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Fluorescence-guided visualization of soft tissue sarcomas by targeting vascular endothelial growth factor-A: a phase 1 single-center clinical trial

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Key Words: soft tissue sarcoma, fluorescence-guided surgery, tumor targeting, molecular imaging, vascular endothelial growth factor A, near-infrared fluorescence

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ABSTRACT

Rationale

Resection of soft tissue sarcomas (STS) is accompanied by a high rate of tumor-positive surgical margins (14-34%), which potentially leads to decreased disease-free survival. Vascular Endothelial Growth Factor-A (VEGF-A) is overexpressed in malignant tumors, including STS, and can be targeted with bevacizumab-800CW during fluorescence-guided surgery for real-time tumor detection. In this phase 1 clinical trial, we determined the feasibility, safety and optimal dose of bevacizumab-800CW for fluorescence-guided surgery (FGS) in STS for *in- and ex vivo* tumor detection.

Materials and Methods

Patients with a histopathologically diagnosis of STS were included. In the dose-escalation phase, patients received bevacizumab-800CW intravenously 3 days prior to surgery (10, 25 and 50 mg, n=8). In the subsequent dose-expansion phase, 7 additional patients received bevacizumab-800CW at the optimal dose. Fluorescence images *in- and ex vivo* were obtained during all stages of standard of care. The optimal dose was determined by calculating *in-* and *ex vivo* Tumor-to-Background ratios (TBR) and correlating these results with histopathology.

Results

Fifteen patients with STS completed this study. All tumors could be visualized during *in- and ex vivo* imaging. The optimal bevacizumab-800CW dose proved to be 10 mg with a median *in vivo* TBR of 2.0 (±0.58) and an *ex vivo* TBR of 2.67 (±1.6). All seven tumor-positive margins could be observed real-time after surgical resection.

Conclusion

FGS using 10 mg bevacizumab-800CW is feasible and safe for intra-operative imaging of STS, potentially allowing tumor detection and margin assessment during surgery. An additional follow-up phase II study is needed to confirm the diagnostic accuracy.

Key Words: soft tissue sarcoma, fluorescence-guided surgery, molecular imaging, vascular endothelial growth factor A

INTRODUCTION

Soft Tissue Sarcoma (STS) account for less than 1% of all cancers diagnosed, with an estimated incidence in the United States of approximately 16.000 diagnosed annually.(*1*) STS comprises a histopathologically heterogenous group of mesenchymal tumors, consisting of approximately 50 subtypes(*2*), with challenging diagnosis and treatment pathways. Surgical excision remains the cornerstone of therapy for localized primary STS, and is usually combined with neoadjuvant or adjuvant radiotherapy. The main goal of surgery is to resect a STS with sufficient margin, excising the tumor and sufficient surrounding normal tissue. A tumor-negative margin is an important prognostic factor for local recurrence and disease-free survival.(*3*,*4*) A tumor-positive margin, which occurs in 14-34% of the cases, results in local recurrence rates of approximately 35%.(*5*) By histology, a tumor-positive margin is defined by tumor extension in the inked resection margin. However, microscopic tumor extension within 1 mm of the surgical margin may also be considered as a tumor-positive margin, as both conditions are associated with increased risk of local recurrence. In case of a tumor-positive margin an additional re-resection of the surgical cavity has to be considered.(*5*) However, a re-resection can be challenging because of complex anatomy in the surgical field as a result of postoperative fibrosis and the presence of neurovascular structures, which increases risk of morbidity. Therefore, in many cases, proper clinical decision-making during surgery is a clinical trade-off in which achieving a tumor-negative surgical margin and functional outcome must be weighed. It is currently difficult for the surgeon to assess margin status during surgery, emphasizing the need for an intra-operative real-time imaging technique. During STS surgery, a clinician should be able to visualize tumor cells in the rim of the resection margin, both *in*- and *ex vivo*. Fluorescence-

guided surgery (FGS) is an optical imaging method that provides real-time tumor detection and can be used for margin assessment. In the past years, several studies have investigated the potential merits of FGS in different malignancies such as breast cancer, peritoneal metastasis, colon cancer, glioblastoma and head and neck cancer.(*6*–*11*) These studies revealed promising data on the potential clinical benefit of detecting residual disease for intra-operative decision-making. For STS, only preclinical studies and one proof-of-concept study in a single patient using ABY-029 focusing on biomarkers like epidermal growth factor receptor for FGS have been reported.(*12*,*13*)

