is the mainstay of treatment and the only potentially curative modality.[1] Likewise, oligometastatic disease with one or few manifestations in one single organ is treated surgically in selected patients with the aim of prolonging survival and reducing symptom burden. Surgery is however often hampered by large tumor size with infiltration of adjacent structures, and extensive vasculature of the tumor.

For primary and recurrent tumors, various preoperative treatment strategies have been tested in clinical trials.[2] Preoperative doxorubicin/ifosfamide has not been shown to yield any benefit in overall or progression-free survival in a randomised trial when compared with surgery alone.[3] The addition of regional hyperthermia to preoperative chemotherapy has improved local progression-free and disease-free survival.[4] Regarding preoperative cytotoxic chemotherapy, additional concerns exist because the administration of drugs with a lifetime dose limit, such as anthracyclines, in a non-metastatic setting might narrow future treatment possibilities in case of recurrence. Preoperative radiation has been shown to have a slight survival benefit compared to postoperative radiation, but the latter is often preferred because of a lower rate of wound complications.[5] Isolated limb perfusion is an option for selected patients but requires a high logistic effort and is limited to referral centres.[2] In conclusion, there is still no consensus if and which preoperative treatment should be applied in patients with STS,[6,7] and further modalities are to be tested in clinical trials.

The ideal preoperative treatment for STS would be fast-acting and effective in terms of devitalisation of the tumor and disruption of its hypervasculature, thus facilitating resection. It would have a low incidence of side effects, thus not hampering surgery and post-surgery recovery. In addition, a valid possibility of early response assessment would be highly desirable, since this could spare non-responders from ineffective treatment and a potentially harmful delay of surgery.

The multikinase inhibitor pazopanib has been approved as treatment for metastatic or non-resectable STS based on the results a phase III trial which compared pazopanib treatment to placebo in patients with metastatic non-adipocytic STS who were angiogenesis inhibitor-naïve and had progressed on at least one prior chemotherapy regimen.[8] Patients in the pazopanib arm had a significantly longer progression free survival (median: 20 versus 7

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weeks; HR=0.31, 95% CI 0.24-0.40), which was the primary endpoint of the trial. There was no significant difference in overall survival (12.5 months with pazopanib versus 10.7 months with placebo; HR=0.86, 95% CI 0.67-1.11), but the trial was not powered for this secondary endpoint. In the heavily pretreated trial population, treatment was sufficiently well tolerated, with a median dose intensity of 96.3% and 14% of toxicity-related treatment interruptions in the pazopanib group. The most frequent adverse events were fatigue, hypertension, anorexia, and diarrhea. The trial was restricted to patients with non-adipocytic STS because of the previous phase II trial which showed no activity in the stratum of adipocytic STS.[9] Subsequent central pathology review, however, re-classified two patients with stable disease (internal communication by the trial sponsor). Based on this finding activity of pazopanib against liposarcoma is probable

Given this proof of efficacy, its fast and pronounced anti-angiogenic effects mediated by kinase inhibition,[10] and its favorable safety profile,[11] pazopanib might be an ideal candidate for neoadjuvant treatment in STS. Therefore, we decided to conduct a pertinent phase II trial as a “window of opportunity” study.[12,13] In this design, patients receive an investigational agent in a “window period” before commencing the established treatment. The risk that effective treatment is unduly delayed is minimised by keeping the duration of the investigational treatment short. The described window-of-opportunity design is feasible for pazopanib, as shown in a trial for non-small cell lung cancer.[14].

A crucial precondition for an effective use of preoperative treatment is good and early response monitoring. Dynamic F-18 labeled Fluorodeoxyglucose Positron Emission Tomography and Computed Tomography (dFDG-PET-CT) appears to be ideal for this purpose. It has been prospectively validated as a strong predictor of histopathological response and progression-free survival both for neoadjuvant and palliative chemotherapy in patients with STS.[15-18] Unlike histopathological response assessment, which can only be performed retrospectively after surgery, it allows for early selection of patients who benefit from preoperative therapy. Magnetic resonance imaging (MRI) is another viable option for early response assessment.[19,20] However, it has not yet been validated as a predictor of progression-free survival. Therefore, up to now, dFDG-PET-CT can be deemed the best available modality for early prospective response assessment of chemotherapy for STS.

Another reason for conducting a neoadjuvant study in STS patients is that it can provide information on biomarkers for response prediction. Ultimately, such markers would allow distinguishing responders from non-responders, thus facilitating true “targeted therapy”. In the context of anti-angiogenic therapy, there are several circulating angiogenic factors which

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might serve this purpose. Endothelial progenitor cells (cEPCs) and soluble vascular epithelial growth factor (sVEGF) correlate with tumor burden in STS,[21] and might thus serve as early predictors of clinical and histological response.

Here, we present the trial protocol. The trial has been initiated in April 2013 and the first patient has been recruited in May 2013. At present, the trial is ongoing.

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METHODS AND ANALYSIS

Study objectives

The primary objective is to evaluate whether neoadjuvant treatment with pazopanib in patients with STS has therapeutic effects, measured as metabolic response. The secondary study objectives are

- To assess the safety of preoperative pazopanib treatment in patients undergoing resection of STS.
- To evaluate potential correlation between metabolic (dPET-CT), radiological (MRI) and histopathological assessment of tumor response to pazopanib treatment in STS.
- To provide exploratory data on blood levels of circulating endothelial progenitor cells (cEPCs) and soluble vascular epithelial growth factor (sVEGF) as potential predictive biomarkers during pazopanib treatment of STS.

Study outline

This is a multi-center (three recruiting centers and one center for PET-CT), single-arm, open-label phase II trial under the auspices of the German Interdisciplinary Sarcoma Group (GISG). Pazopanib is administered for 21 days followed by resection of the tumor after a 7-14 days break (figure 1).

Patient selection

The study population should consist of patients with STS for which efficacy of pazopanib is assumed and with adequate organ function and performance status to tolerate the treatment. This led to the in- and exclusion criteria listed in table 1.

