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# Predicting radiotherapy response, toxicities and quality-of-life related functional outcomes in soft tissue sarcoma of the extremities (PredicT): a study protocol

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# Predicting radiotherapy response, toxicities and quality-of-life related functional outcomes in soft tissue sarcoma of the extremities (PredicT): a study protocol

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## Abstract

Introduction: Radiotherapy improves local tumor control in patients with soft tissue sarcoma of the extremities (STSE) but also increases the probability of long-term toxicities such as tissue fibrosis, joint stiffness and lymphoedema. The use of radiation dose and volume thresholds, called constraints, may potentially reduce the development of toxicities in STSE. The aim of this study is therefore to predictors of radiotherapy-related side effects for STSE.

Methods and analysis: PredicT is a multi-centre cohort study comprising two cohorts. Predict is open to recruitment and planned to complete recruitment in July 2024. PredicT A, a retrospective analysis of the UK VorteX and IMRiS clinical trials aimed at deriving the dose-volume constraints and PredicT B, a prospective cohort study of 150 patients with STSE aimed at testing the validity of those dose-volume constraints and a statistical model with receiving operator characteristics (ROC) and multivariate analysis to predict radiotherapy side-effects will be generated using prospectively collected radiotherapy-specific normal tissue dose volume histograms and patient and clinician-reported toxicities data. The validity of the model will then be validated in the prospective cohort of patients.

Ethics and dissemination: PredicT has received ethical approval (North West - Liverpool Central Research Ethics Committee, 20/NW/0267). PredicT B is registered at clinicaltrials.gov with the identifier NCT05978024. We will disseminate our findings via publications, presentations, national and international conference meetings and engage with local charities.

## Strengths and limitations

- In the context of radiotherapy for soft tissue sarcoma of the extremities (STSE), there is a lack of knowledge of which normal tissues should be spared and their radiation dose-volume constraints;

- PredicT is the first study that aims to identify specific normal tissues at risk of developing toxicities and to establish their dose-volume constraints in patients with STSE;
- PredicT comprises two cohorts: PredicT A, a retrospective analysis of the UK VorteX and IMRiS clinical trials aimed at deriving the dose-volume constraints; PredicT B, a prospective cohort study of 150 patients with STSE aimed at testing the validity of those dose-volume constraints.

## Introduction & Rationale

Soft tissue sarcomas of the extremities (STSE) accounted for 1% of all malignancies arising in adults, with 3,272 cases in the UK in 2010.[1,2,3] Localized disease is potentially curable, with 5-year survival rates of 60% in high-grade disease.[4]

Radiotherapy, when delivered as an adjunctive treatment with surgery in the management of STSE, improves local tumour control rates. However, it also increases the probability of patients experiencing acute and late toxicities. The development of toxicities may be dependent on anatomical location, tumour size, treated volume, radiation dose and fractionation, as well as the intrinsic radiosensitivity of the normal tissues surrounding the tumour.[5] The scheduling of radiotherapy also plays an important role. Neo-adjuvant radiotherapy is associated with higher chances of post-operative wound complications, whereas adjuvant radiotherapy results in worsening limb function due to the high probability of late toxicities caused by delivering a higher total dose to a larger volume.[6]

High-grade radiation-induced fibrosis of normal tissues surrounding the tumour is an important irreversible late side-effect, manifesting as soft tissue contracture, pain in the treated area and associated gait (lower limb) or dexterity (upper limb) problems. This can significantly impact normal activities of daily living and health-related quality-of-life. It can occur months or years after treatment and may worsen over time. The SR.2 phase III trial, conducted by from the National Cancer Institute of Canada Clinical Trials Group, compared pre-operative to post-operative radiotherapy in STSE and reported the incidence on late toxicities. Tissue fibrosis occurred in 48.2% and 31.5%, joint stiffness was observed in 23.2% and 17.8% and lymphoedema was reported in 23.2% and 15.5% of the patients receiving post-operative and pre-operative radiotherapy, respectively.[5]

Radiotherapy techniques have evolved to attempt to improve the therapeutic index. However, there is a dearth of dose-volume constraint guidelines relating to normal tissue structures within the extremities linking them to the incidence and severity of acute and late toxicites.[7] For example, modern radiotherapy planning for STSE is still done by sparing the normal tissue corridor, which is defined by a variably-sited, longitudinal strip of skin and subcutaneous tissue. Historically, in order to avoid lymphoedema or normal tissue fibrosis, it

has been recommended that no more than 50% of the normal tissue corridor volume receives 20 Gy.[8] The definition of this avoidance structure lacks consistency and does not correspond to a specific, anatomically-defined normal tissue.

Dose-volume constraints to reduce the dose to the whole femur have been proposed by Dickie and colleagues and have been used for radiotherapy plan optimisation for over a decade.[9] As a result, an incidence of bone fractures lower than 5% has been reported in recent studies.[10,11]

Addressing uncertainties and developing dose-volume constraints to specific normal tissue structures, such as neurovascular bundle, subcutaneous tissue and muscle compartments, is likely to reduce the severity of toxicities and improve functional outcomes of patients with STSE.

PredicT is an observational non-interventional study to create a platform for collating toxicity and dosimetric data and to determine the relationship between radiation dose delivered to specific normal tissue structures and the incidence and severity of toxicities.

## Design

The PREDICT study is composed of two patient cohorts: Predict A and Predict B (Figure 1).

PredicT A is a retrospective analysis of 384 patients recruited in the VorteX and IMRiS UK trials. It aims to develop a radiation dose-volume constraint model to predict pre-defined late toxicities. PredicT B is a multicentric, prospective observational cohort reporting the intensity and frequency of toxicities in 150 patients with STSE. This cohort will be a validation cohort to test the radiation dose-volume constraints defined in Predict A .

## PredicT A

PredicT A represents the largest radiotherapy STSE cohort of patients accrued as part of two UK clinical trials, VorteX and IMRiS.

The VorteX (NCT00423618) phase III randomised trial investigated whether a reduced volume of post-operative radiotherapy improved limb function without compromising local control for STSE.[12] Two hundred and sixteen patients were recruited between 2008 and 2013. The co-primary endpoints were limb function (measured with the patient-reported Toronto Extremity Salvage Score (TESS)) and time to local recurrence. Secondary endpoints included evaluation of soft tissue and bone toxicity (RTOG clinician-reported scoring system), overall level of limb function (measured with two general questions in TESS), as well as disease-free and overall survival. Patients were randomized to either the control arm to receive a 2-phase radiotherapy technique of 50 Gy in 25 fractions followed by a boost of 16 Gy in 8 fractions or

the research arm, which consisted of 66 Gy in 33 fractions to the reduced boost volume. 3D conformal radiotherapy or intensity-modulated radiotherapy (IMRT) were permitted.

The IMRiS (NCT02520128) phase II trial studied the feasibility of delivering IMRT in three sarcoma cohorts. Specifically, the STSE cohort recruited 168 patients, with 112 and 56 receiving pre-operative and post-operative radiotherapy, respectively. Recruitment was completed in July 2017. The primary endpoint was the rate of high-grade fibrosis at 2 years. Secondary endpoints were the incidence of other high-grade toxicities, patient-reported limb function and quality-of-life (measured with the TESS score), time to local recurrence, disease-free and overall survival. IMRT was delivered either pre-operatively as 50 Gy in 25 fractions or post-operatively as 60 Gy in 30 fractions or 66 Gy in 33 fractions (for patients with positive resection margins).

## PredicT A objectives

### Primary objective

- To identify specific dose-volume constraints for anatomical regions of interest, within the normal tissue of the limb but lying outside the clinical target volume, that can predict for the frequency and intensity of radiotherapy-related side-effects in STSE.

### Secondary objectives

- To identify anatomical regions of interest within the normal tissues in limbs in which delivery of high-dose radiotherapy may result in specific toxicities, such as fibrosis and lymphoedema;
- To define dose-volume constraints for:

    a. Lymphoedema
    b. Fibrosis
    c. Bone fracture
    d. Joint stiffness

#### Patient population

PredicT A is a retrospective analysis of all 384 patients recruited as part of the VorteX and IMRiS clinical trials.

# PredicT B

PredicT B (NCT05978024) (Figure 2) is a multi-centric, prospective cohort study. Radiotherapy is delivered per local clinical protocols. Figure 2 summarises the recommended techniques, as well as RT dose schedules. Patients will be followed up during RT and at 3, 6, 12 and up to 24 months post-RT.

Clinicians should aim for RT to start within 4 weeks of registration, and no longer than 12 weeks after surgery. 3D conformal radiotherapy or IMRT techniques are permitted. The radiation dose is delivered:

- pre-operatively as 50 Gy in 25 daily fractions, delivered in 5 fractions per week over 5 weeks;
- post-operatively as 60/66 Gy (R0/R1) in 30/33 daily fractions to the high-dose planning target volume (PTV) and 52.2/43.46 Gy in 30 daily fractions to the low-dose PTV treated concurrently, or in a 2-phase technique of 50Gy in 25 daily fractions then 10-16 Gy in 5-8 fractions delivered 5 times per week over 6 or 6½ weeks;
- palliatively as 30-36 Gy in 10-12 daily fractions; 40 Gy in 15 daily fractions; 36 Gy in 6 fractions delivered once a week; 25 Gy in 5 daily fractions ($\alpha/\beta$= 3 Gy).

## PredicT B objectives

### Primary objective

1. The primary objective is to report the frequency and intensity of radiotherapy-related toxicities in STSE.

### Secondary objectives

2. To test the validity of specific dose-volume constraints for normal tissue structures within the normal tissue of the limb, predicting the frequency and intensity of radiotherapy-related toxicities for:

    a) Lymphoedema
    b) Fibrosis
    c) Bone fracture
    d) Joint stiffness

### Exploratory objectives

3. To explore the effect of RT technique, schedule, tumour size and anatomical location in the development of acute and late toxicities;

To determine quality-of-life related functional outcomes and explore correlations with dose-volume parameters for patients who received pre-operative, post-operative or palliative radiotherapy for STSE.

### Patient population

PredicT B inclusion, exclusion and subject withdrawn criteria are listed in the following sections.

*Inclusion criteria*

- Histopathological diagnosis of soft tissue sarcoma of the upper or lower limb or limb girdle;
- Patients receiving pre-operative (neo-adjuvant), post-operative (adjuvant) or palliative radiotherapy;
- Patients receiving radiotherapy planned as per local protocols (neoadjuvant chemotherapy will be allowed);
- WHO performance status 0-2;
- Aged ≥16 years;
- Patients fit enough to undergo radiotherapy and willing to attend follow-up visits, during two years;
- Female patients of child-bearing potential and male patients with partners of child-bearing potential must agree to use adequate contraception methods, which must be continued for 3 months after of treatment;
- Capable of giving written informed consent.

*Exclusion criteria*

- Previous radiotherapy to the same site;
- Pregnancy;
- Patients with concurrent or previous malignancy that could compromise assessment of primary and secondary endpoints of the trial;

*Subject withdrawal criteria*

- Patients expressing a wish to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with procedures for reporting study withdrawal. Withdrawal data instigated by the investigator or the patient will be collected and reasons for withdrawal will be recorded during this study;
- The investigators will try to replace patients who are withdrawn whilst study recruitment
- is open, in order to allow for a two-year follow-up. After recruitment is completed, patients who are withdrawn will not be replaced by other individuals;
  As this is an observational study, patients will not experience a change in their regular standard of medical care if there is a study withdrawal.

## Radiotherapy quality assurance (RT QA)

### PredicT A

A comprehensive RT QA programme for VorteX and IMRiS trials was designed and implemented by the National Radiotherapy Trials Quality Assurance (RTTQA) Group, including pre- and per-accrual components. For pre-accrual, QA centres completed the following exercises prior to site activation: 1) Process documents, 2) Benchmark outlining cases, 3) Benchmark planning cases 4) Dosimetry audit (subject to prior RTTQA accreditation). On-trial QA included: Prospective case review of contouring and planning for the first pre-operative and post-operative case from each centre, followed by retrospective review of all cases.

### PredicT B

PredicT B is a non-interventional, prospective cohort study where patients receive standard-of-care radiotherapy. Therefore, an RT QA programme is not required. However, radiotherapy CT scan, structures, dose plan, re-plan, cone-beam CT and diagnostic imaging (where applicable) is anonymised and centrally uploaded in RTTQA systems.

## Statistical analyses

#### Hypothesis

We hypothesise that patients in PredicT B whose radiation treatment plans indicate that the dose to organs-at-risk in the extremities will fail the dose constraints derived in PredicT A will have higher incidences of toxicities than patients whose treatment plans meet the constraints.

### PredicT A: Development of dose-volume constraints predictive model

Patients' characteristics, tumour locations, histologies, toxicities and PROMs will be collated in a database for the VorteX and IMRiS trial datasets.

Normal tissue structures will be defined anatomically for lower limb STSE using anatomy references for:[13,14,15]

- Muscle compartments (MC);
- The neurovascular bundle (NVB);
- Subcutaneous tissue;
- Bones;
- Joints.

All cases will have the aforementioned normal tissues outlined retrospectively. An outlining training programme is ongoing and this is backed up by outlining peer-review and an ongoing inter-observer variability study.

The radiotherapy dose-volume parameters for each predefined normal tissue structure will be exported from the treatment planning system (TPS). The database will be merged with a

toxicity database including the following per patient entries: patient identification, dose-volume histogram parameters for each normal tissue, treatment technique, side-effect measures (clinician-reported RTOG and PROMs), patient characteristics and co-morbidities (e.g. diabetes, hypertension, smoking habits).

An atlas of complication incidence (ACI) described by Jackson et al[16] will be used in order to visualise the incidence of grade 2 and above (grade 2+) toxicities. A specific ACI will be created for each normal tissue, summarising the dose-volume histogram (DVH) for all patients and the incidence of grade2+ toxicities. Each ACI will show a grid of boxes with increments of volume (in cc or percentage of volume) on the y-axis and dose (in Gy) on the x-axis. The DVH for each patient will be overlaid and the number of patients whose DVHs pass through each box will be calculated. The incidence of toxicity related to the patients whose DVH passes through each box will also be calculated. This analysis will identify dose-volume regions associated with toxicities.[17,18,19] Sub-group analyses will be performed to identify if different associations are detected between patients undergoing post-operative versus pre-operative radiotherapy, and patients undergoing IMRT or 3DCRT.
Receiving operator characteristics (ROC) analysis will be used to find optimal dose-volume thresholds (also known as constraints) that best discriminate between patients with and without toxicity (eg. lymphoedema, fibrosis) in the PredicT A dataset. The volumes receiving each specified dose will be ranked (smallest to largest) for all patients and each possible threshold will be considered, generating the areas under the curve (AUC). Only AUC with lower confidence intervals >0.5 ($p<0.05$ compared with chance) will be used to find optimal thresholds using the Youden index. In parallel, multivariate analysis (MVA) will be performed.[20] The associations between dose-volume variables, co-morbidities and radiation-induced grade 2+ toxicities will be tested.

## PredicT B: Prediction validity of frequency and intensity of radiotherapy toxicities in STSE

The validity of the dose-volume constraints identified in the PredicT A cohort will be tested in PredicT B. Specifically, patients will be divided into those meeting or not meeting constraints and comparisons of toxicity levels between both groups will be carried out.[21] In parallel, a similar MVA will be performed in this cohort to assess if dose-volume and toxicity relationships are similar in the validation cohort when compared to PredicT A analyses. The recruitment of the 150 patients is planned for 27 months across three centres. Patients will be followed up during RT and at 3, 6, 12 and up to 24 months post-RT.