In STS, a patient-tailored surgical plan is designed, based on tumor location and histopathological tumor classification, using immunohistochemistry and molecular genetics, and tumor grading.(*14*) High-grade STS with extra-compartmental invasive growth has relatively decreased overall survival and event-free survival, by which one strives for an adequate wide en-bloc resection.(*15*) In particular, myxofibrosarcomas are associated with tumor-positive margins, which is due to its diffuse reticular growth in subcutaneous fat or muscle, whereas other STS, e.g. myxoid liposarcomas tend to have a pushing invasive border, by which they are easier to excise, with narrow but clear margins.(*16*)

Vascular Endothelial Growth Factor-A (VEGF-A) is involved in angiogenesis and lymph angiogenesis and is overexpressed in many solid tumors.(*17*) Overexpression of VEGF-A in STS has been reported.(*18*–*20*) The therapeutic monoclonal antibody Bevacizumab (Avastin®) binds to VEGF-A. By conjugating bevacizumab to the organic fluorophore IRDye800CW, a tumor-specific tracer (bevacizumab-800CW) could be developed.(*9*,*21*,*22*) In previous studies using this tracer, bevacizumab-800CW was shown to be safe for use in humans.

The aim of this FGS study was to determine the feasibility, safety and the optimal dose of bevacizumab-800CW for *in-* and *ex vivo* detection and margin assessment of STS, using a standardized fluorescence imaging workflow.(*22*,*23*)

MATERIALS AND METHODS

This phase 1 single-center feasibility study was performed at the University Medical Center of Groningen. The study was approved by the Institutional Review Board (IRB) of the University Medical Center of Groningen (IRB number 2017/302) and conducted according to the principles of Helsinki (adapted version Fortaleza, Brazil, 2013) and the laws and regulations of the Netherlands. The trial was registered at www.clinicaltrials.gov (NCT03913806). All patients provided written informed consent before participation in the study.

Patients

Patients aged older than 18 years with histopathologically proven STS and appropriate imaging (CT/MRI), scheduled for surgical excision, were included. All patients had a World Health Organization performance score of 0-2. Patients with concurrent invasive malignancy were excluded. Other exclusion criteria were: medical or psychiatric conditions compromising the patient's ability to give informed consent, pregnant or lactating women, a history of infusion reactions to bevacizumab, inadequately controlled hypertension or a history of myocardial infarction, transient ischemic attack, cerebrovascular accidents, pulmonary embolism, uncontrolled chronic hepatic failure or unstable angina pectoris six months prior to inclusion. All patients were discussed in the multidisciplinary sarcoma team meeting prior to surgery.

Study Design

The primary objective of the study was to determine the feasibility of bevacizumab-800CW in STS for *in vivo* and *ex vivo* tumor detection. Secondary objectives were to identify the optimal dose

for visualization of STS tissue and to obtain information on safety aspects of the tracer in STS patients. A classical 3 x 3 dose-finding study design was used, consisting of two parts. In the dose-escalation phase (part I) three dose cohorts were tested. Three subjects per cohort received intravenously administered flat doses of respectively 10mg, 25mg and 50mg three days before surgery. In the dose-expansion phase (part II), the optimal dosing group was expanded up to 10 subjects. The optimal dose group was chosen based on *in-* and *ex vivo* tumor-to-background ratio (TBR). The study design was adapted after the conclusion of part I to exclude patients who underwent neoadjuvant treatment in the dose-expansion phase.

Safety Assessment

Vital signs were measured before, directly after and 1 hour after tracer administration. Follow-up was performed up to two weeks after tracer administration to assess adverse events which were scored according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Bevacizumab-800CW Production

Bevacizumab-800CW was produced in the Good Manufacturing Practice (GMP) facility of the Department of Clinical Pharmacy and Pharmacology at the University Medical Center of Groningen as reported previously.(*21*)

Intra-operative Fluorescence Imaging

Fluorescence imaging of the tumor during surgery was performed with the SurgVision Explorer Air as described earlier.(*22*) Once excision of the tumor was completed, fluorescence imaging of all resection planes of the surgical specimen was performed immediately (i.e.< 5 minutes) after excision with the SurgVision Explorer Air coupled to a closed-field imaging box (i.e. the Vault, SurgVision BV., Groningen, The Netherlands) and within 1 hour after excision with the PEARL Trilogy Imaging system (LI-COR BioSciences, Lincoln, NE, USA). Image acquisitions settings for Explorer Air and Pearl Trilogy were as described previously by our group for both *in*- and *ex vivo* imaging.(*22*) No surgical decisions were made based on fluorescence imaging.