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Table 1: Major in- and exclusion criteria of the trial

Inclusion criteria

- Non-metastatic primary tumor or locoregional recurrence of histologically confirmed high-risk (G2/3, diameter ≥5 cm) soft tissue sarcoma (STS) of any location; or metachronous solitary metastasis of STS for which surgical resection is planned according to the individual choice of the multidisciplinary treatment team (no grade or size restrictions apply for metastasis).
- Resectable and solitary tumor, as assessed by the investigator based on staging exams (CT scan of the chest, CT or MRI of the abdomen, MRI of the limb in case of extremity STS). Measurable disease according to RECIST 1.1
- STS except the following subtypes: embryonal rhabdomyosarcoma, chondrosarcoma, osteosarcoma, Ewing tumor / PNET, Gastrointestinal stromal tumors, dermofibromatosis sarcoma protuberans, inflammatory myofibroblastic sarcoma
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
- Adequate organ system function
- Effective contraception
- Written informed consent
- Age ≥ 18 years

Exclusion criteria

- Prior malignancy other than the STS under study with active disease within the last 5 years
- History or clinical evidence of central nervous system (CNS) metastases
- Prior or concurrent systemic chemotherapy or molecularly targeted therapy or radiotherapy for STS or other malignancies within five years before study entry
- Clinically significant gastrointestinal abnormalities that may increase the risk for gastrointestinal bleeding
- Clinically significant gastrointestinal abnormalities that may affect absorption of pazopanib
- Corrected QT interval (QTc) > 480 ms
- Presence of uncontrolled infection.
- History of severe cardiovascular conditions within the past 6 months:
- Cardiac angioplasty or stenting
- Poorly controlled hypertension [defined as systolic blood pressure (SBP) of ≥140 mmHg or diastolic blood pressure (DBP) of ≥ 90mmHg)
- Cerebrovascular accident including transient ischemic attack (TIA), pulmonary embolism or untreated deep venous thrombosis (DVT) within the past 6 months
- Major surgery or trauma within 28 days prior to first dose of pazopanib
- Evidence of active bleeding or bleeding diathesis
- Known endobronchial lesions and/or lesions infiltrating major pulmonary vessels that increase the risk of pulmonary hemorrhage)
- Any serious and/or unstable pre-existing medical, psychiatric, or other condition that could interfere with subject's safety, provision of informed consent, or compliance to study procedures
- Any ongoing toxicity from prior anti-cancer therapy that is > grade 1 and/or that is progressing in severity, except alopecia

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Treatment plan

All patients are routinely discussed in an institutional multidisciplinary sarcoma board, and a recommendation for the best available treatment and possible inclusion into the trial is jointly determined.

Pazopanib treatment

The treatment consists of the oral administration of 800 mg (two tablets of 400 mg or four tablets of 200 mg) pazopanib once daily. The treatment will be administered for 21 days. Pazopanib should be taken orally without food at least one hour before or two hours after a meal. The tablets should be swallowed whole and must not be crushed or broken. The time of day the tablets are taken should be relatively constant. If a dose is missed, the subject should take the dose as soon as possible, but only if there are 12 or more hours remaining before the next dose is due. If the next dose is due in less than 12 hours, the subject should skip the missed dose and take the next dose as scheduled. If vomiting occurs after taking pazopanib, the subject should not take a replacement dose on that day. The subject should resume taking pazopanib at the next scheduled dose on the following day. If vomiting persists, the subject should be instructed to notify the investigator. Pazopanib will be provided by the manufacturer. It should be stored at room temperature up to 25^{o}C. When stored at these temperatures and in unopened bottles, pazopanib tablets will remain stable until the expiration date indicated on the bottle label. For detailed information on the administration of pazopanib refer to the Investigator's Brochure for pazopanib.[22]

Given the short duration of 21 days of planned treatment, in case of drug toxicity only two dose reductions are permitted in a stepwise fashion: initially to 600 mg once daily, and subsequently to 400 mg once daily if necessary. Given the short treatment period, no dose re-escalation is foreseen. Recommendations for investigational product dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the online supplementary material.

In each individual patient, pazopanib treatment should be discontinued if one of the following events occurs:

- The patient withdraws consent to the study.
- The investigator is of the opinion that continuation of treatment would jeopardize the health status of the patient.
- A Serious Adverse Drug Reaction (SADR).

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- A specific AE requiring treatment discontinuation as defined in the online supplementary material.
- In female subjects: pregnancy.
- Severe non-compliance of the subject which jeopardizes validity of the data in a relevant way.

Surgery

Surgery should be performed 7-14 days after the end of the study treatment. This interval corresponds to at least five half lives of pazopanib. It was chosen in order to minimize the potential risk of surgical complications (mainly wound or anastomotic complications). Evidence from patients receiving anti-VEGFR treatment with bevacizumab suggests an elevated risk which is inversely correlated to the time interval between treatment discontinuation and surgery.[23] Surgery itself is not part of the study protocol and is performed according to the discretion of the treating surgeon. Although not mandatory, for reasons of quality assurance regarding pathological assessment, it is strongly recommended that surgery is performed at one of the study centres.

Postoperative treatment / radiotherapy

A recommendation for possible postoperative treatment is again jointly determined in an institutional multidisciplinary sarcoma board. If adjuvant radiotherapy is carried out, it is not part of the study protocol and can be performed according to the choice of the treating physician. However, for subjects participating in the translational study it should not start before blood samples to determine postoperative cEPC / sVEGF levels were taken (14 days after surgery).

Evaluation, laboratory tests, follow-up

A signed, written informed consent form must be obtained from the subject prior to any study-specific procedures or assessments. Procedures conducted as part of the subject's routine clinical management and obtained prior to signing of informed consent may be utilized for screening or baseline purposes provided these procedures are conducted as specified in the protocol. The study assessments schedules and visit windows are summarized in Table 2. A certified laboratory is to conduct all clinical laboratory assessments. Assessment at laboratories other than the site laboratory (e.g. at a laboratory collaborating with the patient's general practitioner) is possible. Laboratory assessments should be performed as indicated in table 3. All laboratory tests with values that become abnormal and clinically significant while the subject is participating in the study or within 28

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days after the last dose of study drug should be repeated until the values return to normal or baseline.

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Table 2: Time and Events Table

Required measures	Screening (≤14 days before study entry)	d7	d14	d22-28	≥d29	3 monthly until end of follow-up (36 months/ recurrence/ death)
Informed Consent	♦					
History, ECOG PS, toxicity / compliance assessment	♦	♦	♦	♦		♦
Physical assessment incl. pulse, blood pressure, weight, cancer symptoms	♦	♦a	♦	♦		♦
Complete blood count, sodium, potassium, creatinine, bilirubin, alkaline phosphatase, AST, ALT, glucose, albumin, PT/INR, PTT, fT4, TSH	♦		♦b	♦		
UPC	♦					
12-lead ECG	♦					
Pregnancy test	♦					
Chest CT	♦					♦
MRI of tumor region (upon screening and on d22-28 according to specific protocol; during follow-up according to local clinical practice; see section **Error! Reference source not found.**)	♦[c]			♦[c]		♦
FDG-dPET-CT of tumor region (according to protocol; see section **Error! Reference source not found.**)	♦			♦		
Surgery (not part of study protocol)					♦	
cEPC / sVEGF levels	♦			♦	♦ (14 days after surgery)	

[a]Monitoring of BP: A measurement of BP should be taken at day 7+/- 3 days. BP can be assessed by any method (i.e., at home or by another physician) as long as the study physician is informed of the measurement, verifies any measurement that is not normal and takes appropriate action.

