SUPPLEMENTARY INFORMATION

Investigating the role of functional imaging in the management of soft-tissue sarcomas of the extremities

Supplementary Table 1. Abbreviations used in the text.

Abbreviation	Description
STS	soft-tissue sarcomas
PET	positron emission tomography
MRI	magnetic resonance imaging
FDG	^{18}F-Fluorodeoxyglucose
FMISO	^{18}F-Fluoromisonidazole
DW-MRI	diffusion-weighted magnetic resonance imaging
DCE-MRI	dynamic contrast-enhanced magnetic resonance imaging
SUV	standard uptake value
ADC	apparent diffusion coefficient
K^{Trans}	permeability constant
IAUC	initial area under the signal enhancement curve
Lung Mets	Patients that developed lung metastases
No Lung Mets	Patients that did not develop lung metastases
UPS	undifferentiated pleomorphic spindle cell sarcoma
ML	myxoid liposarcoma
LM	leiomyosarcoma
MF	myxofibrosarcoma
RCML	round cell/myxoid liposarcoma
PL	pleomorphic liposarcoma
SS	synovial sarcoma
FM	fibromyxoid sarcoma
PS	pleomorphic spindle cell sarcoma
MIF	myxoinflammatory fibroblastic sarcoma
GTV	gross tumour volume
CTV	clinical target volume
PTV	planning target volume

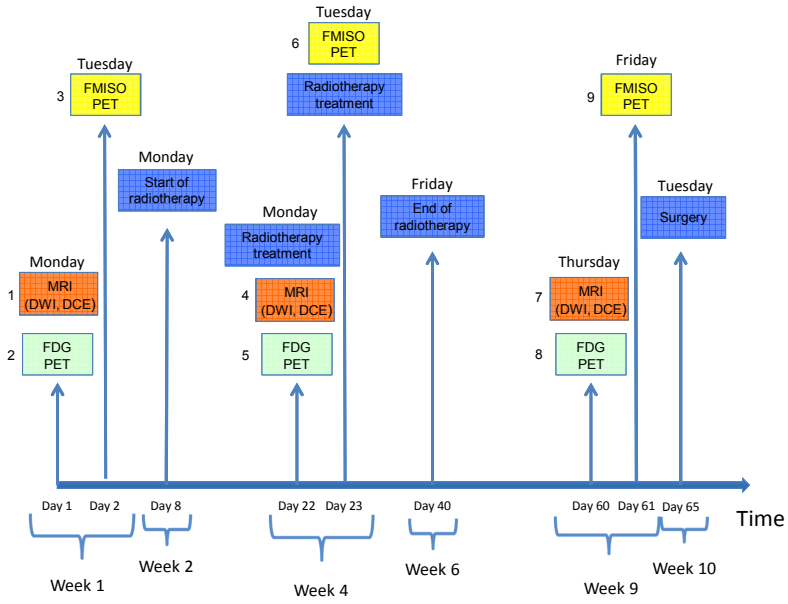
Supplementary Table 2. Clinical characteristics of patients.