- ### PredicT B sample size & power calculation

The primary endpoint is descriptive of toxicity rates as observed in patients recruited in the study. Published studies[6,7] reported toxicity rates of around 40%, the rate in our patient sample n=126 is expected to be within two-sided 95% confidence interval +/- 8.6%. The study has also, therefore, been powered for the main secondary endpoint (endpoint 2). It has been assumed that the incidence of RTOG grade 2+ subcutaneous

tissue fibrosis would be greater for patients whose radiation treatment plan did not meet the radiotherapy dose-volume constraints compared to those for whom it did. We, therefore, aim to find differences in toxicity levels between two groups of patients: (1) patients not exceeding the previously defined dose-volume constraint and (2) patients exceeding the previously defined dose-volume constraints. Dose-volume constraints of interest will be defined in the Predict A cohort. To detect a difference in toxicity rate of 30% between patients treated with a dose below and above a specific constraint (assuming 70% probability of grade 2+ toxicity in patients above constraint and 40% for patients below), using the 126 patients sample size with assumed ratio of 2:1 between patients below (84 patients) and above (42 patients) the constraint. This secondary endpoint will have 90% power to detect difference in the toxicity rate between the two groups at 2-sided 5% significance level. The difference in toxicity rate between groups (30%) has been assumed by using a preliminary review of available VorteX trial toxicity outcome reports. Assuming 19% drop-out rate during the 2 years follow-up, recruitment will continue until 150 (additional 24) patients are recruited.

## Interim analysis

A preliminary analysis including the calculation of ACIs, ROC and MVA for PredicT A is planned when data from 30% (115 patients) of the sample has been retrospectively outlined.

# Planned timeline

PredicT A analysis started in June 2021 and is planned to be finalised by May 2024.

PredicT B recruited its first patient on 16 April 2021. As of 19th of September 2023, the study is open in 3 centres and is expected to complete recruitment by July 2024.

# Ethics approval

PredicT A retrospective analyses have been reviewed and received approval by the Cancer Research UK Clinical Trials Unit (University of Birmingham, Birmingham) and the trial Cancer Research UK & UCL Cancer Trials Centre (London). Collaborations have been established with the sponsors for both VorteX and IMRiS clinical trials, data-sharing agreement have been put in place.

PredicT B has received ethical approval (North West - Liverpool Central Research Ethics Committee, 20/NW/0267).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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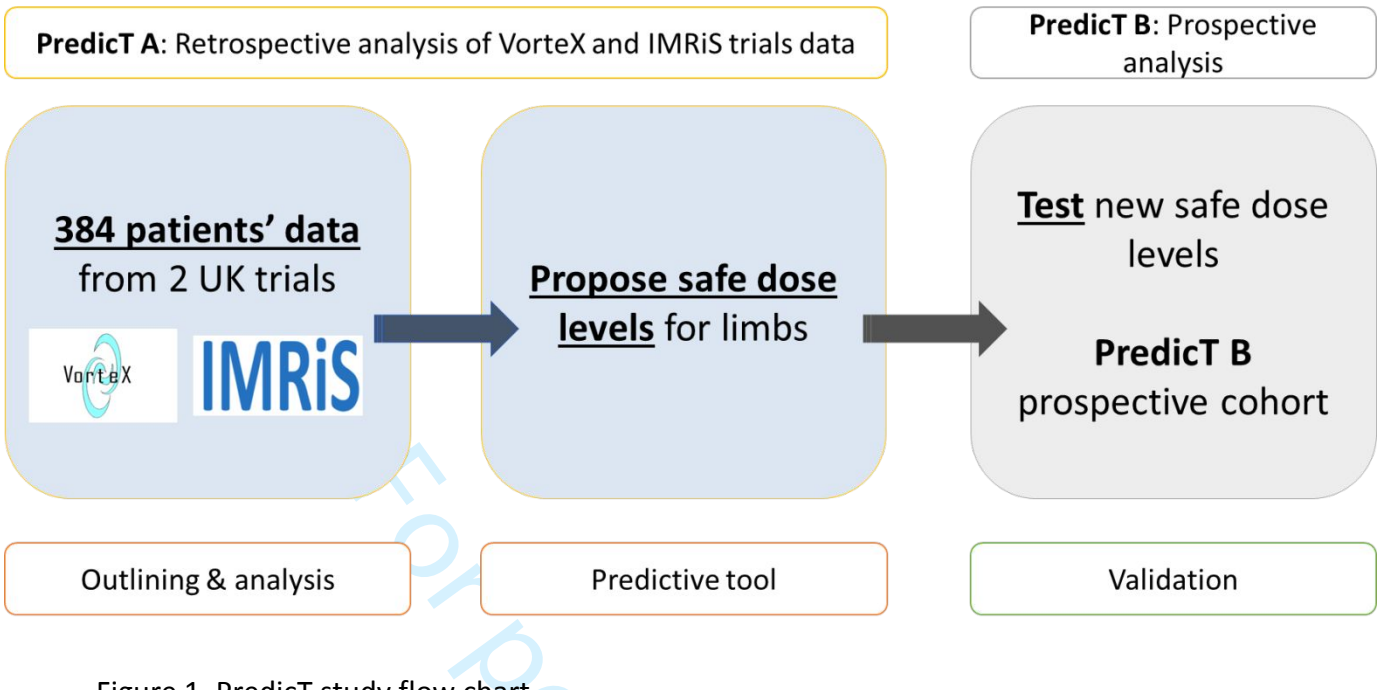


Figure 1. PredicT study flow chart.

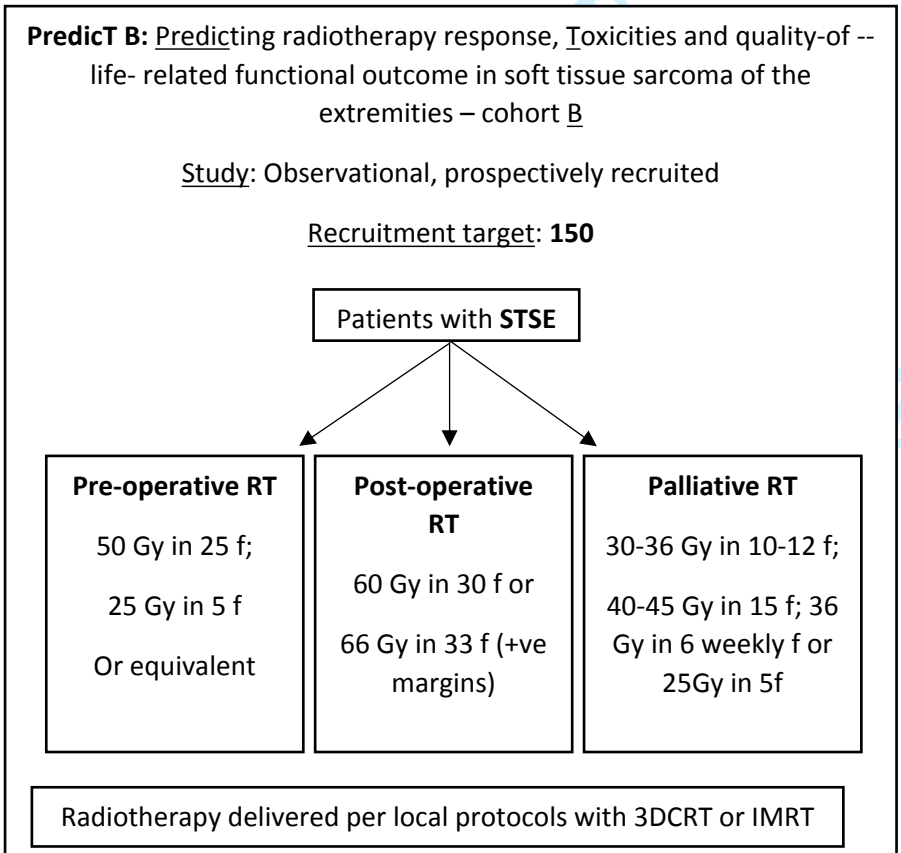

Figure 2. PredicT B summary.

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# Protocol CCR 5166

**Title**: Predicting radiotherapy response, toxicities and quality of life related functional outcome in soft tissue sarcoma of the extremities: a prospective observational cohort study

**Short Title:** Predicting radiotherapy response and Toxicities in soft tissue sarcoma of the extremities – cohort B (PredicT B)

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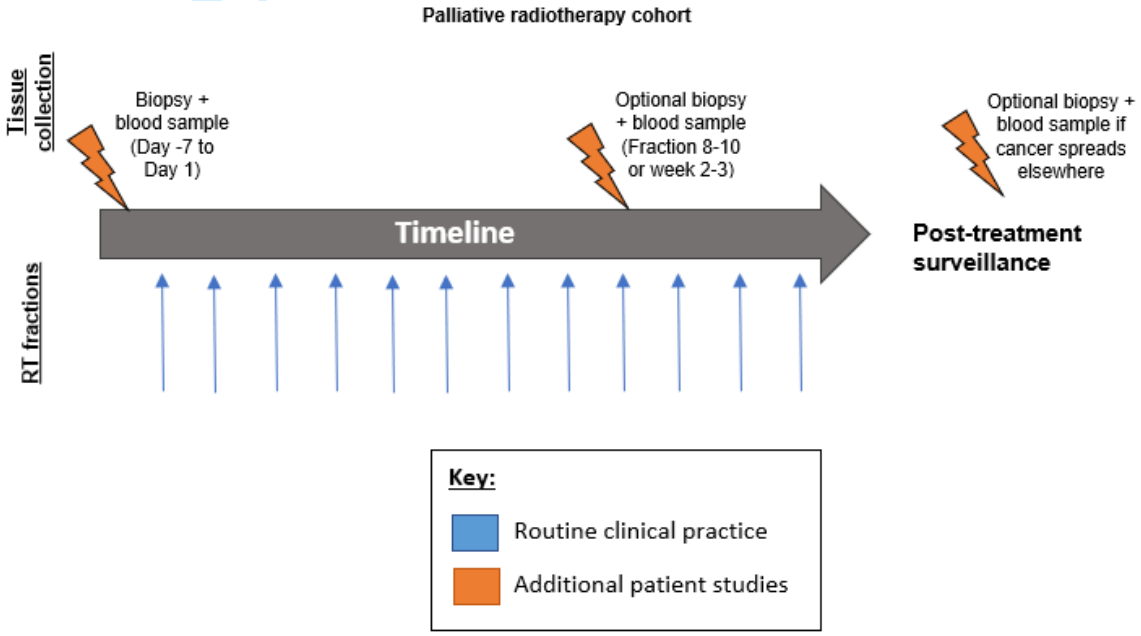

# Summary of changes

Protocol version 2.0 <to add date>

- Study summary, rationale section 2a (page 10): added 'primary' / palliative;
- Section 4, secondary objective point 6 (page 18): added 'primary' / palliative;
- Section 5, dose volume constraints validity testing (page 18): dose prescription updated;
- Section 7, inclusion criteria (page 20): further guidance provided on the inclusion criteria for patients receiving Neoadjuvant chemotherapy;
- Section 8, radiotherapy (23): dose prescription updates in pre-operative and palliative settings;
- Section 9, pre-treatment assessments (page 25): pre-registration and follow-up assessments udated;
- Section 15 (page 35): MRI data exportation to XNAT file repository updated details;
- Appendix 1, objectives (page 43): primary and econdary objectives updated;
- Appendix 1, sub-study design (page 44): recruitement and volunteer sample size updated;
- Appendix 1, volunteer study (page 44): MR scan charateristics updated;
- Appendix 1, patient study (page 45): Details on multi-parametric MRI studies provided;
- Appendix 1, timeline (page 46): timeline updated and sample size corrected;
- Appendix 1, secondary endpoint number 3 (page 47): Bland-Altman analysis updated;
- Appendix 1, exclusion criteria (page 48): patient contraindications updated and patient sample size updated;
- Appendix 1, MR-imaging and tissue collection (pages 49-50);
- Appendix 2, Biopsies, research blood samples, tissue collection and radiotherapy dose fractionation schedules updated (page 55-60);

## Summary

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| Title of the study | Predicting radiotherapy response, toxicities and quality of life related functional outcome in soft tissue sarcoma of the extremities: a prospective observational cohort study (PredicT B) |
| Study description | This is a multicentre prospective cohort study, primarily aimed at reporting the frequency and intensity of radiotherapy side-effects of patients with soft tissues sarcoma of the extremities (STSE).<br><br>Two sub-studies are proposed within this study:<br><br>• *MRI radiation response assessment*<br><br>Aimed at establishing whether changes in median apparent diffusion coefficients (ADC) are predictive of pre-operative STSE response measured using histopathology.<br><br>• *Biomarker development and Immune mediators associated with radiotherapy*<br><br>Aimed at establishing prognostic markers which may refine selection of cases for pre-operative, palliative or no radiotherapy.<br>Also, aimed at determining if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in anti-tumour immunity and if certain subtypes could potentially benefit from the addition of immunotherapy with radiation.<br><br>Patients participation in the sub-studies is optional. |
| Objectives | **Main study**<br><br>***Primary objective***<br><br>The primary objective is to report the frequency and intensity of radiotherapy side-effects in STSE.<br><br>***Secondary objectives*** |

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| | 1. To test the validity of radiotherapy constraints as derived in the analysis of the IMRiS and VorteX, that predict for the incidence of grade 2+ of:<br>a. Lymphoedema<br>b. Fibrosis<br>c. Fracture<br>d. Joint stiffness<br>e. Delayed wound healing following pre-operative RT<br><br>2. To report the incidence of grade 2+ toxicities at 3, 6, 12, 18 and 24 months for:<br>a. Subcutaneous tissue fibrosis<br>b. Lymphoedema<br>c. Bone fractures<br>d. Joint stiffness<br>e. Delayed wound healing following pre-operative RT<br><br>3. To determine the time to developing early and late side-effects.<br><br>4. To determine radiological and histological response rates to radiotherapy and where applicable chemo-radiotherapy for STSE of different histological subtypes.<br><br>5. To determine quality of life-related functional outcomes and explore correlations with dose-volume parameters for patients who have received pre, post-operative or palliative radiotherapy for STSE.<br><br>6. To determine predictive and prognostic factors for local and distant recurrence and overall survival for patients receiving pre-operative and palliative RT.<br><br>**Sub-Studies**<br><br>*MRI radiation response assessment sub-study*<br><br>*Primary objective*<br><br>To establish whether baseline measurements in apparent diffusion coefficient (ADC) measured at baseline, and/or changes in ADC measured midway through fractionation (after fraction 8) |

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| | or following treatment are predictive of soft-tissue sarcoma response measured using histopathology.<br><br>*Secondary objectives*<br><br>1. To develop, optimise and test clinical OE-MRI and MRE protocols for use of STSE imaging using healthy volunteer and MRI test-object studies.<br><br>2. To quantify the single-centre reproducibility of OE-MRI and MRE in STSE tumours.<br><br>3. To demonstrate that sub-regions identified using AI-segmented MRI demonstrate different biological phenotypes through molecular profiling and regional histopathology in soft-tissue sarcoma.<br><br>4. To demonstrate whether heterogeneous sub-volumes identified from AI segmentation models correlate with histological STSE response to radiotherapy.<br><br>5. To identify a correlation between (i) pre-treatment measurements, (ii) mid-RT changes and (iii) post-RT changes of tissue hypoxia (measured using OE-MRI) and tissue stiffness (measured using MRE) with post-radiotherapy changes in tumour cellularity (measured using DW-MRI)<br><br>6. To determine whether MR-imaging parameters measured within this sub-study are predictive of healthy tissue toxicity in STSE.<br><br>7. To develop AI-models for identifying texture features in X-ray CT images that correlate with MRI-derived sub-volumes.<br><br>*<u>Biomarker development sub-study and Immune mediators associated with radiotherapy sub-study</u>*<br><br>*Exploratory objectives*<br>1. Prognostic markers which may refine selection of cases for pre-operative radiotherapy versus palliative radiotherapy and no radiotherapy.<br><br>2. To determine if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in |