[b]Only LFTs

[c] optional according to local availability

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Table 3: Clinical Laboratory Assessments

Clinical Chemistry	
Renal function	Urea, Creatinine[a]
Liver function test (LFT) Panel	Albumin, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-GT and Bilirubin (total)[b]
Electrolytes and others	Calcium, Potassium, Sodium, Magnesium, Inorganic phosphate, Glucose, and Lactate Dehydrogenase (LDH)
Hematology	Hematocrit, Hemoglobin, White Blood Cell Count, Red Blood Cell Count, Neutrophils, and Platelets
Coagulation Tests	Activated partial thromboplastin (aPTT) and International Normalization Ratio (INR)[c]
Urinalysis for Proteinuria	UPC[d]
Thyroid Function Test	TSH[e]

a) Estimated creatinine clearance should be calculated using the Cockroft and Gault method (appendix D). Alternatively, creatinine clearance can be measured directly by 24-hour urine collection.
b) A direct bilirubin level should be obtained if the total bilirubin level is greater than 1.5 X upper limit of normal (ULN). See Section 5 for stopping criteria and dose modification guidelines for treatment-emergent liver function abnormality.
c) Coagulation tests may also be performed in response to an AE/SAE of bleeding and as clinically indicated.
d) UPC should be evaluated as described in appendix E or by 24-hour urine protein. If UPC ≥ 3 or if urine protein is ≥3g, then the dose modification table guidelines should be followed (Section 5).
e) Unscheduled thyroid function tests [TSH and thyroxine (free T_4)] should be performed if clinically indicated (e.g., if a subject develops signs and symptoms suggestive of hypothyroidism).

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Study evaluation

FDG-dPET-CT

At the time points day -14 to 0 and day 22-28, patients will undergo dPET-CT with FDG over the tumor area followed by a baseline whole-body PET-CT. All exams will be performed centrally at the same PET-CT-scanner. A topogram and a low dose CT are used for the positioning of the patient to include the primary volume of interest for the dPET-CT. Following the intravenous application of FDG, a dynamic data acquisition (4D mode) is performed for 60 minutes. Then a whole body PET-CT is acquired using 2 minutes per bed position. The iteratively reconstructed images are evaluated using dedicated software. Besides the SUV (standardized uptake values) further parameters of the tracer kinetics are assessed using compartment and non-compartment models. The SUV_{mean} 60 minutes after the FDG injection is used to assess the therapeutic effect. Exploratory analyses of dynamic PET-CT response will be based on a Volume of Interest (VOI) analysis. The following parameters of the FDG kinetics will be calculated: 1. SUV (mean, max) 55-60 min post injection; 2. Influx rates and transport rates based on a support vector machine algorithm and a two-tissue compartment analysis; and 3. Calculation of the fractal dimension of the FDG kinetics based on fractal analysis. The percentage change of all parameters of the FDG kinetics as well as discriminant analysis based on the absolute values of the FDG kinetics and their changes will be determined along with confidence intervals. In particular, the SUV_{mean} will be used primarily for the assessment of response. Metabolic response is assumed with a change of 50 % following pazopanib treatment (21 days). The dynamic parameters are used as secondary parameters for the assessment of treatment.

MRI

MRIs upon study entry and on day 22-28 are optional according to local availability and should be performed as follows: A 1.5T scanner with phased-array coil will be used. T1W (2D or 3D) pre- and post-contrast sequences with fat suppression will be undertaken. After localizer sequences (T2 weighted HASTE sequences), T1 weighted turbo Spin Echo (TSE) sequences with and T2 weighted turbo Spin Echo (TSE) without fat suppression pre-contrast, followed by an EPI DWI sequence with four b-values (0, 50, 400, 800) and quantification of ADC maps will be performed. Afterwards, T1 weighted fat-saturated gradient echo sequences in breathhold technique before and 30, 60, and 90 sec after contrast media application (0,1 mmol/kg bw Gadolinium chelates), covering at least 20 cm of the tumor size, will be done. The matrix size should be at least 256, and the slice thickness should be 3 mm or less.

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All quantitative analysis of dynamic and diffusion exams as well as evaluation according to the RECIST and modified Choi criteria will be performed by an experienced board certified radiologist at the study centre Mannheim. Data material will be provided for all included patients on CD or be electronic data export.

Evaluation of changes of tumor size during therapy will be performed in accordance with RECIST criteria v1.1 [24]). *Complete response (CR)* is defined as the disappearance of all lesions. *Partial response (PR)* is defined as a decrease of at least 30 % of the longest diameter of the tumor, taking as reference the baseline longest diameter; *progressive disease (PD)* is defined as an increase of at least 20 % in the longest diameter of the tumor, taking as reference the baseline longest diameter, or the appearance of one or more new lesions; *stable disease (SD)* is defined as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the baseline longest diameter. For evaluation during therapy, partial response, stable disease and progressive disease will also be defined according to Choi criteria modified for MRI.[19] Here, *CR* is defined as the disappearance of all lesions. *PR* is defined as ≥10% decrease in the greatest maximal diameter or a ≥15% decrease in contrast enhancement; *PD* is defined as a ≥10% increase in the greatest maximal diameter while the criteria for PR by using contrast enhancement are not met, or a ≥15% increase in contrast enhancement while criteria for partial response by using tumor size are not met, or the occurrence of one or more new lesions; *SD* is defined as all cases who do not meet criteria for CR, PR, or PD.

If technically feasible at the local MRI unit, 2D and 3D measurements of the tumor will be performed. The apparent diffusion coefficient (ADC) values of two reproducible tumor localizations as well as the plasma flow (PF) and mean transit time (MTT) of the tumor at the same localizations evaluated by FDA approved Tissue 4D software or another adequate perfusion quantification tool will be documented and compared between measurements. MRI response will be quantified as the change in percent between pre- and post-therapeutic apparent diffusion coefficient (ADC).[20]

During the follow-up period, all MRIs can be performed according to local clinical practice without adhering to a specific protocol.

Chest CT

For screening, all patients require a multi-slice chest CT with a slice thickness ≤5mm (reconstruction interval) covering the whole area from the lung apices to the diaphragm. The

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use of intravenous contrast medium is recommended unless contraindicated. The images will be assessed by the responsible radiologist, according to local standards.

During the follow-up period (after surgery), all chest CTs can be performed according to local clinical practice without adhering to a specific protocol.