Fluorescence-guided Pathology

The fresh surgical specimen was inked and serially sliced following standard protocols for pathological specimen handling. Fluorescence images of both sides of the tissue slices were obtained using the PEARL Trilogy. Next, tissue slices were formalin fixed for 1-4 days and imaged with the PEARL Trilogy before embedding (Fig. 1). A pathologist, blinded for fluorescence, selected clinically relevant regions for further embedding in formalin-fixed and paraffin embedded (FFPE) blocks. Based on fluorescence imaging, additional regions of interest were selected which were also embedded in additional FFPE blocks. A standard fluorescence-guided workflow was used to cross-correlate the fluorescent tissue slices to final histopathology.(*22*) Hematoxylin and eosin (H/E) stained slides were reviewed by a pathologist blinded for the results of fluorescence imaging. The complete workflow of the study is depicted in Figure 1.

Fluorescence Quantification

The *in vivo* TBR was calculated as mean fluorescence intensity (MFI) of the tumor / MFI of the background. The tumor was delineated on macroscopic visualization. The background MFI was calculated on all non-tumor tissue surrounding the tumor in the surgical field. Mean Fluorescence Intensities (MFI, arbitrary units) were calculated on SurgVision Explorer Air images (exposure time 25 or 50 ms, gain 10 to 100). For the whole specimen imaging immediately after excision the maximum fluorescence intensities (MaxFI) were calculated on the whole resection margin to correlate signal intensity to margin depth.

Ex vivo TBRs were calculated on fresh serially sliced tissue slices. The tumor and the surrounding non-tumor tissue were precisely delineated on standard H/E histopathology by a pathologist blinded for fluorescence. An overlay with fluorescent tissue slices was made based on anatomical landmarks. Afterwards, an *ex vivo* TBR was calculated as mean fluorescence intensity (MFI) of the tumor / MFI of the background. The background MFI was calculated on all non-tumor tissue for every tissue slice.

Statistics

Descriptive statistics were reported as means with standard deviation (SD) in case of normal distribution, whereas median with interquartile range was used in case of a skewed distribution. Fluorescence signals in tumor and normal tissue were compared using the Mann-Whitney test. A P-value of <0.05 was considered statistically significant. For descriptive statistics SPSS (version 23.0; SPSS Inc, Armonk, NY) was used.

RESULTS

Between April and December 2019, fifteen patients with seven different histopathological subtypes of STS, including 7 myxofibrosarcomas, were enrolled in this dose escalation trial. Patient, safety and tumor characteristics are depicted in Table 1 and Supplemental Table 1. Bevacizumab-800CW was administered three days prior to surgery to all patients. No tracer related (serious) adverse events were observed (Table S1).

Phase 1 – Dose Escalation

A total of eight patients were included in the dose escalation phase (10 mg N=3, 25 mg N=3 and 50 mg N=2). All tumors were adequately visualized *in vivo* regardless of dose (10mg: TBR 2.0±0.58; 25mg: TBR 2.5±0.32; and 50mg: TBR 2.0±0.10) (Fig. 2). After excision, tumor tissue was adequately visualized on the excised specimen (10mg: TBR 2.0±1.1; 25mg TBR 2.5±0.31). No reliable *ex vivo* TBR of 50mg patients could be calculated due to lack of non-tumor background tissue. Since no increase in *in vivo* TBR was obtained for the 50mg cohort after two patients, only two patients were enrolled in this cohort. Based on no statistical differences between the in vivo 10 and 25mg cohort, (p=0.18), the 10mg was considered to be the optimal dosing group and was expanded with 7 additional patients.

Ex Vivo Fluorescence and Quantification on Tissue Slices

To quantify fluorescence, quantification on fresh tissue slices was performed. All seven histopathological STS subtypes could be visualized *ex vivo*, displaying a sharp, clearly delineated fluorescent signal in all tissue slices containing tumor (Fig. 3). Significantly higher fluorescence

signals were seen in tumor tissue compared to non-tumor tissue in the 10 mg group (Fig 3., n = 22, *P*<0.001, median MFI of 0.013 for tumor tissue and median MFI of 0.004 for non-tumor tissue). The median *ex vivo* TBRs of the 10mg and 25mg dose cohort were 2.67±1.6 (n = 9, range 1.5-6.5) and 4.6±0.31 (n=2, range 4.5-4.7) respectively. In the STS series, all myxofibrosarcomas (n=7) could be visualized using fluorescence during *ex vivo* imaging (Supplemental Fig. 1). Due to the imaging resolution of the PEARL Trilogy, the border zone with strands of diffusely infiltrating tumor cells could not be individually identified with visual fluorescence inspection. In this study, we found that more solid and cellular tumor areas are easily identifiable.