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| | anti-tumour immunity, to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation. |
| Rationale | The rationale for this study is to develop a personalised approach to recommending radiation treatment for STSE. The study will follow and support the patient pathway to help define:<br><br>1. The incidence and severity of radiation related side effects following a review of dose-volume parameters generated from the radiotherapy treatment plans. VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes. It is anticipated analysis for the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side effects and describe the relationship between radiation dose delivered to these specified areas and short and long-term toxicities. The results of from the first study (PredicT A) will be tested in PredicT B.<br><br>2. The effect of radiation on specific STSE subtypes using standard and novel functional MR imaging techniques and also the pathological and molecular changes associated with pre-operative radiation. In this exploratory study, we hope to identify potential markers of radiation response and radiation sensitivity alongside biomarkers to evaluate potential prognostic markers to predict for early development of metastatic disease. These data could lead to greater personalisation in the management of sarcoma patients, by:<br>a. Prognostic markers which may refine selection of cases for pre-operative radiotherapy, primary/palliative radiotherapy and no radiotherapy<br>b. Guiding radiation dose escalation/ de-escalation strategies (with possible hypofractionation) for different histological subtypes |

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| | c. Evaluate radiation response and whether addition of systemic therapy could enhance the therapeutic index<br>d. Determine the sequence of changes during radiotherapy, evaluating tumour perfusion and vasculature with functional MRI and histological and genomic changes.<br>e. Determine if radiotherapy stimulates the tumour microenvironment to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation. |
| Number of patients | 150 patients |
| Patients inclusion criteria | Inclusion criteria<br>• Histopathological diagnosis of soft tissue sarcoma of the upper or lower limb or limb girdle<br>• Patients receiving pre-operative (neo-adjuvant), post-operative (adjuvant) or palliative radiotherapy<br>• Patients receiving radiotherapy planned as per local protocols (neo-adjuvant chemotherapy will be allowed)<br>• WHO performance status 0-2<br>• Aged ≥16 years<br>• Patients fit enough to undergo radiotherapy treatment and willing to attend follow up visits, during two years<br>• Female patients of child-bearing potential and male patients with partners of child-bearing potential must agree to use adequate contraception methods, which must be continued for 3 months after completion of treatment<br>• Capable of giving written informed consent |
| Patients exclusion criteria | Exclusion criteria<br>• Previous radiotherapy to the same site<br>• Pregnancy<br>• Patients with concurrent or previous malignancy that could compromise assessment of primary and secondary endpoints of the trial |
| Study population | Patients with STSE undergoing to radiotherapy in the pre-operative, post-operative or palliative settings |
| Study design | Prospective observational cohort study |

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| Study duration | Recruitment will have the duration of 18 months;<br>Patients will be followed-up during 24 months. |

# 1. Background

Soft tissue sarcomas of the extremities (STSE) are rare cancers, accounting for 1% of all malignancies arising in adults, with 3,272 cases in the UK in 2010.[1,2] Localized disease is potentially curable, with 5-year survival rates of 60% in high-grade disease.[3]

Radiotherapy (RT) is often used in the management of STSE, either in the pre-operative, post-operative or definitive settings.[4] For large, deep-seated high-grade tumours, RT is recommended as an adjunct in the pre-operative or post-operative settings to improve local control rates of >80%.[5] An international consensus has been made to deliver RT in the pre-operative setting. This allows for smaller volumes to be treated to a lower total dose, which translates into similar local control rates as post-operative RT and a lower incidence of long-term complications, but at the expense of increased acute toxicities, specifically wound complications. However, certain tumours may demonstrate an intrinsic partial radio-resistance with marginal growth during RT which may hinder optimal surgical resection. Under these circumstances, post-operative radiotherapy is recommended with the expectation that a patient may experience increased incidence of long-term side-effects.

RT delivery has improved considerably by the use of various techniques, including intensity-modulated radiotherapy (IMRT) and image-guided radiotherapy (IGRT) to minimise acute toxicities but little progress has been made in stratifying radiation treatment according to histological subtypes (as is practised in the delivery of systemic therapy). Routine and more consistent use of radiotherapy has improved local tumour control rates, but there is still scope for improving outcomes, especially with respect to predicting the response to radiation treatment and treatment-related side-effects.

**Radiotherapy related toxicities**

Acute RT side-effects are defined as an adverse effect of radiation, which can occur up to three months after treatment has completed. For STSE, these include fatigue, skin toxicity (inflammation, erythema and desquamation), and increased risk of surgical wound complications. Late radiotherapy side-effects occur beyond three months, and include soft tissue fibrosis of irradiated normal tissues, limb oedema, joint stiffness and bone fractures. Acute and late side-effects are dependent on anatomical location, tumour size, treated volume, radiation dose and fractionation, as well as the radiosensitivity of the normal tissue surrounding the tumour.[5] Adjuvant radiotherapy leads to better tumour control when compared to limb-sparing surgery alone.[6] However, it may also worsen limb function, with an increased risk of clinically significant oedema and of poorer limb motion range resulting in a negative impact on quality-of-life of sarcoma survivors.[1]

High-grade fibrosis of normal tissues surrounding the tumour is an important side-effect, manifesting as soft tissue contracture, pain in the treated area, limb weakness, affected gait or dexterity problems, with resultant difficulty in undertaking normal activities of daily living. It can occur months or years after treatment and may worsen over time. A previous randomised phase III trial comparing pre-operative and post-operative radiotherapy in STSE has provided important information on late toxicity. High-grade toxicities at two years following treatment are described below:[5]

- Fibrosis in 48.2% and 31.5% of patients, who received post-operative and pre-operative radiotherapy, respectively;
- Joint stiffness in 23.2% and 17.8% of the patients receiving post-operative and pre-operative radiotherapy;
- Lymphoedema (swelling of the limbs) in 23.2% and 15.5% of patients receiving post-operative and preoperative radiotherapy, respectively.

The UK multicentre phase III VorteX trial compared standard post-operative radiotherapy for STSE against an experimental arm using reduced target volumes, aiming to reduce treatment-related side-effects while maintaining local tumour control. High-grade toxicities reported were subcutaneous (47% and 41% in the standard and experimental arms respectively), bone (11% and 15%) and joint (18% for both arms) toxicities.[7]

**Normal Tissue dose-volume constraints**

For some normal tissues, specific dose-volume constraints have been associated with the development of clinically significant toxicities. For example, xerostomia (dry mouth) can be avoided if one parotid gland is spared to a mean dose of less than 20Gy or if both parotids receive a mean dose below 25Gy.[8] Clinicians often use cut-offs in the volume of normal tissues receiving specific doses to avoid or minimise intolerable side-effects after radiotherapy.

There is a knowledge gap in predicting side-effects of radiotherapy for STSE, compared to other tumours. As mentioned above, evidence-based dose-volume constraints can be used by clinicians to predict patients at risk of toxicity and strategies to minimize side-effects can then be implemented during treatment planning.

The most relevant resources relating to radiotherapy toxicity and dosimetric constraints are the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) papers, and the work by Emami and colleagues.[9,10] The latter was drawn from a consensus of expert experience and limited historical toxicity data from local clinics.[10] However, it did not define dose-volume constraints for STSE. The QUANTEC effort, which summarised all available evidence on relationships between radiation dose and normal tissue response, identified relevant dose-volume constraints for specific healthy tissues, but again, did not include information on STSE.[9] However, there is some limited information available. Dickie and colleagues[11] studied a retrospective cohort of 21 and 53 patients with and

without fractures, respectively, who had received radiotherapy for STSE. They defined that treated bones should not receive a mean dose higher than 40Gy and that volume of bone receiving 40Gy should be kept below 64% to reduce radiotherapy-related fractures.[11] The RTOG protocol 063020, a phase II trial investigating the role of pre-operative image-guided radiotherapy for STSE in 98 patients, stated that no more than 50% of a longitudinal strip of skin and subcutaneous tissue, arbitrarily defined by the clinician, should receive more than 20Gy.[12] However, this was a protocol recommendation and was not evidence-based.

The standard radiotherapy technique for STSE uses multi-field 3D-conformal radiotherapy (3DCRT). 3DCRT techniques rely on conformal treatment plans, which use several radiation beams that are shaped to conform to the target volume.[13,14] Intensity-modulated Radiotherapy (IMRT) has built upon this paradigm to deliver increasingly conformal radiotherapy by modulating the radiotherapy dose across the treatment volume, allowing better conformality than 3DCRT. Therefore, IMRT has the potential to allow higher doses to be delivered to the target volume while sparing normal tissues. Improvements in conformality achieved by IMRT may lead to a reduction in side-effects, but there is also a higher volume of normal tissues treated to lower radiation doses. For STSE, the result is that the entire circumference of the limb may receive significant radiation doses, and currently there is not a good understanding of consequences in terms of long-term toxicities. It is therefore important to determine dose-volume constraints to inform radiotherapy planning.

**Predict A**

The VorteX trial investigated whether a reduced volume of post-operative radiotherapy improved limb function without compromising local control.[7] Two hundred and sixteen patients were recruited between 2008 and 2013. The majority were treated with modern 3DCRT; the standard radiotherapy technique for STSE and still widely employed clinically. Approximately 11% of patients in VorteX were treated with IMRT. High-quality radiotherapy planning data and long-term toxicity information were collected. Primary endpoints were limb function (measured with the patient-reported Toronto Extremity Salvage Score (TESS)) and time to local recurrence. Secondary endpoints included the evaluation of soft tissue and bone toxicity (RTOG clinician-reported scoring system), overall level of disability (measured with two general questions in TESS), as well as disease-free and overall survival.

The UK IMRiS phase II clinical trial tested IMRT in three sarcoma cohorts, namely one STSE and two bone sarcoma cohorts. The STSE cohort recruited 168 patients in total, with 56 and 112 receiving post-operative and pre-operative radiotherapy respectively. Recruitment was completed in July 2017. The primary endpoint is the rate of high-grade fibrosis at 2 years. Secondary endpoints are the incidence of other high-grade toxicities, patient-reported limb function and quality-of-life (measured with the TESS score), time to local recurrence, disease-free and overall survival.

VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Although 3DCRT is the standard technique for treating STSE, IMRT is expected to become standard of care in the future. Moreover, VorteX only included post-operative radiotherapy, whereas most patients treated in IMRiS received pre-operative treatment. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes.

It is anticipated that the analysis of the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side-effects and the relationship between radiation dose delivered to these specified areas and short and long-term toxicities.

The results of PredicT A will derive potential dose-volume constraints which could be used in radiotherapy planning in order to reduce radiotherapy-related toxicities. The validity of dose-volume constraints generated in PredicT A will be tested in the observational cohort of patients recruited prospectively in this study (PredicT B).

*This study will be conducted in compliance with the protocol, standard operating procedures, policies, local R&D management guidance, Good Clinical Practice including the Research Governance Framework 2005 (2nd edition) and other applicable regulatory requirement(s) including but not limited to the Human Tissue Act 2004, Human Tissue (Quality and Safety for Human Application) Regulations 2007, the Medical Devices Regulations 2002, Ionising Radiation (Medical Exposures) Regulations 2000 as amended from time to time.*

## 2. Rationale

The rationale for this study is to develop a personalised approach to recommending radiation treatment for STSE. The study will follow and support the patient pathway to help define:

1. The incidence and severity of radiation related side-effects following review on dose-volume parameters generated from a radiotherapy treatment plan. VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes. It is anticipated analysis for the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side effects and describe the relationship between radiation dose

delivered to these specified areas and short and long-term toxicities. The results of the first study (PredicT A) will be tested in PredicT B.

## 3. Hypothesis

- Patients whose radiotherapy treatment plans indicate that the dose to OAR of the extremities fail the dose constraints derived in PredicT A will have higher incidence of toxicity than patients whose treatment plan met the constraints.

## 4. Study objectives

***Primary objective***

The primary objective is to report the frequency and intensity of radiotherapy side-effects in STSE.

***Secondary objectives***

1. To test the validity of radiotherapy constraints as derived in the analysis of the IMRiS and VorteX, that predict for the incidence of grade 2+ of:
    a. Lymphoedema
    b. Fibrosis
    c. Fracture
    d. Joint stiffness
    e. Delayed wound healing following pre-operative RT

2. To report the incidence of grade 2+ toxicities at 3, 6, 12, 18 and 24 months for:
    a. Subcutaneous tissue fibrosis
    b. Lymphoedema
    c. Bone fractures
    d. Joint stiffness
    e. Delayed wound healing following pre-operative RT

3. To determine the time to developing early and late side-effects.

4. To determine radiological and histological response rates to radiotherapy and where applicable chemo-radiotherapy for STSE of different histological subtypes.

5. To determine quality of life-related functional outcomes and explore correlations with dose-volume parameters for patients who have received pre, post-operative or palliative radiotherapy for STSE.

6. To determine predictive and prognostic factors for local and distant recurrence and overall survival for patients receiving pre-operative and primary/palliative RT.

## 5. Study design

This is a multicentre prospective cohort study, primarily aimed at validating the dose-volume parameters identified in the analyses of the VorteX and IMRiS trials datasets.

- **Delineation of healthy tissues**

  Pre-defined outlining guidelines of normal tissues as bones, muscle compartments, joints, lymph drainage basins and subcutaneous tissue from Predict A will be delineated in radiotherapy planning computed tomography (CT) images. All cases will be delineated by a single observer (Rita Simoes). Verification of all outlines will be carried out by Dr Aisha Miah (clinical supervisor).

- **Dose-volume constraints validity testing**

  Patients will be treated as per local protocol treatment technique.
  Radiotherapy, clinical and toxicities data will be collected, with no new intervention on the treatment. Patients enrolled will receive standard radiation prescription doses as described below:
  - Pre-operative radiotherapy- 50 Gy in 25 fractions equivalent (pre-operative radiotherapy). Where appropriate hypo-fractionated schedules as per institutional guidelines can considered: eg. 25 Gy/ 5 daily fractions. In myxoid liposarcomas, 36 Gy in 18 fractions can be considered where suitable;
  - Post-operative radiotherapy- 60 Gy in 30 fractions or 66 Gy in 33 fractions (positive resection margins); alternative hypo-fractionated schedules as per institutional guidelines can be considered;
  - Palliative radiotherapy- 30-36 Gy in 10-12 daily fractions, 40-45 Gy in 15 fractions, 30-36 Gy in 5-6 once weekly fractions or 25 Gy in 5 daily fractions.

  Toxicity will be assessed with the TESS and RTOG scoring instruments and Stern score for lymphoedema. Patients enrolled in the study will fill in a specific quality-of-life questionnaire to assess quality of life related functional outcomes following treatment for STSE. This questionnaire is based on validated questions for assessing quality-of-life. Patients will be followed up at 3, 6, 12, 18, and 24 months post-radiotherapy.

  The recruitment of the 150 patients is expected to occur during a period of 18 months (as detailed in the sample size section below).

The dose-volume constraints generated in PredicT A will be tested in the validation cohort using odds ratios (OR) as previously reported by Gulliford et al.[15] Patients will be divided into those meeting or not meeting constraints and comparisons of toxicity levels between both groups will be carried out. In parallel, a similar MVA will be performed in this cohort to assess if dose-volume and toxicity relationships are similar in the validation cohort when compared to the IMRiS and VorteX dataset.

## 6. Endpoints

***Primary endpoint***

The incidence of any RTOG grade ≥2 toxicities following treatment for STSE at 24 months.

***Secondary endpoints***

1. The ability of the fitted model from dose-volume constraints from PredicT A (IMRiS and Vortex analysis) to correctly predict the incidence of grade 2+ among PredicT B patients.

2. The incidence of radiotherapy-induced late toxicities at 3, 6, 12, 18 and 24 months for:
   a. Subcutaneous tissue fibrosis
   b. Lymphoedema
   c. Bone fractures
   d. Joint stiffness
   e. Delayed wound healing (following pre-operative RT)

3. Time elapsed between day 1 of radiotherapy and the day to develop early and late side-effects. Early side effects recorded up to 3 months and late side effects recorded from 3 months to 24 months.