Histopathological procedures

The histopathological report should include the following information:

- Tumor size in three dimensions
- Resection status (free margins, margins microscopically infiltrated, margins macroscopically infiltrated)
- Smallest distance between resection margin and vital tumor tissue found in the specimen, together with the localisation where it was found
- Histological subtype
- Grading according to the FNCLCC system (G1-3) [25]
- Overall regression grading after slicing of the specimen (in percent, semi-quantitative value)
- Most prevalent type of regression: hyalinous necrosis, apoptosis, scar tissue, hemorrhagic necrosis

Evaluation of efficacy

Metabolic response and metabolic response rate (MRR)

Metabolic response is defined as the achievement of an at least 50% reduction of the mean standardized uptake value (SUV_{mean}) over the tumor area in the post-treatment compared to the pre-treatment FDG-dPET-CT. The *metabolic response rate (MRR)* is defined as the proportion of patients achieving a metabolic response.

Other outcome measures

dPET-CT response is defined as the change of FDG influx as well as of transport rates k1-k4 and distribution volume VB and fractal dimension. Absolute values of all parameters of FDG kinetics will also be used for discriminant analysis evaluation.

Resection status is determined by the assessing pathologist in the respective report.

Recurrence-free survival is defined as the time from resection to the date of diagnosis of recurrence or date of death or the last day when the patient was known to be disease-free or alive in the case of loss to follow-up (censoring). The date of diagnosis of recurrence is defined as the first day when the below mentioned criteria for recurrence are met. In case of

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a diagnosis by imaging, the day when the imaging procedure was performed is recorded as the date of recurrence. In case of a clinical diagnosis, the date of the visit is recorded.

Local recurrence-free survival is defined as survival free from local recurrence, which in this context is defined as a newly occurred lesion within a range of 10 cm of the margin of the original tumor. The assessment if a recurrence is considered local or distant should be made by the investigator at the study centre based on the physical examination and the patient's file. Patients with R2 resection are automatically assigned a local recurrence-free survival of zero.

Distant recurrence-free survival is defined as survival free from any recurrence not fulfilling the criteria of local recurrence.

For both local and distant recurrence, prior death will be counted as a competing interest and not included. The date of diagnosis of local / distant recurrence is defined as the first day when the criteria for recurrence are met. In case of a diagnosis by imaging, the day when the imaging procedure was performed is recorded as the date of recurrence. In case of a clinical diagnosis, the date of the visit is recorded

Overall survival is computed from the date of resection to the date of death (whatever the cause). Patients not known to be dead at the time of the analysis will be censored at the date of last follow-up.

Toxicity: All adverse events will be recorded according to CTCAE, version 4.0. (http://evs.nci.nih.gov/ftp1/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf).

Delay in planned time to resection: Any occurring delay between the actual date of resection and the planned date of resection will be quantified by the treating surgeon and categorized into "treatment-related" and "non treatment-related".

Study design, sample size and analysis plan

Study design and sample size

The trial is designed as a non-comparative single-arm phase II trial. Its primary end-point is the metabolic response rate (MRR), defined as the proportion of patients achieving a 50% reduction of the mean standardized uptake value (SUVmean) in the post-treatment compared to the pre-treatment FDG-dPET-CT. The trial uses an exact single-stage design based on the exact binomial distribution.[26] The MRR below which the treatment is considered ineffective is set at 0.2 (H0: MRR≤0.2). The MRR above which the treatment warrants further exploration in a subsequent phase III trial is set at 0.4 (H1: MRR≥0.4). This magnitude of response can be expected after preoperative therapy with a supposedly active agent for STS, as shown in several non-controlled trials.[15,27-29] With a predefined significance level of 5% and a power of 80%, the sample size is calculated to be 35

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evaluable patients with an actual significance level of 0.034 and an actual power of 0.805 with a critical value of 12 patients at the upper proportion limit (STPLAN, Version 4.5, 2010, The University of Texas, Houston, USA). If after treatment of 35 patients, the primary endpoint MRR cannot be assessed for one or more patients, e.g. for technical or medical reasons, up to 3 additional patients can be enrolled.

Analysis plan

The final analysis of the primary endpoint MRR will be carried out after all patients have received their post-treatment FDG-PET-CT. All patients registered in the study will be included (intention-to-treat analysis). The number of patients who are not evaluable, who died or who withdrew before treatment began or during treatment will be specified. According to the exact single-stage design based on the exact binomial distribution, H_0 will be rejected if 12 or more patients show metabolic response.[26] If at the end of treatment of all enrolled patients, the number of individuals who can be evaluated for metabolic response is below 35, the border for acceptance of H_1 will be modified accordingly with a type I error not exceeding 5%. The MRR will be presented as a percentage with a 95% confidence interval. In addition, metabolic response will be visualised as waterfall plot with the change in percent of SUV_{mean} on the y-axis.

At the time of analysis of the primary endpoint, the secondary endpoints MRI response, dynamic PET-CT response, histopathological response, R0-resectability and toxicity will also be analyzed. MRI response will be quantified as the change in percent between pre- and post-therapeutic apparent diffusion coefficient (ADC)[20] as well as the rates of complete response, partial response, stable disease, and progressive disease according to Choi criteria modified for MRI and RECIST 1.1, all with 95% confidence intervals. A potential correlation between MRI and metabolic response will be evaluated by calculating Pearson's correlation coefficient for SUV_{mean} change in percent between the pre- and post-therapeutic PET-CT and the ADC change in percent between the pre- and post-therapeutic MRI. For the categorical variables "response according to modified Choi and RECIST 1.1 criteria", parameters of diagnostic accuracy (sensitivity, specificity, PPV, NPV) for complete or partial response will be calculated relating to the primary outcome metabolic response as "gold standard".

Exploratory analyses of dPET-CT response will be based on a Volume of Interest (VOI) analysis. The following parameters of the FDG kinetics will be calculated: (1) SUV (mean, max) 55-60 min post infusion; (2) Influx rates and transport rates based on a support vector machine algorithm and a two-tissue compartment analysis, and (3) Calculation of the fractal dimension of the FDG kinetics based on fractal analysis. Percentage change of all described parameters of the FDG kinetics as well as discriminant analysis based on the absolute

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values of the FDG kinetics and their changes will be presented along with confidence intervals.

R0-resectability will be presented as proportion with corresponding 95% confidence interval. Histopathological response will be displayed as the rate of responders with corresponding 95% confidence interval and range as well as a waterfall plot with the percentage of viable tumor tissue on the y-axis. Parameters of diagnostic accuracy for histopathological response will be calculated relating to the primary outcome metabolic response as “gold standard”. The incidence of adverse events (AEs) will be presented for the safety population, in a descriptive analysis. Delay in planned time to resection due to the treatment will be reported in a descriptive way with appropriate summary measures. The analysis of the secondary endpoints overall survival, recurrence-free survival, local and distant recurrence-free survival will be performed when data are mature. Survival curves will be estimated with the Kaplan-Meier method and displayed graphically.