In the tissue slices, a false-positive fluorescent signal could be observed in areas with a high macrophage content. We suspect this occurred due to an inflammatory response after tumor necrosis induced by neoadjuvant radiotherapy (Supplemental Fig. 1, a-c, m-o). For this reason, patients who received neoadjuvant radiotherapy were not eligible to participate during the dose expansion phase, to minimize false-positive non-tumor tissue signal.

Fluorescence Margin Assessment

After histopathological assessment, seven patients were diagnosed with a tumor-positive surgical margin (47%), containing three high-grade myxofibrosarcomas, two high-grade undifferentiated sarcomas, and one synovial sarcoma. In one retroperitoneal well-differentiated and focally dedifferentiated liposarcoma the margin was considered positive due to size of the disease and limitations in surgical excision but was not included in our fluorescence analysis. All tumor-positive surgical margins were detected using fluorescence imaging of the resected specimen (7/7). Three patients had a histological proven tumor-negative surgical margin (20%),

of which fluorescence predicted 1/3 correct (33%). One margin was false-positive due to neoadjuvant treatment while the other margin was false-positive due to a highly fluorescent cyst. Five patients had a histological proven close surgical margin (33%) ranging from 0.6 mm to 1.4 mm, of which one was false-positive for fluorescence due to neoadjuvant treatment and therefore excluded in our analysis (n=4). Quantification of MaxFI on whole specimen imaging with a close surgical margin (0.6 - 1.4 mm) showed a non-significant trend in MaxFI on resection margins with the closest surgical margin (Fig. 3). For all patients (n = 4, 13 resection margins evaluated) with a close surgical margin (range from 0.6 mm – 1.4 mm) the surgical side with the closest surgical margin had the highest maximum fluorescence intensity, suggesting a relation between maximum fluorescence intensity and margin depth (Fig. 4).

DISCUSSION

In this pilot study, we have shown that that fluorescence-guided surgery using bevacizumab-800CW is feasible and safe in a variety of histological subtypes of soft tissue sarcomas using both *in-* and *ex vivo* fluorescence imaging. 10 mg bevacizumab-800CW proved to be safe and sufficient for adequate *in-* and *ex vivo* imaging, whereas a dose of 25mg or 50mg did not significantly improve fluorescence visualization. Moreover, we show that FGS has great potential value for tumor detection and margin assessment during STS surgery. This is evident after studying depth assessment for close surgical margins using *ex vivo* fluorescence-guided specimen imaging immediately after excision, as shown in previous FGS studies.(*22*,*24*) We believe that this technique has the potential to optimize surgical quality and decrease the amount of tumor-positive surgical margins.

Tumor histotype and grade as well as anatomical location of STS are important determinants of local recurrence. Ideally, FGS should reflect tumor infiltration patterns (diffuse vs. pushing infiltrative borders) during surgery. Our data on fluorescence visualization of myxofibrosarcomas confirm that this tumor is accompanied by frequent positive surgical margins due to diffuse, reticular invasive growth in surrounding soft tissue. Despite the fact that the solid cellular tumor areas could be easily identified, future studies should determine whether FGS can enhance intra-operative margin assessment in diffusely infiltrative STS.

In our previous studies in breast cancer we confirmed the co-localization of bevacizumab-800 with VEGF-A IHC. In 90% of all patients there was adjacent/complete overlap of bevacizumab-800CW and VEGF-A immunohistochemistry staining, which also could be observed in immunohistochemistry analysis in STS tissue (Supplemental Fig. 2).(*10*) As VEGF-A staining is heterogenous in different STS subtypes and would only be reliable if executed thoroughly

analyzing the whole surgical specimen, we did not include a full IHC analysis in this study. Nevertheless, VEGF-A expression in myxofibrosarcomas has recently been observed in 93% as a suitable marker for FGS and VEGF-A expression significantly correlated with clinical variables like high histological grade and distant metastasis.(*25*,*26*) Our imaging results, showing specific fluorescence activation in areas containing soft tissue tumor as assessed by H/E staining confirm these preclinical results and are in accordance with earlier clinical reported data. Tumor necrosis, frequently observed in high-grade STS, may be induced by neoadjuvant radiotherapy. In general, tumor necrosis is a significant predictor of relapse-free survival and overall survival in STS patients.(*27*) Bevacizumab-800CW is not adequately visualized in necrotic areas, as no adequate penetration and specific binding could be obtained. We observed false-positive fluorescent signals in areas with high macrophage content and reactive fibrosis related to the inflammatory response after neoadjuvant radiotherapy. Therefore, FGS with bevacizumab-800CW for real-time margin assessment seems to be most suitable during primary surgery with the use of radiotherapy in an adjuvant setting. In a phase II study, a cohort with a significant amount of neoadjuvant treated patients should be included to compare the results of fluorescence-guided surgery in patients with soft tissue sarcomas undergoing neoadjuvant or adjuvant treatment.