4. Exploration of factors which may be related to tumour changes (as defined by comparison of tumour volume between planning CT and CBCTs captured during RT) for pre-operative patients as follows:

   4.1. Tumour volume shrinkage after patients have received a total dose of 16 Gy (fraction number 8) and 32 Gy (fraction number 16).
   4.2. Tumour volume enlargement after patients have received a total dose of 16 Gy (fraction number 8) and 32 Gy (fraction number 16).
   4.3. Tumour volume shrinkage after patients have received a total dose of 50 Gy (fraction number 25).

4.4. Tumour volume enlargement after patients have received a total dose of 50 Gy (fraction number 25).

5. To identify if specific dose-volume parameters correlate with poor quality life reported outcomes for patients who have received pre, post-operative or palliative radiotherapy for STSE.

6. Patient-related and tumour related factors associated with response and distant recurrence and overall survival as determined by univariate and multivariate models as appropriate.
The following will be reported:
Local control rates at 2 years
Metastatic disease-free survival rates at 2 years
Overall survival at 2 years

## 7. Study population

The study population to be enrolled in this observational study will have had been diagnosed with STSE and referred to receive a course of radiotherapy either in the primary, palliative, pre-operative or post-operative settings at the two study centres; The Royal Marsden Hospital and University College London Hospitals.

**Inclusion criteria**

- Histopathological diagnosis of soft tissue sarcoma of the upper or lower limb or limb girdle;
- Patients receiving pre-operative (neo-adjuvant), post-operative (adjuvant) or palliative radiotherapy;
- Patients receiving radiotherapy planned as per local protocols (neoadjuvant chemotherapy will be allowed). Neoadjuvant chemotherapy patients may be approached as they commence chemotherapy;
- WHO performance status 0-2;
- Aged ≥16 years;
- Patients fit enough to undergo radiotherapy treatment and willing to attend follow up visits, during two years;
- Female patients of child-bearing potential and male patients with partners of child-bearing potential must agree to use adequate contraception methods, which must be continued for 3 months after completion of treatment;
- Capable of giving written informed consent.

**Exclusion criteria**

- Previous radiotherapy to the same site;
- Pregnancy;

- Patients with concurrent or previous malignancy that could compromise assessment of primary and secondary endpoints of the trial.

**Subject withdrawal criteria**

- Patients expressing a wish to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with procedures for reporting study withdrawal. Withdrawal data instigated by the investigator or the patient will be collected and reasons for withdrawal will be recorded during this study;
- The investigators will try to replace patients who are withdrawn whilst study recruitment is open, in order to allow for a two year follow-up. After recruitment completion, patients that are withdrawn will not be replaced by other individuals in the trial;
- As this is an observational study, patients will not experience a change in their regular standard of medical care if there is a study withdrawal.

# 8. Methodology

An observational cohort of 150 patients with STSE will be recruited over 18 months. This main study is a non-interventional study, therefore there will be no change to patient's standard treatment. Patients will be treated either with radiotherapy, in the pre-operative, post-operative or palliative settings as per local protocols.

**Patient screening**

Patients meeting the inclusion criteria will be identified during routine multidisciplinary team (MDT) meetings for STSE. No public methods for recruitment (e.g. through leaflets or websites) will be adopted in this study. Any patient who meets any of the exclusion criteria will not be invited to participate in this study. Once identified, the named clinical team members of the study or research nurses will discuss options for the patient to join this imaging study. Consent will be taken by named clinical team members including appropriately trained research nurses and radiographers nominated by the CI and local PIs.

**Informed Consent**

Co-Investigators, or, where delegated by the co-investigator, other appropriately trained site staff, are required to provide a full explanation of the study prior to trial entry. During these discussions, the current approved trial patient information should be discussed with the patient. A **minimum of twenty-four (24)** hours should be allowed for the patient to consider and discuss participation in the study. However, in order to prevent unnecessary return visits patients may consent on the same day as being given the information sheet,

provided the member of staff taking consent is satisfied that the patient understands the study.

All suitable patients will be informed on the rationale behind the study, the additional imaging required, and any additional hazards to which they may be exposed. They will be assured that all data retained for the purposes of the study will be kept strictly confidential, and only used by the co-investigators for the purposes of this study. It will be conveyed to patients that this study will involve the use and storage of (i) toxicity, clinical, radiotherapy and follow-up imaging acquired data (ii) data acquired from imaging sub-study to be run at RMH only, and (iii) tissue obtained following resection of their tumours following radiotherapy, as part of the recruitment in the sub-study (RMH patients only). In addition, they will be made aware that this study will have no impact on their subsequent healthcare, and a conventional clinical pathway will be appropriated. Patients will be supplied with a patient information sheet (PIS) for the study, and patients agreeing to participate will be asked to sign and date an informed consent form prior to recruitment (in accordance with GCP guidelines).

Patients will be informed that participation in the sub-studies is optional and that will be given the option to participate in PredicT B if the participation in the sub-studies is declined.

Patients will be informed that data acquired from the sub-study may be used in future retrospective studies, exploring potential imaging biomarkers and correlates with histopathological analyses. In addition, patients will be made aware that they may request transportation funds for the additional imaging time-points explored in this study.

A further discussion of the study will be held when patients return to the hospital to confirm treatment and at least 24 hours to review PIS to consent to study and/or sub-study. Patients will not be expected to make extra visits to the hospital for participation in the main study as all data collection points will be incorporated into their scheduled clinical follow-up appointments.

Centres recruiting patients are responsible for assessing a patient's capacity to give informed consent.

Sites must ensure that all patients have been given the current approved version of the patient information sheet, are fully informed about the trial and have confirmed their willingness to take part in the trial by signing the current approved consent form.

Sites must assess a patient's ability to understand verbal and written information in English and whether or not an interpreter would be required to ensure fully informed consent. If a patient requires an interpreter and none is available, the patient should not be considered for the study.

**Radiotherapy**

Radiotherapy should aim to start within 4 weeks of registration, and no longer than 12 weeks after surgery. For adjuvant RT patients, if wound healing delays start of radiotherapy, this will be permissible and must be discussed co-chief investigators. Any radiotherapy planning technique will be permitted as per local protocols. The radiation dose will be given as follows:

- Pre-operative RT: 50 Gy in 25 daily fractions biologically equivalent dose, delivered Monday to Friday over 5 weeks, 25 Gy/5 daily fractions; 36 Gy in 18 fractions for myxoid liposarcomas. The suitable fractionation is as per clinician guidance and institutional guidelines;
- Post-operative RT: 60 Gy in 30 daily fractions to the high dose planning target volume (PTV) (PTV_6000) and 52.2 Gy in 30 daily fractions to the low dose PTV (PTV_5220) treated concurrently, or 2 phase technique 50 Gy in 25 daily fractions then 10-16 Gy in 5-8 fractions delivered Monday to Friday over 6 weeks;
- Post-operative RT with positive resection margins: 66 Gy in 33 daily fractions to the high dose PTV (PTV_6600), and 53.46Gy in 33 fractions to the low dose PTV (PTV_5346) treated concurrently, delivered Monday to Friday over 6 1⁄2 weeks
- Palliative radiotherapy schedules: 30-36 Gy in 10-12 daily fractions, 40 Gy in 15 daily fractions, 30-36 Gy in 5-6 fractions delivered once a week, 25 Gy in 5 daily fractions. Applying $\alpha/\beta$= 3 Gy.

Radiotherapy planning CT scan and immobilisation should follow recruiting centres local protocols.
Toxicity will be assessed with the TESS, Stern and RTOG scoring instruments. Quality of life will be reported following specific EORTC quality of life validated questionnaires and measures. Patients will be followed up at 3, 6, 12, 18, and 24 months post- radiotherapy.

Radiotherapy CT scan, structures, dose plan, re-plan, cone-beam CT and diagnostic imaging (where applicable) be will be anonymised and uploaded in the National Radiotherapy Quality Assurance (RTTQA) upload platform or in the RTTQA secure file transfer. From the RTTQA system, the RT data will be exported to an excel spreadsheet. This spreadsheet will then be merged with the toxicity MACRO database including the following per patient entries: patient identification, dose-volume histogram parameters, treatment technique, side-effect measures (clinician-reported RTOG and patient-reported TESS toxicity scores), patient characteristics and relevant co-morbidities (e.g. diabetes, hypertension, smoking habits).

For further information please refer to the radiotherapy uploading guidelines.

A data transfer agreement will be completed following the Trust policies.

Patients treated within PredicT B with a pre-operative intention will be asked to take part in the sub-study.

**Patient withdrawal**

Patients wishing to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with centres procedures for reporting study withdrawal. Withdrawal data instigated by the investigator or the patient itself will be collected and reasons for withdrawal will be recorded during this study.

**Study completion**

This main study is part of a doctoral project which is to be completed in March 2024. The sub-study will be including patients for pre-operative radiotherapy only with interventions occurring during treatment and follow-up complete 2 years after treatment.

**End of study definition**

The definition of the end of this study is the date of completion of any follow-up monitoring and data collection described earlier in the protocol. Any change to this definition will be notified as a substantial amendment. Final analysis of the data (following 'lock' of the study database) and report writing will occur after formal declaration of the end of the study.

## 9. Assessments and data acquisition

**Pre-registration evaluation**

Patients must give written informed consent **before** any trial specific screening investigations may be carried out. The following assessments or procedures are required to evaluate the suitability of patients prior to entry into the trial:

- Histological confirmation of disease;
- Diagnostic MRI and/or CT (if there is a contraindication to MRI) of the primary tumour site;
- Patients receiving post-operative radiotherapy the MRI/ CT should ideally have been performed within 1 month prior to surgery;
- Patients receiving pre-operative radiotherapy, the MRI/CT should ideally be performed within 1 month of starting radiotherapy, although decisions on repeating scans older than 1 month will be made at the treating clinician's discretion;
- Chest imaging (CT or chest x-ray) within 3 months of registration, as per routine practice.

<u>Within 14 days prior to registration:</u>

- Clinical review of relevant medical history;
- Completion of the assessment of adverse events (AEs) using CTCAE v5.0;

- Assessment of WHO performance status;
- Pregnancy test (urine or blood) in females of child bearing potential;
- Measurement of height & weight, assessment of smoking status, diabetic status and limb function or mobility.

**Pre-treatment assessments**

Within 28 days prior to starting treatment:

- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- EORTC QLQ-C30 quality of life questionnaire*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Toronto Extremity Salvage Score (TESS) questionnaire*;
- Musculoskeletal Tumour Society Rating Scale;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).

*Patients having palliative RT may opt out the QoL assessments if they wish to.

The following pre-registration assessments do not need to be repeated if done within 28 days prior to starting treatment:

- Clinical review;
- Pre-treatment routine blood tests: FBC, U&Es, LFTs, CRP, LDH and clotting (for pre-operative or palliative radiotherapy patients or as per institutional guidelines);
- Assessment of AEs using CTCAE v5.0;
- Assessment of WHO performance status;

**Post-surgery assessment of wound complications up to 120 days after surgery**

Patients should be assessed for wound complications during assessment visits occurring from surgery and up to 120 days after surgery. Post-Surgery Wound Assessment wound complications are defined as

- 2nd operation under general or regional anaesthesia for wound repair (debridement, operative drainage, unplanned secondary wound closure using free muscle flaps or skin grafts);

- Wound management without 2nd operation (invasive procedure without general or regional anaesthesia, e.g. aspiration of seroma, readmission for wound care such as intravenous antibiotics, persistent deep wound packing for ≥120 days).

**Assessments during radiotherapy**

During treatment patients should be seen weekly or as per local protocol (in an appropriate on-treatment review clinic, which may be run by a doctor, radiotherapy nurse or therapy radiographer). The following assessments will be completed:

Weekly (or equivalent as per local practice)

- Clinical review mid and at end of RT;
- Cone Beam CT imaging review of treatment volume where applicable;
- Additional imaging review where applicable.

**Assessments on completion of radiotherapy**

The following should be carried out at least **28 days (and up to 35 days)** after the last fraction of radiotherapy:

- Clinical review;
- Assessment of ARs using CTCAE v5.0;
- Assessment of acute radiation morbidity using the RTOG Acute Radiation Morbidity Scoring Criteria;
- WHO performance status;
- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- Toronto Extremity Salvage Score (TESS) questionnaire (if applicable)*;
- Radiological response assessment (approximately 4-6 weeks after pre-op RT);
- Pathology response assessment (after resection, approximately 8-10 weeks after pre-op RT);
- EORTC QLQ-C30 quality of life questionnaire*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).

- *Patients having palliative RT may opt out the QoL assessments if they wish to.

In addition to the assessments listed above, we will collect each patient:

- Diagnostic scans which may have been acquired before treatment commences;
- Radiotherapy planning scans;
- Structures, Plans and dose cubes;
- Treatment imaging review, including CBCT which may have been acquired as per imaging verification protocols.

**Follow-up assessments after completion of radiotherapy**

Patients will be followed approximately 4 weeks after pre-op RT or 6 weeks after post-op RT, then at 3 months after completion of radiotherapy, then 3-monthly for up to 2 years after date of registration. All visits should be carried out at the specified time +/- 2 weeks.

N.B. For pre-operative RT patients, following their last fraction of RT, it may be necessary to omit a follow up visit immediately after surgery, as it may be difficult for the patient to attend clinic.

Patients should have the following assessments at each visit unless stated otherwise:

- Clinical review;
- WHO performance status;
- Assessment of radiation morbidity:
  - RTOG Acute Radiation Morbidity Scoring Criteria up to day 90 after start of treatment;
  - RTOG/EORTC Late Radiation Morbidity Scoring Criteria skin, subcutaneous tissue fibrosis, joint stiffness, bone) from day 91 after start of treatment;
  - Stern's scale for oedema from day 91 after start of treatment
- Clinical assessment of local tumour control at primary site at each 3-monthly visit;
- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- Chest x-ray/ CT imaging (as per local institutional guidelines at each 3-monthly follow up visit;
- TESS questionnaire at 1 year and 2 years after registration*;
- Musculoskeletal Tumour Society Rating Scale at 1 year and 2 years after registration;
- EORTC QLQ-C30 quality of life questionnaire at 1 year and 2 years after registration*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).
- Site of relapse if applicable and subsequent treatment details.
-
- *Patients having palliative RT may opt out the QoL assessments if they wish to.

**Assessments after disease recurrence**

If a patient progresses within 2 years from the date of registration, they should continue to be followed up if possible, fitting in with their routine oncological care. Investigators

should use their judgement on a case-by-case basis to perform follow up on patients according to their circumstances and what is clinically reasonable.

Where possible the following assessments should be performed:

- Clinical review;
- WHO performance status;
- Assessment of radiation morbidity:
  - RTOG Acute Radiation Morbidity Scoring Criteria up to day 90 after start of treatment;
  - RTOG/EORTC Late Radiation Morbidity Scoring Criteria (skin, subcutaneous tissue fibrosis, joint stiffness, bone) from day 91 after start of treatment;
  - Stern's scale for oedema from day 91 after start of treatment (Appendix);
- Clinical assessment of local tumour control at primary site;
- TESS questionnaire at 1 year and 2 years after registration*;
- Musculoskeletal Tumour Society Rating Scale at 1 year and 2 years after registration (Appendix);
- EORTC QLQ-C30 quality of life questionnaire at 1 year and 2 years after registration After the 2 year follow up visit patients should continue to be followed up on a regular basis as per standard oncological care*;
- EORTC QLQ-FA12 fatigue questionnaire*;
  Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location). *Patients having palliative RT may opt out the QoL assessments if they wish to.