A first exploratory analysis of biomarker data (see below), i.e. cEPC and sVEGF blood levels, will be carried out at the time of analysis of the primary endpoint. This will assess if the mentioned biomarkers have a predictive value for metabolic response. For this purpose, pre-treatment levels of cEPC and sVEGF will be plotted against the change in percent of SUV_{mean} in order to display a possible predictive relationship. If possible, linear regression will be performed. Likewise, a potential predictive value of cEPC and sVEGF level changes during treatment and metabolic response will be explored by plotting the change in percent of post-treatment levels (d22-28) compared to pre-treatment levels against the change in percent of SUV_{mean}. If possible, linear regression will be performed. cEPC and sVEGF levels measured at d14 post-surgery will be compared to levels measured after completion of pazopanib treatment (d22-28). The difference will be presented in a descriptive fashion (mean, median, standard deviation), related to resection status in an explorative way, and, if meaningful and feasible, formally compared with appropriate parametric or non-parametric tests between patients who received complete and incomplete resection. At the time of analysis of recurrence-free survival, prognostic properties of cEPC and sVEGF levels will be explored. Biomarker levels will be treated as continuous variables. If possible in a meaningful way, recurrence-free survival of patients in the different quartiles of pre-treatment and postoperative cEPC and sVEGF levels will be compared by means of Kaplan-Meier curves. In addition, pre-treatment and postoperative levels of recurred and recurrence-free patients will be compared with an appropriate parametric test.

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Translational research

There is an increasing amount of evidence suggesting that cEPCs, in conjunction with soluble cytokines such as sVEGF, are involved in neoangiogenic processes of solid tumors.[30] Studies suggest that the recruitment of cEPC, which is probably mediated through cytokines, is an expression of tumor-induced neoangiogenesis in situations of tumor growth. cEPCs have been shown to contribute to vasculogenesis and neoangiogenesis in several tumour entities.[31,32] On the other hand side, tumor response to chemotherapy or kinase inhibition seems also to be related with a rise in cEPC and sVEGF levels.[30,33]

Based on these preliminary findings, it seems probable that blood levels of cEPCs and sVEGF have a certain prognostic and/or predictive value for the course of disease and treatment of STS. We were able to show a positive association between clinical tumor load and cEPCs level in the blood of patients with lung cancer[34] and STS (unpublished data). Consequently, pre-treatment cEPC levels might reflect tumor load and metabolism and thus constitute a prognostic marker. The same might hold true for postoperative cEPC levels, which could be an indicator of residual microscopic tumor load and thus of recurrence risk. On the other hand side, high cEPC pre-treatment levels, indicating an extensive tumor vasculature and thus a high susceptibility to an anti-angiogenic treatment, might be a predictive marker for response. The same might be true for a drop in cEPC level during treatment. Similar associations have been observed between sVEGF levels and treatment response.

In order to explore these hypotheses, the study foresees measurement of cEPC and cVEGF levels at various time points. Patients will be eligible for the translational research project if they are eligible for the clinical trial and have given written informed consent to participate in this project. Patients will have the possibility to accept or refuse participation in the translational research project, or to accept participation in only a part of the project, without affecting their participation in the clinical study.

During the specified visits, 20 ml of full blood will be drawn by insertion of a 20-gauge cannula in a peripheral vein and collected in tubes containing sodium citrate (0.105 M) as anticoagulant. In addition, 5 ml of serum will be collected through the same cannula and stored in an appropriate tube. Full blood samples will be processed within 1 hour after collection.

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PBMCs will be prepared by gradient centrifugation using Ficoll-Hypaque (Amersham Biosciences, Freiburg, Germany). The expression of cell-surface antigens will be determined by four-color immunofluorescence staining. Of each sample, 100µl of PBMC (containing 1 x 106 cells) will be incubated with 10 µl of FcR-blocking reagent (Miltenyi Biotec, Bergisch-Gladbach, Germany) for 10 minutes to inhibit nonspecific bindings. Hereafter the cells will be incubated at 4°C for 30 min with 10µl PE-conjugated anti-human CD133 mAb (Miltenyi Biotec, Bergisch-Gladbach, Germany), 10 µl PerCP-conjugated anti-human CD34 mAb (BD Biosciences, Heidelberg, Germany), 10µl APC-conjugated VEGF R2 mAb (R&D Systems, Wiesbaden-Nordenstadt, Germany) and 10µl FITC-conjugated Annexin V mAb (BD Biosciences, Heidelberg, Germany). PE-, PerCP, APC and FITC conjugated isotype-matched immunoglobulin (Ig)-G1 and IgG2a antibodies (DakoCytomation, Hamburg Germany) will be used for each patient and measurement as negative controls. The cells will be washed three times to remove unbound antibodies and finally re-suspended in 400 µl of FACS solution (BD Biosciences, Heidelberg, Germany). FACS-analysis will be performed on a FACSCalibur flowcytometer (BD Biosciences, Heidelberg, Germany) and the data will be analysed using WinMDI 2.8 software. A minimum of 500,000 events is to be collected. FACS analysis of each probe will be performed in triplicate. The frequency of cEPCs in peripheral blood is determined by a two-dimensional side-scatter / fluorescence dot-plot analysis of the samples, after exclusion of Annexin V-positive cells and appropriate gating. The exclusion of Annexin V-positive cells is performed to rule out contamination with apoptotic cells. cEPC counts will be expressed as percentage of total PBMC in each patient.

sVEGF serum concentrations will be assessed using an enzyme-linked immunosorbent assay kit (R&D Systems, Wiesbaden-Nordenstadt, Germany) in triplicate samples obtained from 5 ml serum. ELISA will be performed according to the manufacturer's instructions. The cVEGF concentration will be measured in pg/ml. Further details will be described in a laboratory manual.

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ETHICS AND DISSEMINATION

After due consideration, all participating investigators are convinced that the trial has a positive risk-benefit ratio. Based on results from a phase III trial, which showed efficacy for pazopanib treatment in STS,[8] patients who are treated according to this protocol can be expected to have a higher chance of complete tumor resection and thus a lower risk of tumor recurrence, which should positively affect progression-free and overall survival. Moreover, the preoperative treatment has the potential to result in a less radical, tissue-sparing resection with fewer side effects. In contrast, the expected magnitude of potential risks and harmful side effects of the study treatment is small. Pazopanib is a registered drug. Overall, it is well tolerated and most side effects are manageable and quickly subside once the dosage is reduced or the therapy discontinued. Therefore, the risk that surgery is delayed or perioperative morbidity is increased due to pazopanib side effects is expected to be small. Given the short preoperative treatment period in the window-of-opportunity design and the demonstrated efficacy of pazopanib in STS, the risk of relevant tumor progression during treatment is judged to be very low.