As previously described in head- and neck specimens, quantification of all resection planes of the whole specimen directly after surgical excision using a fluorescence signal surface map, may identify margin depth with fluorescence intensity peaks. The margin segment with the highest fluorescence intensity was termed the sentinel margin—the location where the closest margin is mostly likely to be located. (*28*) Although we could correlate the maximum fluorescence intensity to a close surgical margin, our study was not powered to determine the invasion depth

on a microscopic level. Currently, it is challenging to determine sentinel margins in sarcoma surgery as fluorescence imaging of especially the deep resection margins is not feasible using the current available large intra-operative imaging devices. Sarcomas are usually in tight anatomical spaces requiring challenging positioning of intra-operative devices. Furthermore, the variation in imaging acquisition settings and the absence of standardization may result in a lack of quality control. Ex vivo fluorescence imaging with so-called closed-field cameras enables better control of image acquisition parameters and removes interference of ambient light. Our *ex vivo* analysis can be performed within 5-10 minutes after surgical excision, as *ex vivo* imaging enables a standardized and reproducible image analysis platform to guide surgical decision making. Standardizing fluorescence imaging could minimize signal inhomogeneity that can lead to erroneous tumor delineation. In a future scenario, on-site examination in the surgical theatre by a pathologist equipped with a fluorescence imaging system may guide the surgeon in adapting surgical treatment decisions. Based on intra-operative imaging findings and the outcome of fluorescence-guided pathology, this may potentially prevent both under- and over-treatment and diminish surgical reinterventions at a later stage.

CONCLUSION

To conclude, this study is a first proof-of-concept that FGS using a flat dose of 10 mg bevacizumab-800CW is feasible in a variety of STS subtypes. Moreover, the potential value of fluorescence imaging during *ex vivo* pathology assessment was evident. FGS appears to be most valuable in patients undergoing primary surgery without neoadjuvant treatment. A subsequent phase-II trial will be performed in our institute to determine the sensitivity and specificity of FGS in patients treated with primary surgery with curative intent.

DISCLOSURE

The research leading to the results was supported by an unrestricted research grant from SurgVision BV. GMvD is consultant for OncoNano Medicine Inc. and CEO, founder and shareholder of the AxelaRx / TRACER BV group. No other potential conflicts of interest relevant to this article exist.

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KEY POINTS

Question

The incidence of tumor-positive surgical margins is soft tissue sarcomas (STS) is high and in this clinical research we investigated whether fluorescence-guided surgery using a targeted imaging approach with bevacizumab-800CW for in- and ex vivo tumor detection has the potential to decrease the amount of tumor-positive surgical margins.

Pertinent Findings

This phase I single-center feasibility study recruited 15 patients with histopathological proven STS who received bevacizumab-800CW intravenously three days prior to surgery and underwent fluorescence-guided surgery. All soft tissue sarcomas could be visualized during in vivo and ex vivo imaging. A flat dose of 10 mg bevacizumab-800CW was proven to be the optimal dose and all (7/7) tumor-positive margins could be observed in real-time by back-table fluorescence imaging immediately after surgical resection.

Implications for Patient Care

Fluorescence-guided surgery using a flat dose of 10 mg bevacizumab-800CW is feasible in a variety of STS subtypes, potentially allowing for improved tumor detection to optimize surgical quality and decrease the amount of tumor-positive surgical margins.

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FIGURES

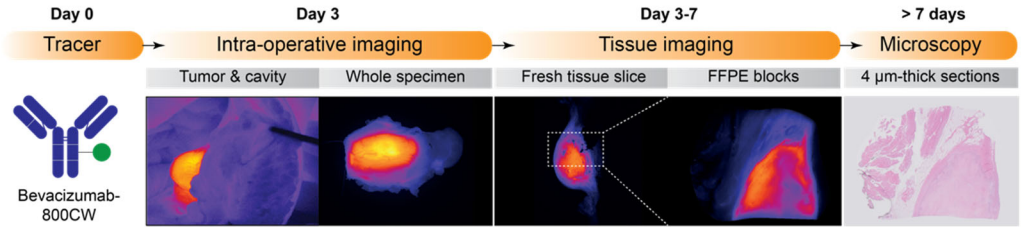


Figure 1 | Standardized fluorescence imaging workflow

Patients received bevacizumab-800CW intravenously three days prior to surgery. During surgery, fluorescence imaging of the tumor *in vivo* was performed using SurgVision Explorer Air. Directly after excision, imaging of the whole surgical specimen was performed using SurgVision Explorer Air and PEARL Trilogy. After serial slicing performed by the pathologist, fluorescence imaging of all steps of histopathological processing was performed and cross-correlation with standard of care H/E slides was done.