Patient #	Gender	Age	Location (depth)	Size (cm)	Type (grade)	RT dose (Gy/#Fx)	Local failure (time-to-event)	Lung mets (time-to-event)	Other mets (time-to-event)	Follow-up (status)
S001	M	69	Shoulder (superficial)	> 10	UPS (high)	14/7	No	Yes (8 months)	No	10 months (PD)
S002	F	76	Arm (deep)	5 to 10	UPS (high)	50/25	Yes (12 months)	Yes (2 months)	LN/Bone	12 months (PD)
S003	M	46	Leg (deep)	> 10	ML (low)	50/25	No	No	Bone (36 months)	37 months (AWD)
S004	M	55	Thigh (superficial)	> 10	LM (intermediate)	50/25	No	No	No	33 months (NED)
S005	F	61	Thigh (deep)	< 5	MF (high)	50/25	No	No	No	32 months (NED)
S006	F	28	Thigh (deep)	> 10	RCML (high)	50/25	No	No	AW/PV (11/23 months)	35 months (NED)
S007	M	73	Thigh (deep)	> 10	ML (low)	50/25	No	No	No	25 months (NED)
S008	M	80	Shoulder (superficial)	> 10	PL (intermediate)	50/25	No	Yes (9 months)	Bone/Liver (2/9 months)	10 months (DOD)
S009	F	58	Arm (deep)	5 to 10	SS (NA)	50/25	No	No	No	28 months (NED)
S010	M	73	Chest wall (deep)	> 10	MF (high)	50/25	No	No	No	27 months (NED)
S011	F	73	Thigh (deep)	> 10	MF (high)	50/25	No	Yes (9 months)	No	23 months (AWD)
S012	F	62	Thigh (deep)	> 10	MF (high)	50/25	No	No	No	22 months (NED)
S013	M	57	Thigh (superficial)	5 to 10	MF (high)	50/25	No	Yes (6 months)	Reg. LN (5 months)	10 months (DOD)
S014	M	56	Thigh (deep)	5 to 10	MF (high)	50/25	No	No	No	22 months (NED)
S015	M	52	Thigh (deep)	> 10	FM (low)	50/25	No	No	No	20 months (NED)
S016	M	29	Shoulder (deep)	> 10	PS (high)	50/25	No	No	No	20 months (NED)
S017	F	34	Leg (deep)	5 to 10	ML (low)	0	No	No	No	15 months (NED)
S018	F	27	Arm (superficial)	5 to 10	MIF (NA)	50/25	No	No	No	13 months (NED)

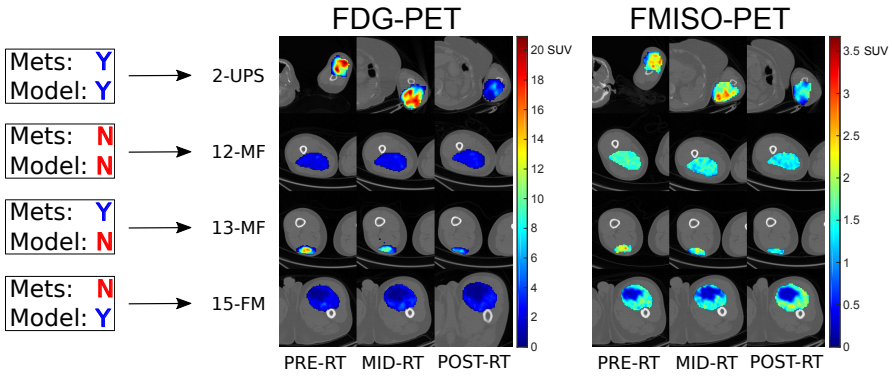
* The tumour type abbreviations UPS, ML, LM, MF, RCML, PL, SS, FM, PS and MIF stands for undifferentiated pleomorphic spindle cell sarcoma, myxoid liposarcoma, leiomyosarcoma, myxofibrosarcoma, round cell/myxoid liposarcoma, pleomorphic liposarcoma, synovial sarcoma, fibromyxoid sarcoma, pleomorphic spindle cell sarcoma and myxoinflammatory fibroblastic sarcoma, respectively.

* AWD: Alive with Disease, DOD: Dead of Disease, PD = Patient Deceased, NED: Alive with No Evidence of Disease.