Study-related diagnostic procedures performed in addition to standard of care include two dPET-CTs. These expose the patient to radiation. However, the overall radiation dose of these two procedures does not exceed the annual dose threshold stipulated by relevant German laws for individuals who are professionally exposed to radiation. Thus, the risk of radiation-induced secondary malignancy is judged to be very low and outweighed by the scientific benefit of the study as well as the individual clinical benefit of each participating subject. dPET-CT, although not yet part of standard clinical algorithms for primary STS, does yield relevant additional information. It can detect occult metastases leading to a change in treatment strategy. Moreover, the planning of resection is facilitated for the surgeon, as PET-CT provides additional information on tumor extension and depicts the metabolism in different tumor areas, which potentially changes the surgical approach.

Before the start of the study, patients are informed in writing and verbally about the nature and implications of the proposed study, and especially about the possible benefits for their health and any risks. Patients document their consent by signing the informed consent form. Medical confidentiality and the provisions of the German Federal Data Protection Act are complied with. Moreover, the German Medicines Act (AMG) and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP) regulations are complied with. The principal investigator can decide to discontinue the entire study if he

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concludes that continuation would pose a relevant threat to the health or wellbeing of the individual subjects, or if he concludes that the risk-benefit-ratio of the study is unfavorable, i.e. if the risks clearly outweigh the potential benefits.

The trial was approved by the ethics committee II of the University of Heidelberg, Germany (Reference number 2012-019F-MA). Furthermore, it was approved by the German Federal Institute for Drugs and Medical Devices (Reference number 61-3910-4038155) and the German Federal Institute for Radiation Protection (Z5-22463/2-2012-007). Prior to initiation, the trial has been registered at clinicaltrials.gov (NCT01543802) and the European Clinical Trials database (EudraCT: 2011-003745-18). The University of Heidelberg is the legal sponsor of this trial.

The originals of all central study documents are archived at the principal study site for at least 10 years after preparation of the final report. The Principal Investigator retains the generated administrative documents (correspondence with Ethics Committee, supervisory authority etc.), patient identification list, signed informed consent forms and copies of the general study documentation (protocol, amendments) for the time period stated above. Original data of study patients (medical source records) are to be retained for the applicable archiving period of the study centre but for not less than 15 years, starting from study completion.

It is aimed to publish results from this study in the form of one or several manuscripts in international medical journals. The Principal Investigator will review all manuscripts to prevent forfeiture of patent rights to data not in the public domain. Publication of the first manuscript reporting study results is planned to take place as soon as possible after analysis of the primary endpoint.

At the time of manuscript submission (September 2015), 18 patients have been recruited into the trial.

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DISCUSSION

We present the protocol of a trial which uses pazopanib for preoperative treatment of STS in a window-of-opportunity study. While pazopanib has shown efficacy in metastatic and irresectable STS[8] and therefore been approved for this indication, the drug has not yet been extensively evaluated in the preoperative setting in resectable STS. Recently, results of a study on preoperative pazopanib combined with radiation therapy were published.[35] It assessed the safety of this combination but was a phase I trial and thus not designed to provide sufficient information on efficacy. An ongoing pilot trial (NCT01446809) evaluates pazopanib induction therapy prior to preoperative chemotherapy. Results are awaited in 2016. Another ongoing phase II/III trial (NCT02180867) compares the combination of pazopanib and chemoradiotherapy or radiotherapy with chemoradiotherapy or radiotherapy without pazopanib. Results are awaited in 2018.

Our trial is unique in testing preoperative pazopanib monotherapy in a window-of-opportunity design. The relatively short duration of pazopanib therapy was chosen to minimize the risk of tumor progression and consequent irresectability in non-responders. Notwithstanding, given the drug's mechanism of action, 21 days of treatment are considered sufficient to detect efficacy by assessing dPET-CT response, which is the trial's primary outcome.[36] The wide range of secondary outcomes allows validating other potential parameters for response assessment. In particular, the trial offers the unique possibility to prospectively compare different modalities of response assessment, i.e. MRI and histopathological features with dFDG-PET-CT, which is considered gold standard.

The timing of metabolic response assessment with dFDG-PET-CT is important for the validity of the results and their interpretation. For several tumor entities, the phenomenon of "rebound growth", i.e. an increase in tumor proliferation and possibly neoangiogenesis shortly after discontinuation of anti-angiogenic therapy, has been described.[37-39] Therefore, to assess the strongest achievable response to an anti-angiogenic drug like pazopanib, it would probably be best to perform metabolic response assessment at the last day of therapy. Following this rationale, one would also perform surgery without previous discontinuation of therapy. However, surgical complications, mainly wound and anastomotic morbidity, have been described in patients with prior anti-angiogenic treatment. Evidence suggests an inverse correlation of their incidence with the time interval between treatment discontinuation and surgery. To minimize this complication risk, we established a wash-out period of at least seven days between end of drug treatment and surgery, corresponding to five half lives of the drug. The same seven day period was used in a recent trial on

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neoadjuvant pazopanib treatment for renal cell carcinoma.[40] We believe that this would also be a rational approach if pazopanib was used as neoadjuvant treatment outside of trials. Consequently, we aimed at assessing metabolic response after treatment discontinuation and close to the date of surgery. This mirrors the metabolic state of the tumor at the time of surgery, thus providing the best estimate of the clinically meaningful therapeutic benefit of pazopanib.

Another important element of the trial is its translational sub-study. The trial design offers the opportunity to measure two potential biomarkers, cEPCs and sVEGF, during antiangiogenic treatment and in the perioperative setting, and to correlate them with several parameters of treatment response.

In summary, this trial will provide initial evidence regarding preoperative pazopanib treatment of STS, and, if efficacy can be demonstrated, lead to a pertinent phase III trial. Moreover, cEPCs and sVEGF are further explored regarding their use as biomarkers for antiangiogenic treatment of STS.

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AUTHORS' CONTRIBUTIONS

UR and PH conceived of and planned the trial.

UR drafted the trial protocol and edited its final version.

ADS wrote the parts of the protocol relating to dPET-CT.

JJ, MS, and PH provided advice on the parts of the protocol relating to surgical treatment.

BK, GE, SF, and HGD provided advice on the parts of the protocol relating to medical treatment.

KN wrote the parts of the protocol relating to the translational study.

LRP did the final trial design, devised the analysis plan and wrote the corresponding parts of the trial protocol.

UA provided advice on the parts of the protocol relating to MRI diagnostics.

TG provided advice on the parts of the protocol relating to pathology.

All authors read and approved the final version of the protocol and this manuscript.