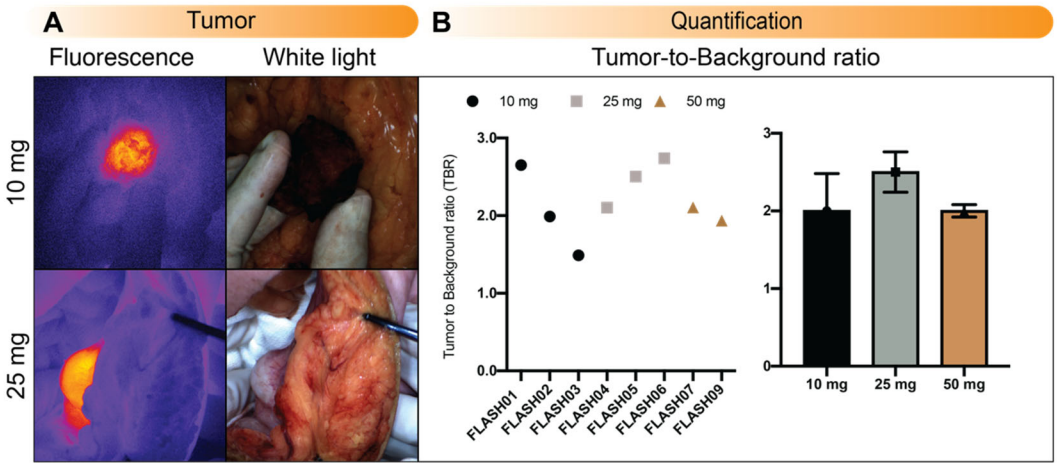


Figure 2 | In vivo visualization of soft-tissue sarcomas

Intra-operative imaging was performed with the SurgVision Explorer Air. Fluorescence and white light images of an intra-abdominal leiomyosarcoma *in vivo* using 10 mg bevacizumab-800CW and a synovial sarcoma using 25 mg bevacizumab-800CW were obtained (A). Median *in vivo* Tumor-to-Background Ratio (TBR) for all patients in the dose escalation phase (n=8) were calculated. Median *in vivo* TBRs are calculated per dosing group (B).