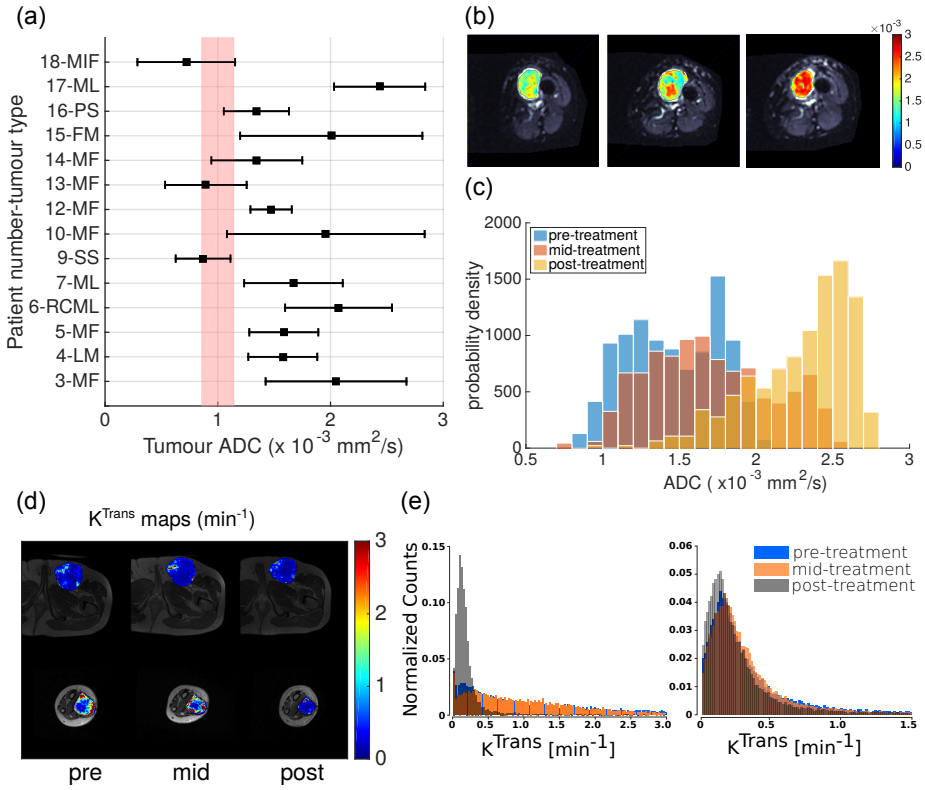
* AW: Abdominal Wall, PV: Paravertebral, LN: Lymph Nodes, NA: Non-Available



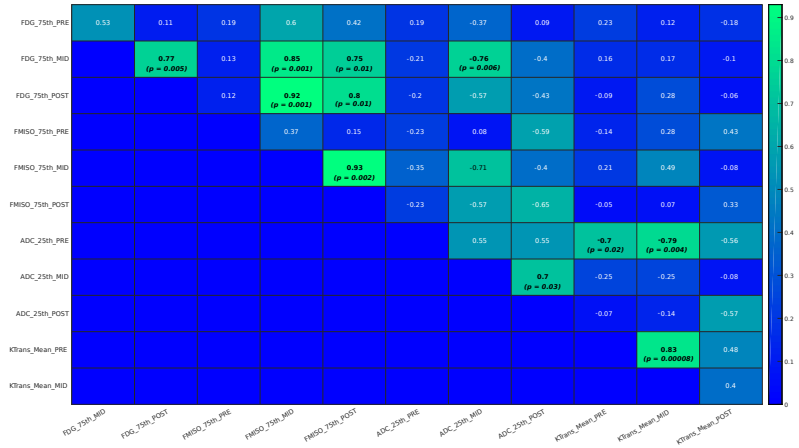
Supplementary Fig. 1. Timeline of the study protocol. Abbreviations are defined in Supplementary Table 1.



Supplementary Fig. 2. Evolution of FDG-PET (left) and FMISO-PET (right) intratumoural uptake of the central slice of the tumour of four example patients over the 3 time points during radiotherapy treatment management (PRE-RT, MID-RT, POST-RT). Note that different colorbars are used for FDG-PET and FMISO-PET. Two patients eventually developed lung metastases (Mets Y: 2-UPS, 13-MF) and two patients did not develop lung metastases (Mets N: 12-MF, 15-FM). The previously developed FDG-PET/MRI texture-based model [1] correctly predicted lung metastases development for two patients shown here (2-UPS, 12-MF) and was incorrect for the other two (13-MF, 15-FM). The tumour type abbreviations UPS, MF and FM stands for undifferentiated pleomorphic spindle cell sarcoma, myxofibrosarcoma and fibromyxoid sarcoma, respectively.