## 10. Data Analysis

*Delineation of normal tissues*

All patients will have GTV, CTV, PTV and OAR outlined in the planning CT scan. Further bones, muscle compartments, joints, lymph drainage basins and subcutaneous tissue will be delineated in radiotherapy planning computed tomography (CT) by a single observer (Rita Simoes). Verification of all outlines will be carried out by Dr Aisha Miah (clinical supervisor) and reviewed with a specialist radiology team at ICR/RMH. A planned exercise to repeat delineation of the normal tissue structures will be performed after 2 months to account for intra-observer and inter-observer variability.

*Radiotherapy database*

A specific database including dose-volume parameters for each pre-defined normal tissue structure d will be merged with a toxicity database including the following per patient entries: patient identification, dose-volume histogram parameters, treatment technique, side-effect measures (clinician-reported RTOG and patient-reported TESS toxicity scores), patient characteristics and relevant co-morbidities (e.g. diabetes, hypertension, smoking habits).

The dose-volume constraints generated in PredicT A will be tested in the validation cohort using odds ratios (OR) as previously reported by Gulliford et al.[15] Patients will be divided into those meeting or not meeting constraints and comparisons of toxicity levels between both groups will be carried out. In parallel, multivariate analysis will be performed in this cohort to assess if the development of radiation-induced side-effects is related with clinical co-morbidities.

Patients will be followed up for two years and the planned timing for analysis will last 6 months from the completion of the last patient follow up appointment.

## 11. Study organisation/ trial monitoring and management strategy

The following study-related responsibilities have been defined:

**Responsibilities**

- The Royal Marsden NHS Foundation Trust is the Sponsor of this study and has responsibility for ensuring appropriate ethics committee opinion and authorisations are obtained.

- The Chief Investigator, Dr Miah, will have overall responsibility for the study and will conduct it in accordance with the UK Policy Framework for Health and Social Care Research and the principles of Good Clinical Practice (GCP).

- Monitoring of study progress will be the responsibility of the CI of this study (Dr Miah). Monitoring of the sub-study progress will also be the responsibility of Dr Matt Blackledge. This role will be supported by co-investigators, trial statistician and additional members of the trial management group. In addition, dedicated PPI representatives will be invited to all quarterly ITC meetings to ensure that all research falls within the remit of patient benefit.

- All participants within the sub-study will complete an MRI safety questionnaire with the research radiographers prior to being scanned. The imaging protocols will be maintained by the research radiographers and Dr Blackledge. The routine sequences of the MRI scans will be reviewed and reported by radiologists and made available to the clinical units in the standard way.

- Statistician, Mr Mohammed, will input into protocol, CRF and database design and will work with the investigators to ensure the quality and completeness of the data.

- Data Analysis will be performed by: Mrs Rita Simoes, Dr Miah, Dr Blackledge, Ms Thrussell, Dr Zaidi and Mr Hayes.

- The trial co-ordinator will be responsible for study administration including progress reporting, amendments and notification of study closure in accordance with ethical and R&D approval procedures.

Following review and notification in writing to the Chief Investigator of approval by the Committee for Clinical Research /Research & Development (CCR/R&D) and by the Health Research Authority (HRA), the study will be activated on the Hospital Information System and the Principal Investigator and trial coordinator notified that the study is open for recruitment. This will be classified as the start date for the R&D database. The study will be deemed to have reached completion at 24 months after the last patient has been recruited. The final questionnaires are due at 24 months and an extra three months has been added to allow for scheduling delays and completion of the investigations.

There will be a quarterly progress meeting for all stakeholders in the study to discuss all aspects of the study's progress and any issues encountered. There will be monthly progress meetings run by the Chief Investigator to review the day to day running and progress of the study. All study personnel will have attended a Good Clinical Practice (GCP) course within the previous three years. The Data Manager will maintain a Study Site File in which all critical study documentation including study protocol, correspondence with Local Research and Ethics Committee (LREC) and the RMH CCR, study amendments, copies of Adverse Event Forms, CVs and GCP certificates for all study personnel will be held.

## 12. Safety reporting

The following definitions have been adapted from Directive 2001/20/EC, ICH E2A "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" and ICH GCP E6:

**Adverse Event (AE)**

Any untoward medical occurrence in a patient treated on a trial protocol, which does not necessarily have a causal relationship with radiotherapy. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of radiotherapy, whether or not related.

**Adverse Reaction (AR)**

All untoward and unintended responses to radiotherapy treatment related to any dose administered. A causal relationship between radiotherapy and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

**Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)**

A SAE or SAR that at any dose:

- Results in death
- Is life threatening (the term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalisation or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is otherwise medically significant (e.g. important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above)

**Related and Unexpected Serious Adverse Reaction**

An adverse reaction meeting the following criteria:

- Serious – meets one or more of the serious criteria above
- Related – assessed by the local investigator or sponsor as causally related to one or more elements of the trial treatment
- Unexpected – the event is not consistent with the applicable reference safety information (RSI)

It is anticipated that Serious Adverse Events will not occur since this is a non-Clinical Trial (non-CTIMP) study. However, all adverse events defined as those resulting in death, as being life-threatening, requiring hospitalisation or prolongation of hospitalisation, resulting in persistent or significant disability or incapacity to a study participant will be reported in line with Trust's Generic SOP for Adverse Events Reporting for Non-CTIMP Trials sponsored and hosted by RMH/ICR (gSOP-03-03)(gSOP-03-03).

## 13. Statistical analysis

**Sample size**

The primary endpoint is descriptive of toxicity rates as observed in patients recruited in the study. Published studies[6,7] reported toxicity rates of around 40%, the rate in our patient sample n=126 is expected to be within two sided 95% confidence interval +/- 8.6%. The study has also therefore been powered for the secondary endpoint number 1 (The ability of the fitted model from IMRiS and Vortex analysis to correctly predict the incidence of grade 2+ among PredicT B patients in whom dose-volume constraints were exceeded compared to the patients in whom dose/volume constraints were met).

It has been assumed that number of patients developing RTOG grade≥ 2 subcutaneous tissue fibrosis would be greater for patients whose radiotherapy treatment plan did not meet the radiotherapy dose-volume constraints compared to those patients whose radiotherapy treatment plan met the dose-volume constraints. We therefore aim to find differences in toxicity levels between two groups of patients: (1) patients not exceeding the previously defined dose-volume constraint and (2) patients exceeding the previously defined dose-volume constraints.

Dose-volume constraints of interest will be defined in the Predict A study, using previously available data of two clinical trials of patients undergoing radiotherapy for STSE (VorteX and IMRiS).

To detect a difference in toxicity rate of 30% between patients treated with a dose below and above a specific constraint (assuming 70% probability of grade 2+ toxicity in patients above constraint and 40% for patients below), using the 126 patients sample size with assumed ratio of 2:1 between patients below (84 patients) and above the constraint (42 patients). This secondary endpoint will have 90% power to detect difference in the toxicity rate between the two groups at 2 sided 5% significance level.
The difference in toxicity rate between groups (30%) has been assumed by using a preliminary review of available VorteX trial toxicity outcome reports. Assuming 19% drop-out rate during the 2 years follow-up, recruitment will continue until 150 (additional 24) patients are recruited.

***Primary endpoint analysis***

Incidence of any grade ≥2 toxicities at 24 months will be calculated using descriptive methods in the form of number and proportion of patients with grade ≥ 2.

***Secondary endpoints***

Endpoint no 1 - To report the differences in experienced by patients with STSE for whom dose-volume constraints were below or exceeded. Binary logistic regression will be used to quantify the differences using odds ratios (OR) from the fitted models. Univariate logistic regression models will be fitted to predict for the parameters relevant to toxicity grades in the RTOG, Stern's and TESS scales. ORs for each of the parameters will be used to judge for significant differences between the groups.

Endpoint no 2 – Frequencies and proportions of radiotherapy-induced late toxicities at the specified at 3, 6, 12, 18 and 24 months will be reported descriptively at each time point for the overall events and by types of events:

a. Subcutaneous tissue fibrosis
b. Lymphoedema
c. Bone fractures
d. Joint stiffness
e. Delayed wound healing (following pre-operative RT)

Endpoint no 3 – Time elapsed between day 1 of radiotherapy and the day to develop early and late side-effects using Kaplan-Meier methods. Median time to early side-effects with 95% confidence interval will be reported. Patients without any side-effects (on day 90 from end of RT) will be censored. Similarly, median time to late side-effects with 95% confidence interval will be reported. Patients without an event will be censored at the 24 months assessment visit or at last follow-up date known to be on the study without any event.

Endpoint no 4 – Differences in the tumour volumes (in cc and %) will be calculated from CT and CBCT images at fractions 8, 16 and 25 for patients receiving pre-operative RT. Changes in target volumes during radiotherapy will be assessed by computing dissimilarity indices (e.g., Simpson's dissimilarity index) in sequential CBCT images captured during treatment compared to radiotherapy planning CT. Tumour factors are tumour histotype, staging and tumour size.

Endpoint no 5 – Correlations between functional outcomes (expressed in TESS and EORT-QLQ- C30 and QLQ-FA12 fatigue questionnaire scores) with dose-volume parameters (expressed in Gy/volume, where volume will be defined in % of total volume and cc) will be calculated using Spearman's correlation method. Patients will be categorised according to dose volume constraints and compare the groups using t-test or Mann-Whitney non-parametric test as appropriate.

Endpoint no 6 – Percentage of patients responding to treatment will be calculated, this will be reported in the overall patients and for dose-volume constraints groups. Differences in the response rates will be assessed according to: histopathological semi-quantitative scores (pathological: % viable cells). Radiological: % volume change (RECIST), % cystic sold component). Tumour factors are: tumour subtype, staging and size. Local control rates, disease-free survival rates and overall survival at 2 years will be calculated using Kaplan-Meier methods. Disease free survival events are local or distance disease recurrence and death from disease related causes. Overall survival events are death from any causes.

# 14. Regulatory & Ethics Committee Approval

**Ethical Considerations**

The study will be carried out in accordance with the Declaration of Helsinki (2013), and local R&D and Ethics Committee approval will be sought. The study will also be reviewed and approved by HRA. It is the responsibility of the Chief Investigator to obtain a favourable ethical opinion prior to recruiting patients and to conduct the study in accordance with the conditions of ethical approval.

**Informed Consent**

Written informed consent will be obtained from each patient by clinical members of the study team or named research nurses/radiographers with appropriate training and nominated by the CI. It is the responsibility of the Chief Investigator (or designated representative) to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. Sufficient time will be allowed for the patient to decide on study entry, and they will be informed about their right to withdraw from the trial at any time. The patient information sheet, which will be given to each patient before enrolment, will be an approved patient information sheet according to national guidelines, with support from our dedicated PPI representatives.
All delegated staff consenting patients will have had appropriate consent training.

**Data Protection and Patient Confidentiality**

Patient confidentiality will be maintained in accordance with the Data Protection Act 1998 and local confidentiality code of practice and data protection policy and procedure.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

Participants will be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

## 15. Data Handling and Record Keeping

Patients and volunteers will be assigned a study number, record of this and personal patient data will be anonymized and stored within the Royal Marsden Hospital on a secure server, supported by the NIHR imaging clinical research facility team. Imaging studies will be anonymized and archived at the RTTQA platform and on our secure imaging repository at the Royal Marsden Hospital (XNAT).

MRI data will be exported to XNAT, which is an image file repository located behind the RMH firewall. All data will be anonymised with a study number prior to analysis to ensure patient confidentiality. Anonymised MRI data will be transferred to another XNAT system (designed for anonymised images, and located behind the ICR firewall) for analysis.

The investigators will make source data and documents available for the purposes of trial-related monitoring, audits, IRB/IEC review and regulatory inspections as required, without compromising patient confidentiality.
The chief investigator carries overall responsibility for ensuring that data handling and record keeping for this study is in accordance with the Data Protection Act and Caldicott principles. Only the named investigators will have access to study data.

## 16. Financing, Indemnity & Insurance

Main study primary, secondary endpoint analyses will be conducted as part of a doctoral fellowship programme, funded by Health Education England and the National Institute for Health Research (HEE/NIHR ICA Programme Clinical Doctoral Research Fellowship, Mrs Ana Rita L Simoes dos Reis Ferreira, known as Rita Simoes, ICA-CDRF-2018-04-ST2-004).

MRI sub-study endpoints analyses will be conducted by Dr Blackledge. Additional MRI scans required for the endpoint number sub-study analyses are funded by Sarcoma UK, along with travel costs for patients participating in these scans. Funding from the ICR will support the PhD programme of Ms Thrussell.
Clinical trial infrastructure is supported by the NIHR imaging clinical research facility at the RMH.

The NHS Litigation Authority will cover standard clinical negligence by employees, staff and health professionals employed by the Royal Marsden NHS Foundation Trust. For more information visit the following website: http://www.nhsla.com/Claims/Pages/Clinical.aspx

There is unlimited liability and no excess. Insurance is provided under the Clinical Negligence Scheme for Trusts and there is no cover for non-negligence claims.

For all notification of claims please contact the Board Secretariat.

## 17. Publication Policy

All results will be submitted for peer-review at 2-3 international scientific meetings (funding already obtained from NIHR/HEE clinical doctoral programme for Mrs Simoes and from the ICR for Dr Blackledge). Incorporating feedback from these meetings, research articles will be published in open-access journals in relevant fields, to be led by Dr Miah (main study), Dr Blackledge ((MRI radiation response assessment sub-study,

Appendix A) and Dr Zaidi and Mr Hayes (Biomarker development and immune mediators associated with radiotherapy sub-study (BIODATA) Appendix B) . Publications will be shared amongst our STSE working group, who will meet on a quarterly basis, along with our nominated PPI representatives.

We will identify opportunities for writing guidelines and delivering a STSE software tool that could be used for clinical purposes within the health sector. The research conducted throughout this programme of work will involve development of new computer algorithms, for which patent protection and potential commercial exploitation will be considered. In addition, copyright protection will exist in material prepared for presentations, and when scientific articles are published in peer-review journals. The Enterprise Unit (EU) of the Institute of Cancer Research will work with the investigators of the study (A. Miah and M. Blackledge) to review outputs and identify any potential commercial or copyright materials. The EU representative (Dr Alan Stuttle) will manage IP for both the ICR and RMH, where the clinical studies will be performed.

The Biomedical Research Council (BRC) will be acknowledged in all the publications arising from this study.



## 18. Abbreviations

| | |
|---|---|
| 3DCRT | 3D-Conformal Radiotherapy |
| ADC | Apparent Diffusion Coefficient (from DWI) |
| AE | Adverse Event |
| AI | Artificial Intelligence |
| AR | Adverse Reaction |
| CBCT | Cone-Beam Computed Tomography |
| CI | Chief Investigator |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CT | Computed tomography |
| CTV | Clinical Target Volume |
| DCE-MRI | Dynamic Contrast Enhanced MRI |
| DWI | Diffusion-Weighted Imaging (MRI) |
| EF | Enhancement Fraction |
| EORTC | European Organisation for Research and Treatment of Cancer |
| EoT | End of Treatment |
| GCP | Good Clinical Practice |
| GIST | Gastrointestinal Stromal Tumour |
| GTV | Gross Tumour Volume |
| Gy | Gray (radiation unit) |
| ICR | Institute of Cancer Research |
| IGRT | Image-Guided Radiotherapy |
| IMRT | Intensity Modulated Radiotherapy |
| MRE | Magnetic Resonance Elastography |
| MRI | Magnetic Resonance Imaging |
| NIHR | National Institute for Health Research |
| OE-MRI | Oxygen Enhanced MRI |
| OR | Odds Ratio |
| PE | Phase-Encode MRI gradient direction |
| PI | Principal Investigator |
| PD | Proton Density MRI scan |
| PPI | Patient and Public Involvement |
| PTV | Planning Target Volume |
| QLQ | Quality of Life Questionnaire |
| $R_1$ | Longitudinal Tissue Relaxivity |
| $R_2$* | Transverse Tissue Relaxivity |
| RMH | Royal Marsden Hospital |
| RO | Readout MRI gradient direction |
| RT | Radiotherapy |
| RTOG | Radiation Therapy Oncology Group |
| SAE | Serious Adverse Event |
| SAR | Serious Adverse Reaction |
| SPAIR | SPectral Attenuated Inversion Recovery |
| SS | Slice-Select direction |
| STS(E) | Soft-Tissue Sarcoma (of the Extremities) |

| | |
|---|---|
| TESS | Toronto Extremity Salvage Score |
| WHO | World Health Organization |

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# Appendix 1.