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FUNDING STATEMENT

This trial was initially funded by GlaxoSmithKline Oncology. Funding is continued by Novartis, who acquired pazopanib from GlaxoSmithKline Oncology in 2015.

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COMPETING INTERESTS STATEMENT

UR, BK, JJ, and PH have received travel reimbursements from GlaxoSmithKline Oncology / Novartis.

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Figure 1: Study flowchart. d: day. PET-CT: Positron Emission Tomography and Computed Tomography. MRI: magnetic resonance imaging.

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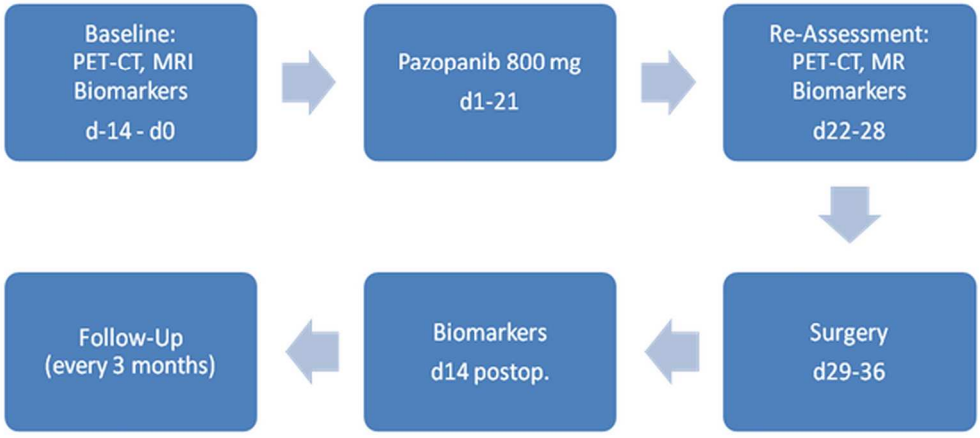

Study flowchart. d: day. PET-CT: Positron Emission Tomography and Computed Tomography. MRI: magnetic resonance imaging.
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Online supplementary material: Recommendations for investigational product dose interruptions/modifications

AE Terms & Descriptions	Dose Modification Algorithms
Hypertension	
(A). Asymptomatic and persistent SBP of ≥140 and <170 mmHg, or DBP ≥90 and <110 mmHg, or a clinically significant increase in DBP of 20 mmHg (but still below 110 mmHg).	Step 1. Continue pazopanib at the current dose. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled[a] blood pressure (BP). If BP is not well-controlled within 2 weeks, consider referral to a specialist and go to scenario (B).
(B). Asymptomatic SBP ≥170 mmHg, or DBP ≥110 mmHg, or failure to achieve well-controlled BP within 2 weeks in scenario (A).	Step 1.Consider reducing or interrupting pazopanib, as clinically indicated. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled BP. Step 4. Once BP is well-controlled, restart pazopanib dose-reduced by 200 mg if pazopanib was interrupted.
(C). Symptomatic hypertension or recurring SBP ≥170 mmHg, or DBP ≥110 mmHg, despite modification of antihypertensive medication(s)	Step 1. Interrupt pazopanib. Step 2. Adjust current or initiate new antihypertensive medication(s). Step 3. Titrate antihypertensive medication(s) during next 2 weeks as indicated to achieve well-controlled BP. Referral to a specialist for further evaluation and follow-up is also recommended. Step 4. Once BP is well-controlled, restart pazopanib dose-reduced by 200 mg.
(D). Refractory hypertension unresponsive to above interventions.	Discontinue pazopanib and continue follow-up per protocol.
Prolongation of QTc Interval: If the QTc is prolonged, the ECG should be manually read to ensure accuracy of the reading. The values below refer to manually-read ECGs (see section **Error! Reference source not found.**).	
QTc ≥ 480 < 500 msec	Continue pazopanib; monitor as clinically indicated.
QTc ≥500 msec	Discontinue pazopanib and continue follow-up per protocol.
Proteinuria	
UPC <3	Continue pazopanib at the current dose; monitor as clinically indicated.
UPC ≥3 or 24-h urine protein ≥3g	Step 1. Interrupt pazopanib. Step 2. Weekly UPC or 24-hr urine protein monitoring until UPC is <3 or 24-hr urine protein is <3 grams. Then restart pazopanib dose-reduced by 200 mg. Step 3. If UPC ≥3 or 24-h urine protein ≥3g recurs, repeat steps 1 and 2. Step 4. If UPC ≥3 or 24-hr urine protein ≥3 recurs and the pazopanib dose can no longer be reduced, discontinue pazopanib and continue follow-up per protocol.
Hemorrhage /Bleeding: Investigate and document underlying etiology of the bleeding	

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AE Terms & Descriptions	Dose Modification Algorithms
Grade 1	For hemoptysis, interrupt pazopanib and contact the GSK Study Physician to discuss whether further treatment with pazopanib is appropriate. For other Grade I hemorrhage/bleeding events, continue pazopanib at the current dose; monitor as clinically indicated.
Grade 2	Step 1. If pulmonary or GI bleed (other than hemorrhoidal bleeding), discontinue pazopanib and continue follow-up per protocol. Otherwise, interrupt pazopanib until the AE resolved to ≤ Grade 1. Step 2. Restart pazopanib; consider reducing dose and monitor as clinically indicated.
Grade 3 or 4, or Recurrent ≥ Grade 2 event after dose interruption/reduction.	Discontinue pazopanib and continue with follow-up per protocol.
Venous Thrombosis (DVT, PE)	
Grade 2	Continue pazopanib at the current dose; monitor as clinically indicated
Grade 3	Step 1. Interrupt pazopanib. Step 2. Initiate and monitor anticoagulation as clinically indicated. Step 3. Resume pazopanib at same dose only if all of the following criteria are met: • The subject must have been treated with anticoagulant at the desired level of anticoagulation for at least one week. • No Grade 3 or 4 or clinically significant Grade 2, hemorrhagic events have occurred while on anticoagulation treatment. Subject should be monitored as clinically indicated during anticoagulation treatment and after resuming study treatment. When treating with warfarin, international normalized ratio (INR) should be monitored within three to five days after any change in pazopanib dosing (eg, re-initiating, escalating/de-escalating, or discontinuing pazopanib), and then at least weekly until the INR is stable. The dose of warfarin (or its derivatives) may need to be adjusted to maintain the desired level of anticoagulation
Grade 4 and/or PE	Discontinue pazopanib and continue follow-up per protocol.
Arterial Thrombosis/Ischemia	
Any Grade	Discontinue pazopanib and continue follow-up per protocol.
Thrombocytopenia: Investigate and document underlying cause	
Grade 1 or 2	Continue pazopanib with current dose; monitor as clinically indicated.
Grade 3 or 4	Step 1. Interrupt pazopanib until toxicity resolves to ≤ Grade 2. Step 2. Restart pazopanib dose-reduced by 200 mg and monitor as clinically indicated. If no recovery to ≤ Grade 2 or recurrent Grade 3 or 4 thrombocytopenia, discontinue pazopanib and follow-up per protocol.
Anemia: No specific dose reduction rules are indicated for anemia unless due to hemorrhage or bleeding as noted above.	