Supplementary Fig. 3. General observations from MRI.
(a) The ADC (mean ± standard deviation, in) for each tumour. The pink box represents the ADC range (one standard deviation) of muscle, computed from muscle regions across all patients. Patients are identified by their number in the sequence and the tumour type abbreviations are given in Supplementary Table 1.
(b) Top, L to R: Example ADC maps (tumour only, colour overlay on axial DW-MRI) from patient with myxofibrosarcoma (patient 5) shows the increase in ADC from pre- to post-radiotherapy.
(c) The therapy-induced increase in ADC in patient 5 is highlighted in the ADC distribution at pre-, mid- and post-treatment exams.
(d) Example K^{Trans} maps in two patients, showing a single slice for each (mid-tumour) at pre-, mid-, and post-treatment. Top row: patient 4 (leiomyosarcoma in the groin) showing little or no K^{Trans} changes during treatment. Bottom row: patient 9 (synovial sarcoma in the arm), showing evolution of tumour K^{Trans} during treatment.
(e) The histograms show the evolution of K^{Trans} distributions over the treatment course for each of the two patients (left: patient 9, right: patient 4).



Supplementary Fig. 4. Correlation analysis between the metrics presented in Fig. 2 of the main text. Spearman rank correlation coefficients were calculated between all pairs of metric vectors for all time points, where a metric vector for a given time point is defined as the vector of metric values for all patients available for that given metric and time point. "25th", "75th" and "Mean" refers to the 25^{th} percentile, 75^{th} percentile and average metrics, repectively. "PRE", "MID" and "POST" refers to pre-RT, mid-RT and post-RT time points, respectively. Note that the colorbar corresponds to absolute coefficients and that significant correlations are shown in bold.

1 Creation of tumour sub-region contours

Image analysis

In the last paragraph of section 2.4 of the main text, we briefly described how thresholds of the percentage of maximum intensity on imaging scans were manually defined to create discrete high-intensity sub-region contours for each patient. High-intensity sub-region contours were created for two reasons: i) investigate the complementarity between STS tumour sub-regions as defined by functional imaging in FDG-PET, FMISO-PET, DW-MRI and DCE-MRI; and ii) investigate how the different volumes of these sub-regions change over radiotherapy to act as potential markers of radiation response. Here, we provide more details about how the high-intensity sub-region contours were created for each scan:

1. The following scans were chosen for investigation: i) FDG-PET; ii) FMISO-PET; iii) apparent diffusion coefficient (ADC) maps in DW-MRI; and iv) maps of the initial area under the signal enhancement curve (IAUC) from the injection to 60 seconds post-injection in DCE-MRI. For this part of the study, IAUC maps were chosen over permeability constant maps (K^{Trans}) in DCE-MRI due to the lower tendency of IAUC maps to generate outlier intensity noise. Note that IAUC and K^{Trans} are also correlated to each other [2].

2. The maximum intensity value of each scan was found for all pre-RT scans. Thresholds of the maximum intensity value were manually varied around the 50 % value in order to obtain a smooth contour mask as much representative of high intensities as possible, separately for each patient and pre-treatment scan.

3. All pre-RT scans (FDG, FMISO, ADC, IAUC) were brought to a common space (T2-weighted MRI scan) using rigid registration. The overlap between all pairs of high-intensity masks was then calculated using DICE coefficients.

4. High-intensity tumour sub-region masks were then created for the mid-RT and post-RT time points using the same thresholds and methods as for the pre-RT time point.

5. The percentage volume of high-intensity tumour sub-regions relative to the whole tumour volume was calculated for each scan and for each time point.

6. Percentage volume of high-intensity tumour sub-regions was finally investigated as a potential marker of radiation response in STS.