## Sub-Study: Artificial Intelligence for automated assessment of multi-parametric MRI in soft-tissue Sarcoma – application to neoadjuvant RadioTherapy (AIMS-RT). (Lead: Dr Matthew Blackledge – The Institute of Cancer Research)

**Background**

Histological changes of STSE following pre-operative radiotherapy have been previously reported in a retrospective series, which also demonstrated that partial MRI response (>50% reduction in tumour volume) was predictive of pathological response (percentage of treatment-related necrosis).[16] The gross tumour volume (GTV) was delineated according to T1-weighted magnetic resonance imaging (MRI) including gadolinium enhancement, and the clinical target volume (CTV) encompassed margins for microscopic disease and peri-tumoral oedema, as visualised on T2-weighted MRI sequences. However, there is still a lack of concordance or understanding of the histological changes occurring within STSE tumours following radiotherapy that lead to the imaging phenotypes observed with T1-weighted or T2-weighted MRI. Moreover, STSE tumours are highly heterogeneous; imaging tools may allow for dose-escalation to resistant tumour sub-regions if the biological underpinning of imaging signatures in STSE could be better understood.

Accurate delineation of the GTV combined with IMRT and IGRT allows for accurate RT dose delivery and has the potential to minimise radiation dose to adjacent normal tissue structures. This could consequently translate to a lower incidence of acute and long-term toxicities. Furthermore, tumours are typically heterogeneous and imaging tools may allow for dose-escalation or de-escalation based on biological information.

Quantitative MRI is highly applicable to monitoring response of STSE, due to its excellent soft-tissue contrast and the lack of ionising radiation. Recent advances in scanner technologies now allow the exploitation of quantitative MRI techniques in clinical trials, as they enable (i) non-invasive investigation of the entire tumour volume and (ii) can provide information about the biological properties of tumours through functional measurements. For example, maps of apparent diffusion coefficient (ADC) derived from diffusion-weighted MRI inform on tissue cellularity, with lower ADC values observed in highly cellular or more aggressive regions within tumour.[17] Using contrast enhanced MRI, the signal enhancement after intravenous injection of a gadolinium-based contrast agent provides information about tumour vascular perfusion and permeability (DCE-MRI)[18], and by using so-called Dixon MRI, the presence of fat in sarcomas can also be measured and quantified.[19] In our previous work, we have demonstrated a negative correlation between ADC and tissue cellularity in STSE, and a positive correlation between MR-derived estimates of fat-fraction with histopathology-derived measurements of fat

content.[20] Furthermore, this study revealed significant increases in median ADC measurements following pre-operative radiotherapy in 14 patients. These imaging methods are now part of the EORTC-STBSG guidelines for radiological examination and reporting of STSE.[21] More recent additions to the collection of functional MRI techniques include Oxygen-Enhanced MRI (OE-MRI), which provides an indirect measure of tissue oxygenation[22], and magnetic resonance elastography (MRE), which measures tissue stiffness by imaging the effects of acoustic shear waves as they transverse through the tissue.[23] The former of these approaches is particularly attractive in the setting of radiotherapy, as previous studies have demonstrated an inverse relationship between tissue hypoxia and radiotherapy effectiveness.[24,25] The ability to quantify and map the hypoxic status within the tumour could provide very powerful predictive biomarkers of radiotherapy response, and allow clinicians to target radiotherapy doses to regions of the tumour that are not expected to respond well. Tissue stiffness as measured by MRE could also provide biomarkers of (i) tumour response to radiotherapy, and (ii) healthy tissue toxicity following irradiation exposure by monitoring the onset of fibrosis and oedema.

With the development of more powerful MR-imaging hardware, it is now possible to acquire all of these quantitative mapping techniques in a single radiological study. By combining these approaches, we can non-invasively gather information about the biology of STSE and how they respond to treatment, allowing oncologists to make informed judgments on personalized, adaptive RT plans. However, radiological evaluation of such multi-parametric information is confounded by the large quantity of data that is delivered by the scanner. This could be particularly important for any future studies of neoadjuvant STSE radiotherapy that involve the MR-linac[26], as functional imaging could be acquired at each treatment fraction, resulting in up to 20-30 imaging studies per patient. It is therefore vital that new image analysis techniques be developed that can capture relevant information from multi-parametric MRI in STS. With the advent of new methods in artificial intelligence (AI) in image analysis, the time for exploiting these techniques when analyzing multi-parametric MRI in STSE is ripe.

This sub-study provides the opportunity to standardise the radiological and histological response criteria used in assessing response to radiation. Although percentage necrosis is a well-established criterion of response to pre-operative chemotherapy in osteosarcoma, currently there are no standardized histological response criteria for pre-operative therapy in soft tissue sarcoma. A consensus has been sought to utilise percentage viable tumour cells as a marker of pathological response. Applying post-treatment imaging to guide the analysis of representative sections of the tumour specimen may provide a methodology in consistency in reporting response. In addition, this study will perform early biopsy and imaging of the tumour to determine if predictive changes can aid adaptation of the RT dose delivered entirely or partially to the tumour.

Our current practice has demonstrated that there are variations in RT response with different histological subtypes, shown radiologically and histologically and no clear standardised response assessment. There may be also the potential to consider pre-

operative chemotherapy in selected histological subtypes which may influence the timing and/or necessity for RT.

**Rationale**

The effect of radiation on specific STSE subtypes using standard and novel functional MR imaging techniques and also the pathological and molecular changes associated with pre-operative radiation. These data could lead to greater personalisation in the management of sarcoma patients, by determining the sequence of changes during radiotherapy, evaluating tumour perfusion and vasculature with functional MRI and histological and genomic changes.

**Hypotheses**

- MR-imaging changes measured within soft-tissue sarcomas observed during radiotherapy fractionation are predictive of final response to radiotherapy.
- Imaging phenotypes of STSE measured using multi-parametric MRI correlate with distinct molecular and histopathological alterations; regions of molecular/histopathological heterogeneity in STSE can be successfully identified using AI-based image analyses of multi-parametric MRI.

**Objectives**

***Primary***

1. To establish whether baseline measurements in apparent diffusion coefficient (ADC) measured at baseline, and/or changes in ADC measured midway through fractionation (after fraction 7-11 inclusive) or following treatment are predictive of soft-tissue sarcoma response measured using histopathology (% necrosis).

***Secondary***

2. To develop, optimise and evaluate clinical OE-MRI and MRE protocols for use of STSE imaging using healthy volunteer and MRI test-object studies.

3. To quantify the single-centre reproducibility of OE-MRI, MRE, T1, T2, magnetisation transfer (MT), DCE-MRI, and dixon imaging in STSE tumours, including all derived quantitative biomarkers.

4. To determine whether sub-regions identified using AI-segmented MRI demonstrate different biological phenotypes through molecular profiling and regional histopathology in soft-tissue sarcoma.

5. To assess whether heterogeneous sub-volumes identified from AI segmentation models correlate with histological STSE response to radiotherapy.

6. To assess the correlation between (i) pre-treatment measurements, (ii) mid-RT changes and (iii) post-RT changes of tissue hypoxia (measured using OE-MRI) and

tissue stiffness (measured using MRE) with post-radiotherapy changes in tumour cellularity (measured using DW-MRI)

7. To determine whether MR-imaging parameters measured within this sub-study are predictive of healthy tissue toxicity in STSE.

8. To develop AI-models for identifying texture features in X-ray CT images that correlate with MRI-derived sub-volumes.

**Sub-study design**

This sub-study is aimed at establishing whether changes in median ADC are predictive of pre-operative STSE radiotherapy response measured using histopathology. This sub-study will involve up to 65 patients treated with pre-operative RT and will only run at the Royal Marsden Hospital.

Recruitment will involve STSE patients receiving pre-operative radiotherapy. Patients (up to N=65) will be invited to have an MRI scan before treatment, following fraction 7-11 of radiotherapy and 4 weeks before surgical resection. Additionally, molecular and pathological assessment will be undertaken on surgical resection specimen. Whole genome sequencing has become standard of care so further correlative analysis with clinical outcome will be performed. On board weekly CBCT imaging will be reviewed to determine changes occurring during treatment.
A set of patients (N=15) will be recruited to volunteer for an repeat imaging baseline study (> 6 days after first baseline before treatment) to perform a repeatability study of the MR-imaging biomarkers. We will collect the ADC measurements of these patients measured at their normal clinical imaging time points. Additionally, molecular and pathological assessment will be undertaken on surgical resection specimen.
Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both. The total number of patients that will be recruited will range 50-65 depending on how many patients take part in both sections.

**Volunteer Study**

In the initial phase, OE-MRI and MRE protocols will be optimized prospectively on a cohort of 20 healthy volunteers at the RMH. Volunteers will undergo conventional clinical scans for STSE in the abdomen, pelvis and extremities (diffusion-weighted, Dixon, MT, Dixon, T1, T2, and DCE-MR imaging; the latter without the use of any contrast agent). For the same fields-of-view, OE-MRI and MRE sequences will be acquired; spatial resolution and geometric distortion of these new protocols will be measured by comparison to clinical imaging protocols. Initial developments of these techniques on MRI test-objects are already underway at the RMH.
All volunteer studies will be performed according to RM healthy volunteer guidelines; volunteer details will be entered into the institutional EPR system, imaging data will be

uploaded onto the RMH PACS system and anonymously stored on a secure research imaging archive hosted within the RMH; all scans will be reported for incidental findings. Anonymised data for analysis will be transferred to another XNAT system, behind the ICR firewall.

**Patient Study**

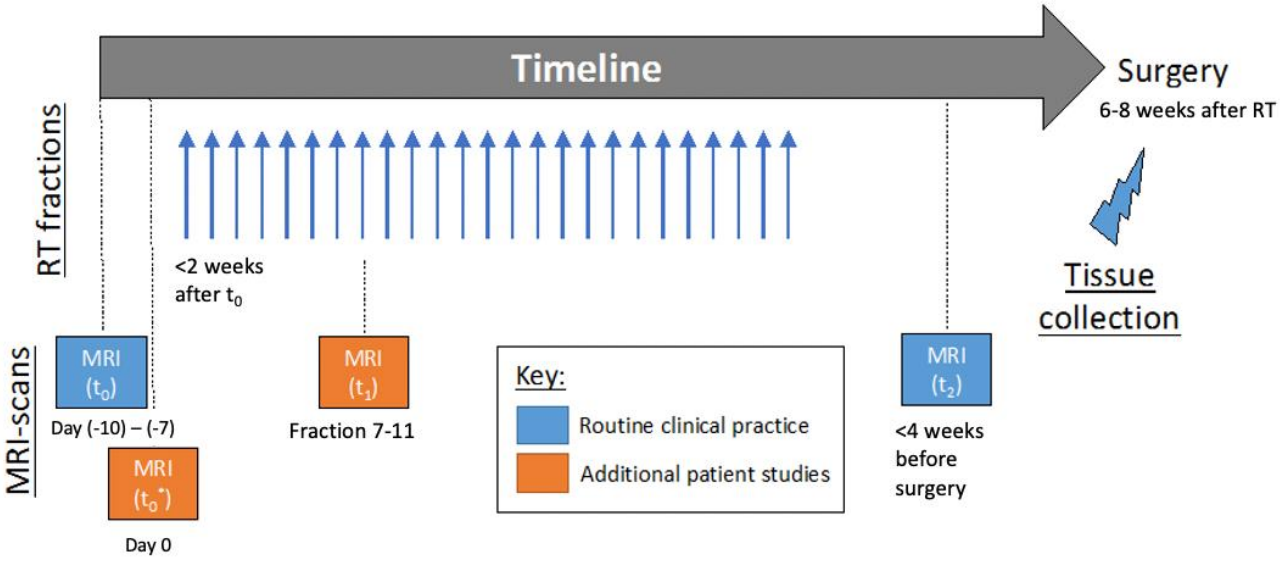
This prospective, observational imaging sub-study aims to recruit adult STSE patients receiving neoadjuvant RT as part of routine healthcare at the Royal Marsden Hospital (single-centre).

Up to 65 patients will receive multi-parametric MRI studies performed within one week prior to RT ($\mathbf{t_0}$), following 7-11 RT fractions inclusive ($\mathbf{t_1}$), and within 4 weeks prior to surgical resection ($\mathbf{t_2}$).

15 patients will receive the multi-parametric MRI study performed prior to RT ($\mathbf{t_0}$), and again 7 days after the first ($\mathbf{t_0}$*) but before treatment begins.

Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both.

An illustration of our proposed patient pathway in shown in the figure below:




In addition to the MR-imaging study, we will collect diagnostic baseline and follow-up X-ray CT data from the same 65 patients recruited on the sub-study, along with radiotherapy planning structures. These data will be used to explore surrogate measures of heterogeneous response using X-ray CT, with developed methodologies for multi-parametric MR acting as the gold-standard (objective 8). A U-NET deep-learning model will be developed and adapted to train a new AI network for this purpose.

**Specific endpoints**

***Primary***

1. ADC measurements at baseline ($t_0$), and ADC changes at mid-fractionation ($t_1$), and following RT conclusion ($t_2$) will be correlated with tumoural response, measured as % necrosis from histopathological analysis of post-surgical specimens, using a linear model.

***Secondary***

2. Clinical feasibility of OE-MRI and MRE protocols for imaging if STSE within the RMH (image quality evaluated by a consultant radiologist, and acquisition time < 20 minutes for both protocols).

3. Bland-Altman analysis of the repeated MR techniques (OE, DCE, MRE, T1, T2, MT and dixon) prior to start of RT. Intraclass-correlation coefficient (ICC) and coefficient of variance (CoV) will be measured.

4. Our system of deriving sub-volumes from STSE tumours using AI will be used to identify regions of interest for immunohistochemistry from tissue samples. Measures of (i) % viable tumour, (ii) % dedifferentiated tumour, (iii) % necrosis, (iv) % fat, (v) % ki67 uptake, and (vi) % fibrosis/hyalinisation will be recorded within these regions.

5. Changes in AI-derived measurements of STSE tumour sub-volumes at mid-fractionation and following conclusion of RT will be correlated with histopathological response.

6. Measurements at baseline, and changes at mid-fractionation, and following RT conclusion in tissue stiffness and tissue hypoxia will be correlated with tumoural response, measured as % necrosis from histopathological analysis of post-surgical specimens, using a linear model.

7. Time-to-report of tissue fibrosis, wound-healing complication and lymphoedema as measured on a 3-monthly interval following surgery (follow-up period of two years).

8. Accuracy of heterogeneous tumour sub-volumes segmented from X-ray CT images compared to our MRI approach (gold standard).

**Sub-study inclusion and exclusion criteria**

Patients receiving pre-operative radiotherapy at the Royal Marsden Hospital will be invited to participate in AIMS-RT sub-study. The specific inclusion and exclusion criteria for this sub-study are listed below.

***Inclusion criteria***

i. Patients with soft tissue sarcomas planned for surgical resection and preoperative radiotherapy at the Royal Marsden Hospital
ii. Patients due to receive pre-RT MR imaging studies as part of routine clinical care.