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AE Terms & Descriptions	Dose Modification Algorithms
Palmar-plantar Erythrodysesthesia Syndrome	
Grade 1 Minimal skin changes or dermatitis without pain (erythema, oedema, hyperkeratosis)	1. Continue pazopanib at present dose
Grade 2 Skin changes with pain; limiting instrumental activities of daily living (ADLs) (peeling, blisters, oedema, bleed, hyperkeratosis)	1. Hold pazopanib 2. Treat as clinically appropriate 3. Upon resolution to Level 1 or better restart pazopanib with a dose reduction to 400 mg 4. If recurrent consider a further dose reduction to 200mg or discontinuation
Grade 3 Severe skin changes with pain and limiting self care ADLs	1. Discontinue pazopanib
Other Clinically Significant Adverse Events[b]	
Grade 1	Continue pazopanib; monitor as clinically indicated.
Grade 2 or 3, if clinically significant	Step 1. Interrupt pazopanib until toxicity resolves to ≤ Grade 1. Step 2. Restart pazopanib dose-reduced by 200 mg and monitor as clinically indicated.
Grade 4	Discontinue pazopanib and continue follow-up per protocol.

a. Well-controlled BP defined as SBP <140 mmHg and mean DBP <90 mmHg.
b. AEs are graded according to NCI Common Terminology Criteria for Adverse Events v4.0 (NCI CTCAE v4)
Abbreviations: BP, blood pressure..

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Event	Dose Modification Algorithms
(A). ALT of ≤ 3.0 x ULN	Continue pazopanib at current dose with full panel LFTs[c] monitored as per protocol.
(B). ALT >3.0 x ULN to ≤8.0 x ULN **without** bilirubin elevation (defined as total bilirubin[d] <2.0 x ULN or direct bilirubin ≤35%) and **without** hypersensitivity symptoms (e.g., fever, rash)	**Liver Event Monitoring Criteria:** (1) Continue pazopanib at current dose levels. (2) Monitor subject closely for clinical signs and symptoms; perform full panel LFTs[a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1.
(C). ALT >8.0 x ULN **without** bilirubin elevation (defined as total bilirubin[b] <2.0 x ULN or direct bilirubin ≤35%) and **without** hypersensitivity symptoms (e.g., fever, rash)	**1st occurrence – Liver Event Interruption Criteria[e]:** (1) Interrupt pazopanib until toxicity resolves to ≤Grade 1 or baseline. Report the event to GSK as an SAE **within 24 hours** of learning of its occurrence and complete the eCRF liver event forms. Make every reasonable attempt to have subjects return to the clinic within 24 to 72 hours for repeat liver chemistries and liver event follow up assessments. (2) Liver imaging and other laboratory investigations should be considered as clinically appropriate. (3) Monitor subject closely for clinical signs and symptoms; perform full panel LFTs [a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1. (4) If the subject is benefiting from the study treatment, contact GSK Study Physician for possible re-challenge. Re-treatment may be considered if ALL following criteria are met: - ALT/AST reduced to Grade 1 - Total bilirubin <1.5 x ULN or direct bilirubin ≤35% - No hypersensitivity signs or symptoms - Subject is benefiting from therapy. If approval for re-treatment is granted, the subject must be re-consented (with a separate informed consent specific to hepatotoxicity). **Recurrence – Liver Event Stopping Criteria[e]:** Discontinue pazopanib permanently and monitor subject closely for clinical signs and symptoms; perform full panel LFTs [a] weekly or more frequently if clinically indicated until ALT/AST is reduced to Grade 1. At the time of the recurrence, complete the eCRF liver event forms.
(D). ALT >3.0 x ULN **with** concomitant elevation in bilirubin[d] (defined as total bilirubin ≥2.0 x ULN; with direct bilirubin >35%) or **with** hypersensitivity symptoms (e.g., fever, rash).	**Liver Event Stopping Criteria[e]:** (1) Discontinue pazopanib immediately, report the event to GSK as an SAE **within 24 hours** of learning of its occurrence , and complete the eCRF liver event forms. Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries and liver event follow up assessments. (2) Consult a gastroenterologist / hepatologist and perform the following assessments to identify potential co-factors: - Eosinophil count - Viral serology for hepatitis A, B, C and E, cytomegalovirus, Epstein-Barr virus (IgM antibody, heterophile antibody, or monospot testing) - Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies. - Serum creatinine phosphokinase for possible muscle injury caused LFT elevation - Liver imaging -Consider toxicological blood screen for possible contributing chemical/medical entities (3) Monitor subject closely for clinical signs and symptoms; record the appearance or worsening of clinical symptoms of hepatitis, or hypersensitivity, such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever rash or eosinophilia as relevant on the AE report form. Perform full panel LFTs [a] weekly or more frequently if clinically indicated until LFTs are reduced to Grade 1.

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Event	Dose Modification Algorithms
For isolated total bilirubind elevation without concurrent ALT increases (defined as ALT <3 X ULN).	(1) Isolated hyperbilirubinemia (i.e., in the absence of elevated ALT or other signs/symptoms of liver injury) does not require dose modification. Pazopanib inhibits UGT1A1 and OATP1B1, which can cause elevation of indirect (unconjugated) bilirubin in the absence of liver injury.. (2) If bilirubin is >1.5 x ULN in the absence of ALT elevation, fractionation of bilirubin elevation should be performed. If bilirubin is >35% direct (conjugated), further evaluation for underlying cause of cholestasis should be performed.

c. Full panel LFTs include: AST, ALT, alkaline phosphatase, GGT, and total bilirubin. Coagulation tests should be performed as clinically indicated.

d. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable and a subject meets the criterion of total bilirubin >1.5 x ULN, then the event should be promptly reported as an SAE.

e. When a liver chemistry event meets the Liver Event Interruption Criteria, or Liver Event Stopping Criteria, blood samples should be obtained for PK and for clinical laboratory testing by the central laboratory (Liver Event Kits will be provided for this purpose).

Abbreviations: ALT alanine aminotransferase; AST aspartate aminotransferase; eCRF electronic case report form; IP investigational product; LFT liver function tests; SAE serious adverse event; ULN upper limit of normal

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