Dose painting

All STS patients included in this study received standard radiotherapy as per the guidelines of our institution: i) The planning GTV was delineated on the MRI

co-registered to the planning CT scan; ii) The CTV margin was +3 cm proximal and distal and +1.5 cm radially, anatomically confined, i.e., not extending into bone or beyond an intact facial barrier or the skin surface; iii) The PTV margin was +5 mm, cropped at 5 mm from the skin; iv) A minimum of 50 Gy in 25 fractions to cover 95 % of the PTV; v) $>$ 99 % of the PTV to receive $>$ 97 % of the prescribed dose; and vi) $<$ 2 % of the PTV to receive $>$ 110 % of the prescribed dose. Then, the dose painting part of this study consisted of re-planning patients with multiple sub-boosts to the GTV in order to evaluate the feasibility of the exercise. For this dose painting exercise, we defined two nested sub-GTVs contained withing the originally planned GTV (GTV_{plan} or $GTV_{53.5Gy}$) to receive dose boosts: i) A sub-GTV defining the hypermetabolic region within GTV_{plan} ($GTV_{hypermetabolic}$ or GTV_{60Gy}); and ii) A sub-GTV defining the hypoxic region within $GTV_{hypermetabolic}$ ($GTV_{hypoxic}$ or GTV_{65Gy}). In this part of the study, the thresholds were set to specific values in order to evaluate the methodological/clinical feasibility of using the same threshold for all patients. To define the $GTV_{hypermetabolic}$, we used a threshold of 30 % of the maximum intensity in FDG-PET scans. Since in our work we observed that FMISO-PET did not prove useful in defining the level of hypoxia in STS as compared to nearby muscles, we used low-perfusion DCE-MRI maps (i.e. highlighting the low-perfusion components of the tumour) created similarly to the work of Stoyanova *et al.* [3] as a hypoxia surrogate. We then used a threshold of 50 % of the maximum intensity in these low-perfusion DCE-MRI maps to define $GTV_{hypoxic}$. These thresholds of 30 % in FDG-PET and 50 % in low-perfusion DCE-MRI were found to produce sub-GTV contours suitable for RT planning for most patients of the cohort (i.e. for 14 patients, the rest were excluded from the dose painting exercise).

2 Initial study protocol

Image acquisition protocols

^{18}F-Fluorodeoxyglucose PET/CT scan

FDG-PET studies were performed on a hybrid PET/CT scanner (Discovery ST, General Electric Medical Systems, Waukesha, WI, USA), which combines a dedicated, full-ring PET scanner with a 16-slice spiral CT scanner. Patients were required to fast for at least 6h before the time of their appointment. Blood glucose levels were recorded immediately prior to FDG administration. If the serum glucose level was greater than 11.1 mmol/l (200 mg/dl) the study was re-scheduled. A volume of 400 ml of barium sulfate oral contrast was administered and between 370 and 500 MBq (10 and 13.5 mCi) of FDG was injected intravenously. Sixty minutes following FDG injection, CT and PET images were consecutively acquired from the base of the skull to the upper thighs, with additional images acquired as needed according to the STS location.

For the CT scan portion of the study, the settings were the following: 120-140 kVp, 90-110 mA (depending on the body weight), a rotation time of 0.8 s, a table speed of 17 mm per gantry rotation, a pitch of 1.75:1, and a detector row configuration of 60.625 mm. For the PET portion of the study, 2-D acquisition was performed and images were acquired using 4-5 min per bed position (depending on the body weight) and 5 to 6 bed positions (depending on the patients height). PET attenuation-corrected, PET non-attenuation-corrected, CT, and fused images were reconstructed in the transaxial plane with an ordered subset expectation maximization (OSEM) iterative algorithm.

^{18}F-Fluoromisonidazole PET/CT scan

FMISO-PET studies were performed on a hybrid PET/CT scanner (Discovery ST, General Electric Medical Systems, Waukesha, WI, USA), which combines a dedicated, full-ring PET scanner with a 16-slice spiral CT scanner. Patients were required to fast for at least 2 hours. Oral contrast (400 ml of barium sulfate) was administered and between 370 and 500 MBq (10 and 13.5 mCi) of FMISO was injected intravenously. One hundred and twenty minutes following FMISO injection, CT and PET images were consecutively acquired from the base of the skull to the upper thighs, with additional images acquired as needed according to the STS location. A period of one day separated the administration of FDG and FMISO. For the CT scan portion of the study, the settings were the same as for the FDG-PET studies.