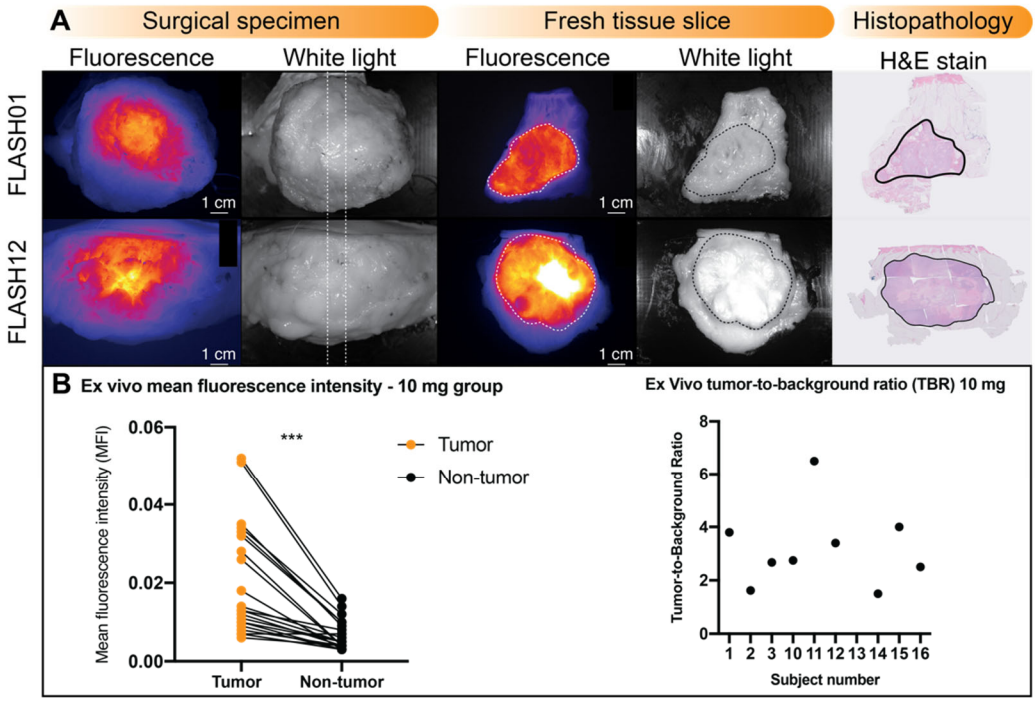


Figure 3 | Ex vivo visualization of soft tissue sarcomas

Leiomyosarcoma and myxofibrosarcoma with whole specimen image of a close surgical margin on the basal resection margin (0.6m) and a close surgical margin on the caudal resection margin (0.6cm). Fluorescence signal on tissue slice with the tumor delineated with a dashed line and a close surgical margin on the basal resection margin and caudal resection margin. The tumors are delineated with a solid black line on standard H/E staining (A). Sub-analysis in the 10 mg group showed a significantly higher mean fluorescence intensity (MFI) in all tumor tissue compared to non-tumor tissue (n = 22, P<0.001) (k) and all *ex vivo* TBRs are presented for all patients in the 10 mg group, n=8, no data available for FLASH03 (false-positive signal) and FLASH13 (angiosarcoma, no reliable calculation possible) (B).

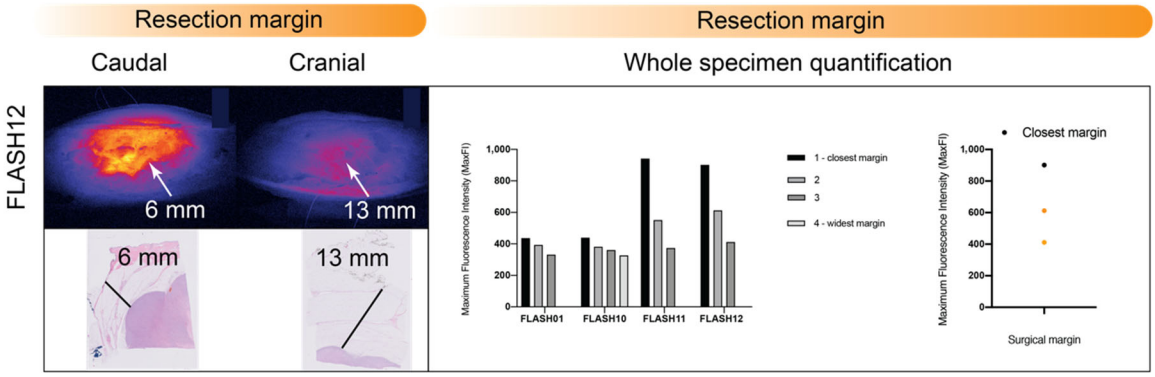


Figure 4 | Ex vivo whole specimen imaging directly after excision

Immediately after surgical excision (< 5 minutes) standardized fluorescence images of all resection planes of the surgical specimen were obtained. In this myxofibrosarcoma, the caudal resection margin showed a high maximum fluorescence intensity (MaxFI=901) which correlated to a close surgical margin of 6mm, whilst the cranial resection margin showed a lower MaxFI of 612 correlating with a deeper resection margin of 13mm. Maximum fluorescence intensity signals were calculated on all close resection margins from four patients. 1 is the closest surgical margin as described by standard histopathology and 4 is the widest surgical margin. Per patient analysis for patient FLASH12 is presented.