***Exclusion criteria***

i. Patients with contraindications to MRI (e.g. MR Unsafe implant)
ii. Claustrophobia
iii. Patients with renal failure or problems with IV access will not receive intravenous contrast but may still be recruited to undergo the non-contrast enhanced components of the imaging protocol.
iv. Patients who do not tolerate the use of an Oxygen administration mask or have Chronic Obstructive Pulmonary Disease (COPD) will not undergo OE-MRI
v. Patients who would find it difficult/uncomfortable to position the tissue of interest within the central section of the scanner

***Subject withdrawal criteria***

All patients will require MR-imaging to be acquired pre-/post-radiotherapy as part of routine care, prior to tumour removal. Patients may be withdrawn from the study if there are any reason MR-imaging becomes contra-indicated during their treatment (e.g. new implantation of an MR-unsafe implant). Should at any time they feel unable to participate in a third MR-imaging time-point, they would also be withdrawn from the study.

Up to 65 pre-operative patients will be recruited for the exploratory study from patients enrolled in the main study at RMH. However, as pre-operative patients participating in the main study may decline participating in the secondary study, this number may not be accrued during the planned 18 months of accrual of the main study. In that case, specific recruitment of pre-operative patients for the sub-study only (i.e., who would not participate in the main study) will be extended by a maximum of 12 months.

***Sub-study participation***

Patients treated within PredicT B with a pre-operative intention will be asked to take part in the sub-study.
This sub-study can be summarised in two parts: i) a

50 patients will be recruited for this sub-study which will involve administration of one additional dose of MRI contrast agent (Gadolinium-chelate), midway through radiotherapy fractionation (standard dosage of 0.1 mmol/kg). Subject compliance to our additional imaging protocols will be recorded through the use of MRI checklists, to be completed by the research radiographer during each MR-acquisition time-point.

15 patients recruited for the repeatability study will be asked to volunteer for an additional imaging baseline study (> 6 days following first baseline but prior to radiotherapy) to perform a repeatability study of the MR-imaging biomarkers explored in this cohort. This will include another MR contrast injection (0.1 mmol/kg). Once the required numbers for this secondary objective have been recruited (N = 15), we will not pursue further recruitment for repeatability assessment.

Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both.

**Data-acquisition**

***MR-Imaging***

Imaging studies at all time-points (including the repeatability sub-set), will include the imaging sequences listed in **Table A1**. Each imaging study will not last longer then 1-hour, including patient set-up, positioning and removal from the scanner couch. All sequences will be acquired in free-breathing, with the patient lying in a supine position; in all patients, a cannula will be used to administer the MRI contrast agent required for DCE sequences. In addition, patients will be required to wear an oxygen mask for pure O2 breathing during OE-MRI sequences, and 3-4 small (~ 3 x 5 $cm^2$) applicators will be placed on the surface of the STSE to emit shear waves required for MRE imaging. Our MRE equipment is not CE-marked for medical use. We have approached the medical devices team at the Medicines and Healthcare products Regulatory Agency (MHRA) regarding its use within this trial, and they have informed us that official application to the MHRA is not required in this instance as we will be using the equipment for research purposes only. All sequence parameters will be collated into an imaging manual for the study, to ensure consistency of imaging results across all patients and to provide research radiographers with detailed instructions on the timing of all imaging sequences. In addition, radiographers will be requested to complete an imaging check sheet, which will be archived to as a record of all completed scans.

| Sequence Name | Quantitative Imaging Biomarker | Exogenous Contrast Required? | Approximate Time (minutes) |
|---|---|---|---|
| **1) Localizer** | N/A: Used for positioning of subsequent sequences | - | 1 |
| **2) Dixon imaging** | Fat fraction (%) | - | 2 |
| **3) MT imaging** | Magnetic Transfer Ratio (MTR) | - | 2 |
| **4) Diffusion-weighted imaging** | ADC: Surrogate marker of tissue cellularity ($mm^2/s$) | - | 10 |
| **5) T1/T2 Mapping** | T1/T2 | - | 10 |

| | | | |
|---|---|---|---|
| **6) 3D gradient echo (PD mapping)** | N/A: Provides an estimate map of water density | - | 1 |
| **7) Dynamic contrast-enhanced imaging** | Enhancement Fraction: Surrogate marker of vascularity (%) | Gadolinium-chelate | 10 |
| **8) Magnetic Resonance Elastography** | Speed of sound: Surrogate marker for tissue stiffness (m/s) | - | 10 |
| **9) Oxygen Enhanced Imaging** | $\Delta R_1$, $\Delta R_2$*. Map of tissue hypoxia ($ms^{-1}$) | Pure $O_2$ breathing | 10 |

**Table A1.** Approximate timings for the MRI protocol investigated in this study. Including 20 minutes set-up time, a 1-hour scanning slot will be necessary for each imaging time-point (approximately 15 minutes longer than conventional MRI protocols for STS).

***Tissue Collection***

Tumours will be surgically removed following radiotherapy (approximately 6-8 weeks after RT) and orientation of tumour matched to orientation of MRI by the surgical team in consultation with the radiology team. Tissue blocks will be extracted from excised tumours and sectioned in the same plane as for MRI-acquired images. We will acquire a number of representative blocks, covering the axial field-of-view of the tumour in two concurrent sections, and for each record (i) % viable tumour, (ii) % dedifferentiated tumour, (iii) % necrosis, (iv) % fat, (v) % ki67 uptake, and (vi) % fibrosis/hyalinisation and any further histopathological and immunohistochemical measurements. Following AI-based segmentation of tumour sub-regions from MRI, we will manually locate spatially similar regions on histology samples consulting both modalities simultaneously (1 location per tumour sub-type identified). Within $1cm^2$ regions of these samples, we will measure cell/stroma ratio and stromal morphology (fibrous/hyalinised, myxoid or fibromyxoid) and perform molecular profiling using RNA-Seq/proteomics.

The management of tissue samples related to this aspect will be conducted by Mrs Emma Perkins from the sarcoma research team at the Royal Marsden. All persons involved in the collection, transportation and handling of human tissue will be adequately trained for their involvement in this work. Formalin-fixed paraffin embedded (FFPE) tumours blocks will be held in the secure storage facility in the Molecular and Systems Oncology lab, ICR. In addition, frozen surgical specimens will be stored at the appropriate temperature in freezers at the ICR. Both the RMH and ICR are Human Tissue Authority (HTA) licensed premises, with transfer of significant material between sites covered by overarching Material Transfer Agreement (MTA). All human tissue received into the lab and covered under the auspices of the HTA will be prospectively logged onto FreezerPro (Brookes Automation), a web-based lab management system to ensure HTA compliance and assist with sample tracking. To test our tissue processing pathway for this sub-study we will

leverage existing excess tissue blocks, stored within the RMH tissue archive, that was obtained as standard of care and as part previous prospective trials for which consent has been given.

**Sub-study endpoint analysis**

***Endpoint 1***

We will generate two linear models of response for predicting changes in tumoural response (measured semi-quantitatively using pathology) using (i) changes in median ADC at our mid-fractionation imaging time point ($\Delta ADC_1$), and (ii) change in median ADC following completion of RT ($\Delta ADC_2$). The accuracy of both models for predicting RT response, measured as % necrosis from histopathological analysis of post-surgical specimens, will be compared to identify whether changes occurring mid-fractionation provide substantive evidence for final treatment outcome. 50 patients would suffice for such a study; there are no conventional methods for powering linear model studies.

***Endpoint 2***

A radiologist with experience in STSE imaging will review the quality of all new imaging techniques and assess them for contrast-to-noise, presence of imaging artefacts and spatial resolution. Imaging will be acquired in the thigh of each volunteer to obtain a similar field-of-view to that required for STSE patients. A time-cap of 20 minutes will be required for these imaging techniques, but where possible this will be reduced.

***Endpoint 3***

Bland-Altman analysis will be used to test the repeatability of the metrics derived from MRE and OE-MRI (tissue stiffness and ΔR2* respectively). We will calculate the median of both parameters within the tumoural extent (regions of interest outlined by I. Thrussell, and confirmed by a radiologist), and compare the change repeat measurements with the average to obtain a coefficient of variance (CoV) measure. In addition, we will compute the intra-class correlation (ICC: measure of 1 representing ideal repeatability).

***Endpoint 4***

Our AI methodology will be developed on all patients split into a training and test datasets on a 4:1 ratio. Using the first 40 patients, we will train state-of-the-art deep-learning techniques (e.g. U-Net) to automatically segment heterogeneous sub-regions within STSE tumours (using quantitative measures from all available MRI modalities). Radiologist defined regions will provide a gold-standard for these sub-regions, using a technique we have previous developed. The cross-validation accuracy of this new methodology will be compared with our existing approach. The remaining 10 patient datasets will be used to provide the final accuracy of the trained models. We will also explore the use of AI for automatic delineation of the entire tumour extent using U-Net models or equivalent deep-learning technology as it becomes available. It should be noted that for each patient, we expect to generate data from at least 4-5 different heterogeneous regions within the tumour (both in terms of imaging and histopathology), thus increasing the effective sample size in this endpoint.

Regional histopathology and molecular profiling will be used to validate the classifications made to each tumour sub-type identified through imaging acquired prior to surgery (**$t_2$**).

***Endpoint 5***

Volumes will be calculated for each tumour sub-region detected using our AI approach. Changes in these volumes at $t_1$ and $t_2$, denoted $\Delta V_1$ and $\Delta V_2$ respectively, will be correlated with histopathological response.

***Endpoint 6***

Linear models in the same vein as for the primary endpoint of the study will be explored for MRE, OE-MRI and DCE-MRI measures of tissue stiffness, hypoxia and tissue vascularity. Models will include ADC measures, to identify which parameters are most predictive of response, and whether baseline measures or changes at t1 provide the highest predictive power.

***Endpoint 7***

Cox proportional hazard models will be developed for changes in tissue stiffness and ADC with time to toxicity related outcomes following surgery (tissue fibrosis, wound-healing complication and lymphoedema). We will focus on the utility of simple statistics from MR-derived parameters within the entire tumour volume for this endpoint (i.e. median MRI measures).

***Endpoint 8***

We will investigate the use of U-Net models and Generative Adversarial Networks (GANs) for developing models of segmentation of heterogeneous STSE regions using X-ray CT. Our MRI methodology (endpoints 4 and 5), will act as gold-standard for this technology; we will compare volumes of measured sub-regions derived from both CT and MRI.

# Appendix 2. Biomarker development and immune mediators associated with radiotherapy sub-study (BIODATA). (Leads: Dr Shane Zaidi and Mr Andrew Hayes)

## Background

### Biomarker Development

Recent technological advances in radiotherapy planning and delivery may lead to more individualised radiotherapy by improving the therapeutic ratio based on tumour anatomy.[27] Adapting radiotherapy by applying tumour biology also has the potential to contribute to improving the therapeutic gain.

Soft-tissue sarcomas are characterised by considerable genetic and epigenetic heterogeneity driven by genomic instability.[28] Patients with the same histological subtype demonstrate a wide range of natural progression of disease and response to treatments, including radiotherapy. A major goal of personalised cancer medicine includes the use of biomarkers to tailor treatment to individual patients. Prognostic biomarkers provide information regarding disease outcome regardless of treatment, and predictive biomarkers determine which patients will derive benefit from a specific treatment.

There are few predictive biomarkers that have made the transition to clinical use in soft-tissue sarcomas. Gastrointestinal stromal tumours (GIST) harbor specific mutations in *KIT* and *PDGFRA* correlating with clinical response to the oral tyrosine kinase inhibitor imatinib.[29] Although there are other examples of predictive biomarkers for directing systemic therapy in soft-tissue sarcomas, there is an urgent need to develop markers predicting outcome to radiotherapy.

Many factors are known to influence tumour response to radiation including total dose, fractionation, hypoxia and intrinsic radiosensitivity. Pre-clinical tests to determine tumour hypoxia and intrinsic radiosensitivity may correlate with clinical outcome but are not available for routine use. Tumour response to radiation includes activation of the DNA damage response pathways providing additional potential candidate markers for evaluating radiosensitivity.[30]

Development of new molecular profiling techniques now allows us to identify specific tumour gene expression signatures and attempts have been made to develop prognostic models. Techniques include analysis of circulating tumour cells, gene expression from tumour samples, analysis of common somatic mutations and rearrangements in specific genes and analysis of blood serum proteins. Although several candidate markers have been identified there is a need to validate these prospectively in the clinical setting.

### Immune mediators associated with radiotherapy

Radiotherapy has been used to treat cancers based on the ability to cause DNA-damage leading to cell death. Recent preclinical and clinical data suggest radiation may also mediate an anti-tumour immune response. Although we need to further investigate the underlying mechanisms, radiation has an effect on the immune response to antigens including up-regulating MHC- class I antigen presentation, creating neo-antigens and activating cytotoxic T cells.[31] Analysis of paired soft tissue sarcoma tumour samples pre- and post-radiotherapy have demonstrated changes in tumour microenvironment, triggering immunogenic cell death, enhances immune-related signatures suggesting scope for combining radiotherapy with immunotherapy. Clinical studies are now combining immunotherapy with radiation in patients with soft-tissue sarcoma. There is a need for refined focus on the use of radiotherapy as an immune-modulator in patients with soft tissue sarcoma. Biomarker analysis of candidate markers may identify which patients will benefit from personalized care combing radiation with modern immunotherapy agents including checkpoint inhibitors.

**Rationale**

In this exploratory study, we hope to identify potential markers of radiation response and radiation sensitivity alongside biomarkers to evaluate potential prognostic markers to predict for early development of metastatic disease, which may

a. Refine selection of cases for pre-operative radiotherapy, palliative radiotherapy and no radiotherapy
b. Guide radiation dose escalation/ de-escalation strategies (with possible hypofractionation) for different histological subtypes
c. Evaluate radiation response and whether addition of systemic therapy could enhance the therapeutic index
d. Determine if radiotherapy stimulates the tumour microenvironment to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation (see laboratory manual).

Participation in this sub-study will be offered to patients receiving pre-operative and palliative radiotherapy.

**Hypothesis**

Biological markers will provide predictive information for tumour response for specific histological subtypes following radiotherapy and scope for combining radiation with immunotherapy.

**Exploratory objectives**

1. Determine if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in anti-tumour immunity, to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation.

a. Quantify the change in infiltration of immune cell populations.
b. Measure activation and exhaustion of therapeutically relevant populations.
c. Profile expression of therapeutically tractable immune checkpoint markers.
d. Investigate TCR clonality, neoantigens and immunoediting due to radiotherapy.

2. Prognostic markers which may refine selection of cases for pre-operative radiotherapy, palliative radiotherapy or no radiotherapy.

**Sub-study design**

This prospective sub-study is aimed at developing biomarkers that are predictive of response following radiotherapy delivered in the pre-operative / palliative setting.

This single centre sub-study (Royal Marsden Hospital, London) will aim to recruit 50 patients treated with pre-operative and 10 patients treated with palliative radiotherapy.

Recruitment will involve patients receiving pre-operative or palliative radiotherapy (minimum n=5/group) classified into the following groups:

1. Undifferentiated pleomorphic sarcoma (minimum number n =20),
2. Synovial sarcoma
3. Myxofibrosarcoma
4. Myxoid liposarcoma
5. Malignant peripheral nerve sheath tumour
6. Leiomyosarcoma
7. Other

**Biopsies**

Patients receiving pre-operative radiotherapy:

Freehand (surgical) tumour biopsies will be performed at baseline (during screening between day -7 and day 1) and fraction 8-10 (optional). Representative samples of the final resection specimen will also be collected for analysis. If patients are no longer scheduled for surgery (due to development of new or progressive metastatic disease or other factors) will collect a freehand (surgical) tumour biopsy (optional) following the post- radiotherapy MRI scan (after Day 40-50), ideally day 54-64. There is a separate consent form for this biopsy.

Patients receiving primary (palliative) radiotherapy:
Freehand (surgical) tumour biopsies will be performed at baseline (during screening) with an additional optional biopsy performed during treatment (see table 1 below).