Diffusion-weighted MRI

In order to obtain ADC maps, standard echo-planar imaging MRI sequences were acquired using three different diffusion b-values of 0, 100 and 800 s/mm^2. DWI was obtained using the following parameters: FOV of 26 cm, matrix size of 160-256, TR $>$ 3500, TE minimum, NSA (number of signal averages) of 6, section thickness of 5mm /1 mm gap with fat suppression.

Dynamic contrast-enhanced MRI

MRI perfusion images were obtained after administration of Gadolinium contrast using 3D FSPGR sequence with the following parameters: TE and TR minimum, Flip angle 25, Bandwidth 42, Matrix size 256 x 128, single excitation and FOV of 24-28 cm.

Image interpretation

^{18}F-Fluorodeoxyglucose PET/CT scan

Qualitative criteria were used to correlate with the histologic response as follows: the tumor SUVmax at baseline, the percentage of decrease of SUVmax at mid-treatment, the SUVmax at mid-treatment, the percentage of decrease of SUVmax at the end of treatment, and the SUVmax at the end of treatment.

^{18}F-Fluoromisonidazole PET/CT scan

SUVmax was considered for target activity (T) and mean SUV for blood activity (B). A target lesion was considered hypoxic if T is above or equal to the mean B of all patients + 2 SDs (95 % confidence interval [CI]). The hypoxic volume was computed using a threshold value of T/B = 1.2 as per previous studies. Qualitative criteria were used to correlate with the histologic response: any hypoxia at baseline, hypoxic volume at baseline, any hypoxia at mid-treatment, percentage of decrease of SUVmax at mid-treatment, percentage of decrease of hypoxic volume at mid-treatment, hypoxic volume at mid-treatment, any hypoxia at the end of treatment, percentage of decrease of SUVmax at end of treatment, percentage decrease of hypoxic volume at the end of treatment, and hypoxic volume at end of treatment.

Diffusion-weighted MRI

The following qualitative criteria were used to correlate with response: changes in the tumor region of mean ADC, maximum ADC, minimum ADC and ADC histogram descriptors from baseline scans to mid-treatment and post-treatment scans.

Image registration

In order to investigate the spatial overlap of PET and MR imaging findings, rigid registration (rotations and translations) was used to spatially transform the MR scans into the reference frame of the PET scans. To perform co-registration, we used the commercial software MIM® (MIM software Inc., Cleveland, OH). MIM® provides an assisted alignment tool that uses normalized mutual information (NMI) as the similarity measure. Practically speaking, to achieve co-registration of MR scans onto PET scans, we first rigidly registered the MR images onto the CT images of the combined PET/CT scans. Subsequently to this spatial transformation, we could directly overlay the MR images onto the PET images since the PET and CT images come from the same combined PET/CT scans and are thus in the same reference frame.
http://www.mimsoftware.com/products/mimfusion

References

[1] Vallières, M., Freeman, C.R., Skamene, S.R. & El Naqa, I. (2015). A radiomics model from joint FDG-PET and MRI texture features for the prediction of lung metastases in soft-tissue sarcomas of the extremities. *Phys. Med. Biol.*, **60**(14), 5471-5496.

[2] Walker-Samuel, S., Leach, M.O. & Collins, D.J. (2006). Evaluation of response to treatment using DCE-MRI: the relationship between initial area under the gadolinium curve (IAUGC) and quantitative pharmacokinetic analysis. *Phys. Med. Biol.*, **51**(14), 3593-3602.

[3] Stoyanova, R. *et al.* (2012). Mapping tumour hypoxia in vivo using pattern recognition of dynamic contrast-enhanced MRI data. *Transl. Oncol.*, **5**(6), 437-447.