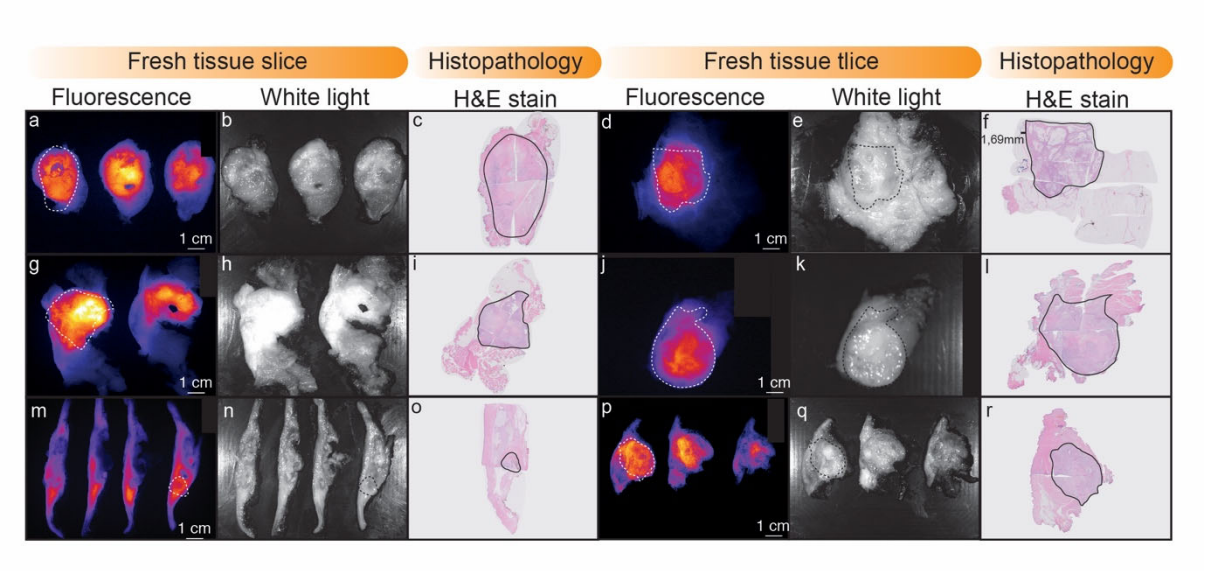
TABLES

Table 1. Clinical and pathological characteristics of patients who underwent fluorescent guided surgery for soft tissue sarcoma	
	All patients (*n* = 15)
Characteristic	
Age at surgery (years), *mean (range)*	66 (34-84)
Sex: males, *n (%)*	8 (53)
Weight (kg), *mean (range)*	82 (64-103)
Height (cm), *mean (range)*	174 (161-189)
Dosing groups, *n (%)* 10mg 25mg 50mg	 10 (67) 3 (20) 2 (13)
Tracer related adverse events, *n (%)*	0 (0)
Tumor type primary tumor, *n (%)* Myxofibrosarcoma High grade Mixed type Liposarcoma Synovial sarcoma Leiomyosarcoma Angiosarcoma Undifferentiated pleomorphic sarcoma	 7 (47) 6 (86) 1 (14) 3 (20) 2 (13) 1 (7) 1 (7) 1 (7)
Tumor diameter (mm), *mean (range)*	112 (18-320)
Localization tumor, *n (%)* Arms Legs Intraabdominal Hip/flank Breast	 4 (27) 4 (27) 4 (27) 2 (13) 1 (7)

Table 1 | Clinical and pathological characteristics

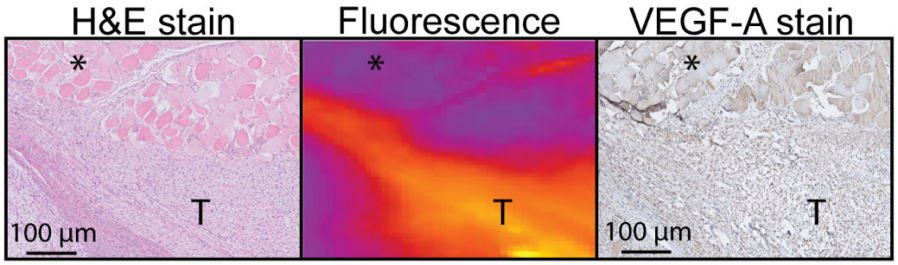
Data are given in numbers with percentages (%) or means with range

SUPPLEMENTAL FIGURES



Supplemental Figure 1 | Fluorescence of myxofibrosarcomas

Representative *ex vivo* images of six myxofibrosarcomas imaged with PEARL Trilogy in this study. The last myxofibrosarcoma is presented in Figure 3 panel f-j. The tumor is delineated on H&E staining with a black line, the white and black dotted lines represent the tumor on fluorescence and white light images. In two patients, a false-positive signal is observed possibly due to tumor necrosis and macrophage infiltration due to neoadjuvant treatment (a-c, m-o).



Supplemental Figure 2 | VEGF-A staining

Microscopic localization of bevacizumab-800CW in a myxofibrosarcoma. The T represents tumor tissue and the Asterix represents muscle tissue. High intensity of Vascular Endothelial Growth Factor-A is observed in tumor tissue.

SUPPLEMENTAL TABLE

S Table 1. Safety data of patients who underwent intravenous administration of bevacizumab-800CW prior to surgery for soft tissue sarcoma

Dose group	*(S)AE*	*CTCAE*	*Description*	*Events*	*Moment*	*Relation to tracer*
10mg	AE	1	Postoperative pain	6x	Post-operative	Not related
	AE	1	Hematochezia	1x	*	#
	AE	2	Opioid intoxication	1x	*	#
25mg	AE	1	Postoperative pain	2x	*	#
	AE	2	Mild pancreatic injury after surgery	1x	*	#
50mg	AE	1	Postoperative pain	2x	*	#
	AE	1	Hot flashes	1x	*	#
	AE	1	Fever	1x	*	#
	AE	1	Disorientation	1x	*	#

*All post-operative. #All not related.Abbreviations: (S)AE, (serious) adverse event; CTCAE, common terminology criteria for adverse events

Supplemental Table 1 | Safety data

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