Patients entering the BIODATA sub-study who develop local recurrence and/or metastatic progression during routine post-operative surveillance will be asked to consent for collection of a standalone biopsy. Tumours will be easily and safely amenable to freehand or image guided biopsy. There is a separate consent form for this biopsy. If they have surgery including metastasectomy to resect metastatic disease, patients will be asked if they consent for samples to be taken from the resected tissue for research purposes.

**Research blood samples**

Blood samples will be used to store PBMC and plasma: a total of 60 mL blood will be collected at each time-point.

Patients receiving pre-operative radiotherapy:
Blood samples will be collected at baseline (between day -7 and day 1), fraction 8-10 (optional) and at surgery to enable biomarker analyses.
If patients are no longer scheduled for surgery (due to development of new or progressive metastatic disease or other factors), will collect optional blood samples following the post- radiotherapy MRI scan (after Day 40-50), ideally day 54-64.

Patients receiving palliative radiotherapy:
Blood samples will be collected at baseline (during screening prior to surgery) and selected time-points (optional) to enable biomarker analyses (see table 1).

Patients entering the BIODATA sub-study who develop local recurrence and/or metastatic progression during routine post-operative surveillance will be asked to consent for collection of a standalone set of blood samples.

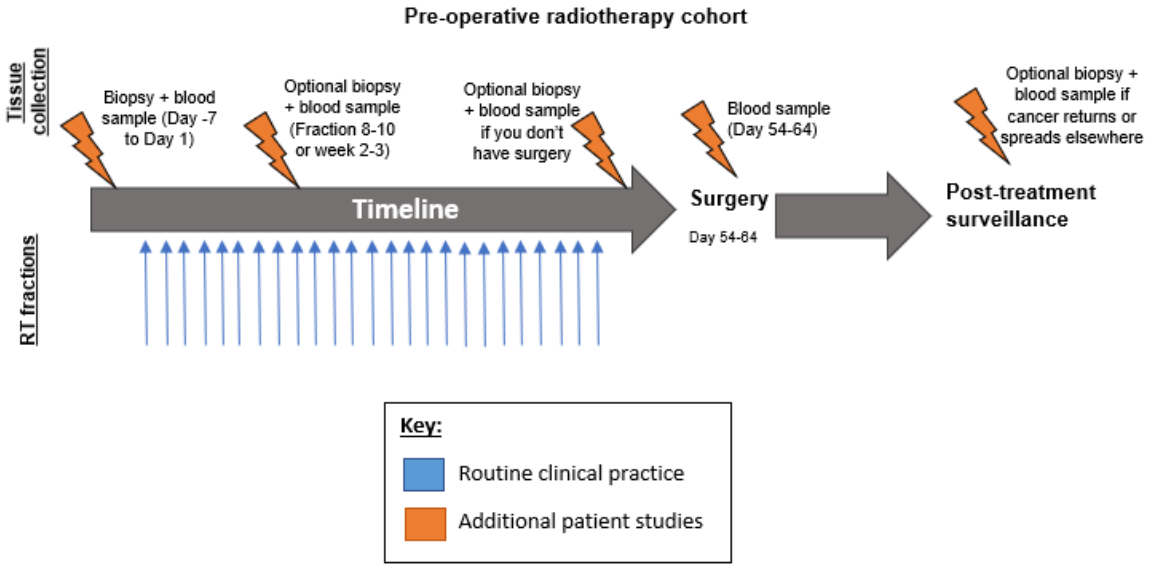



**Figure 1.** Illustration of proposed biomarker pre-operative radiotherapy STSE study.

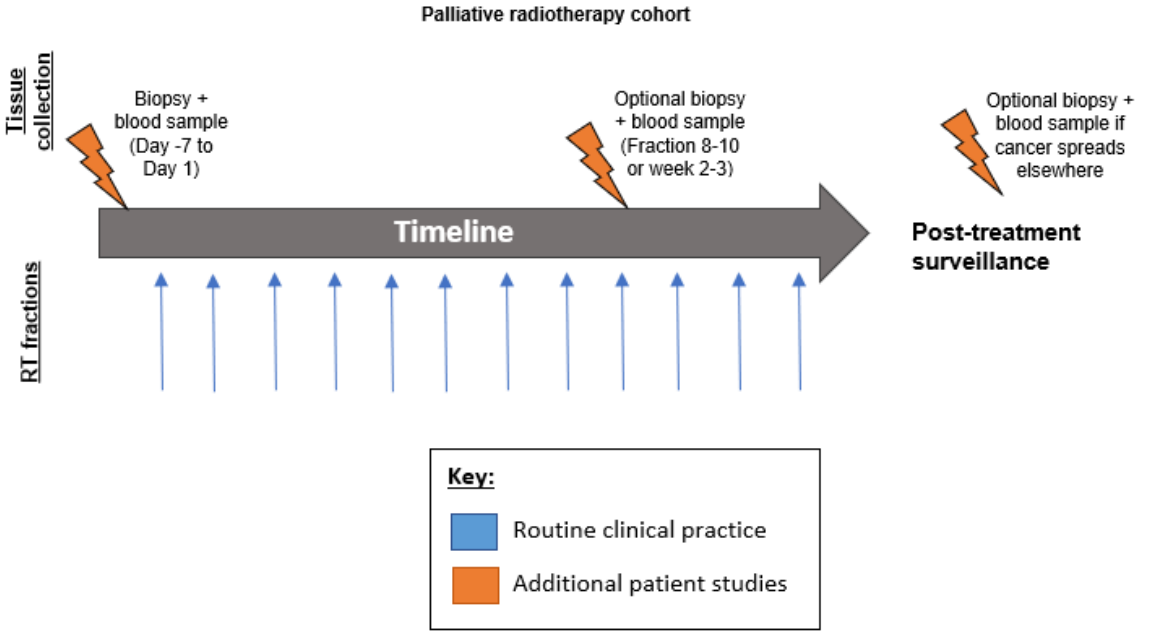



**Figure 2.** Illustration of proposed biomarker palliative radiotherapy STSE study.

| Dose-fractionation | RT delivery | Baseline research biopsy (during screening) | Second (optional) research biopsy | Baseline research bloods | Second (optional) research bloods | Third Research bloods |
|---|---|---|---|---|---|---|
| **Pre-operative radiotherapy cohort (tumour samples collected from resected specimen)** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 Gy/25# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | At surgery 6-8 weeks after completing RT |
| 36 Gy/18# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | At surgery 6-8 weeks after completing RT |
| **Palliative radiotherapy cohort** | | | | | | |
| 40-45 Gy/15# | Daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 36 Gy/12# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 30 Gy/10# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 30 Gy/5# | Once Weekly | Between D-7 and D=0 | Just before #3 | Between D-7 and D=0 | Just before #3 | NONE |
| 36 Gy/6# | Once weekly | Between D-7 and D=0 | Just before #3 | Between D-7 and D=0 | Just before #3 | NONE |
| 25 Gy/5# | daily | Between D-7 and D=0 | After #5 | Between D-7 and D=0 | After #5 | NONE |

**Table 1:** RT dose fractionation schedules and timing of tests.

### *Tissue Collection*

Tissue collection will be coordinated by Eniola Ayeni, and Emma Perkins in consultation with the Sarcoma Unit of the RMH, and the research cancer nurse specialists who will be acquiring blood samples. Tissue samples will be stored in The Royal Marsden and The Institute of Cancer Research in accordance with the Human Tissue Act 2004. We have already tested our tissue processing pathway for this sub-study as part of the RMH sponsored CCR4640 APPLE Study (REC Ref 18/LO/0240).

### Specific endpoints

1. To establish whether pre-operative or palliative radiotherapy results in a measureable change in tumour microenvironment

    a. Analyse PD-L1 and PD-L2 expression at baseline and after treatment using IF.

    b. Predict changes to immune cell populations and radiation induced "neoantigen" expression from whole exome sequencing and RNA sequencing on frozen tumour samples

c. To analyse the impact of treatment on phenotype and function of immune cell infiltrates (baseline, during and surgery tumour specimens)

i. Phenotype and activation status by multi-IF for T cells (including but not limited to CD3, CD8, GzmB), B cells (CD20, IgG), Treg (FOXP3) and TAM (CD163)

ii. T cell activation/proliferation through multi-IF for CD8, Foxp3 and KI67 (or costimulatory molecules), and expression of other immune check point receptors.

d. To analyse the impact of treatment on plasma cytokines. The levels of circulating growth factors and T cell cytokines analyzed by Luminex or Elisa technology.

2. Biomarkers identified through laboratory analysis will be correlated with development of local recurrence, Metastatic disease-free survival and overall survival at 24 months.

***Inclusion criteria***

1. Patients with extremity soft-tissue sarcomas who are either due preoperative radiotherapy followed by surgical resection, or palliative radiotherapy, at the Royal Marsden Hospital.
2. Include but not limited to the following soft-tissue sarcoma histological sub-types: leiomyosarcoma, myxoid liposarcoma, liposarcoma, undifferentiated pleomorphic sarcoma, fibrosarcoma, epithelioid, clear cell, and synovial sarcoma.
3. Able and willing to undergo tumour biopsies.
4. Written informed consent.

***Exclusion criteria***

1. Patients on anti-coagulation who are unable to have a safe tissue biopsy performed (to discuss with Chief Investigator on an individual basis). Patients on anti-platelet medication can be entered into the study.
2. Previous radiotherapy within the treatment area.

**Data analysis**

Time course data on immune infiltration, mutations and neoantigens as a result of radiotherapy in sarcoma do not currently exist. This study involves consenting 60 patients with the aim of collecting a set of 3 serial tissue samples per patient (Day 8 biopsy is optional). A complete set of samples which pass quality control for sequencing and imaging is anticipated to be in the region of 20-25. This is a descriptive study with numbers anticipated to be too low for formal statistical tests. Data generated as a result of this

study will be used to guide both the design and the appropriate statistical power calculations for a number of follow-on studies.

Genomic analysis (DNA whole exome sequencing, DNA methylation, RNAseq) and protein analysis (multiplex immunohistochemistry/IF) will be used where necessary to address the following analysis questions. Genomic and protein analysis results will also be correlated with clinical endpoints (Local recurrence, metastatic disease-free survival, and overall survival).

1. DNA analysis will be used to identify defects in DNA damage response and repair pathway genes.
2. IHC/multiplex ImmunohistochemistryIF will be used to assess markers of DNA damage and DNA repair competence.
3. RNAseq and multiplex IHCIF will be used to first identify radiation induced changes to immune populations. This will involve established and extensively validated methods to predict immune populations from RNAseq data (CIBERSORT immune deconvolution). Predicted populations will be validated by multispectralmultiplex IHCIF.
4. Immune expression of clinically tractable immune-checkpoint targets will be determined. Changes on RNAseq will be used to validate immune checkpoint expression on therapeutically important cell populations by multiplex IHCIF.
5. The ability of radiation to alter the expression of mutated proteins which are subsequently predicted to be processed and presented to the immune system (referred to as tumour “neoantigens”) will be assessed from whole exome sequencing and RNAseq data.
6. Peripheral blood can be used to test if circulating immune populations recognise predicted antigens in point 45, and if this recognition has been altered by radiotherapy.
7. Clinical endpoints will be collected (local recurrence, metastatic disease-free survival , and overall survival at 24 months).

**Radiotherapy dose fractionation schedules**

The following radiotherapy dose fractionation schedules are permitted for primary (palliative) radiotherapy.

40-45Gy in 15 fractions, delivered daily, Monday-Friday;
36 Gy in 12 fractions, delivered daily, Monday- Friday;
30 Gy in 10 fractions, delivered daily, Monday- Friday;
36 Gy in 6 fractions, delivered once weekly;
30 Gy in 5 fractions, delivered once weekly;25 Gy in 5 fractions, delivered daily over one week.

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# Appendix 3. Schedule of assessments for main study

| Investigations | pre-registration/ post consent[4] | Within 14 days prior to registration | Within 28 days prior to starting treatment | From surgery and up to 120 days after surgery | During Radiotherapy | 28 days (and up to 35 days) after the last fraction of radiotherapy[5] | Month 1 post RT[6] | Month 2 Post RT | Month 3 Post RT | Month 6 post RT | Month 9 Post RT | Month 12 Post RT | Month 15 Post RT | Month 18 post RT | 2 years post registration (EOT) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Histological confirmation of disease | X | | | | | | | | | | | | | | |
| Diagnostic MRI and/or CT (if there is a contraindication to MRI) of the primary tumour site[1, 2] | X | | | | | | | | X | X | X | X | | X | |
| Chest imaging (CT or chest x-ray) within 3 months of registration, as per routine practice | X | | | | | | | | X | X | X | X | | X | |
| Clinical review of relevant medical history | | X | | | | | | | | | | | | | |
| Completion of the assessment of adverse events (AEs) using CTCAE v4.03 | | X | | | | X | | | | | | | | | |
| Assessment of WHO performance status | | X | | | | X | X | X | X | X | X | X | | X | X |
| Pregnancy test (urine or blood) in females of child bearing potential | | X | | | | | | | | | | | | | |
| Medical History ( smoking, diabetic status) | | X | | | | | | | | | | | | | |
| Measurement of height & weight | | X | | | | | X | X | X | X | X | X | | X | X |
| Physical Exam (limb function/ mobility)[3] | | X | X | | X | X | X | X | X | X | X | X | | X | X |
| EORTC QLQ-C30 quality of life questionnaire | | | X | | | X | | | | | | X | | | X |
| EORTC QLQ-FA12 fatigue questionnaire | | | X | | | X | | | | | | X | | | X |
| Toronto Extremity Salvage Score (TESS) questionnaire (1 and 2 years post registration) | | | X | | | X | | | | | | X | | | X |
| Musculoskeletal Tumour Society Rating Scale | | | X | | | | | | | | | X | | | X |
| Post-Surgery Wound Assessment | | | | X | | | | | | | | | | | |
| Cone Beam CT imaging review of treatment volume where applicable (additional imaging review where applicable) | | | | | X | | | | | | | | | | |
| RTOG Acute Radiation Morbidity Scoring Criteria[7] | | | | | | X | X | X | X | | | | | | |
| RTOG/ EORTC Late Radiation Morbidity Scoring Criteria from day 91 after start of treatment | | | | | | | | | X | X | X | X | X | X | X |
| Stern's scale for oedema from day 91 after start of treatment | | | | | | | | | X | X | X | X | X | X | X |
| Measurement of circumference of the limb | | | X | X | | X | | | X | X | X | X | X | X | X |
| Clinical assessment of local tumour control | | | | | | | | | X | X | X | X | X | X | X |
| Radiological response assessment (approximately 4-6 weeks after pre-op RT) | | | | | | X | | | | | | | | | |
| Pathology response assessment (after resection, approximately 8-10 weeks after pre-op RT) | | | | | | X | | | | | | | | | |
| Assessment of wound related clinical findings up to 120 days after surgery | | | | X | | | | | | | | | | | |

1. Patients receiving post-operative radiotherapy the MRI/ CT should ideally have been performed within 1 month prior to surgery

2. Patients receiving pre-operative radiotherapy, the MRI/ CT should ideally be performed within 1 month of starting radiotherapy, although decisions on repeating scans older than 1 month will be made at the treating clinician's discretion

3. Assessment of wound related clinical findings up to 120 days after surgery (if applicable within 28 days prior to starting treatment)

4. The following pre-registration assessments do not need to be repeated if within 28 days prior to starting treatment: clinical review, pre-treatment routine bloods (FBC, U&Es, LFTs, CRP, LDH and clotting), Assessment of Aes using CTCAEv4.03, WHO performance s

5. we will collect each patient: Diagnostic scans which may have been acquired before treatment commences, Radiotherapy planning scans, Structures, Plans and dose cubes, Treatment imaging review, including CBCT which may have been acquired as per imaging ve

6. All visits should be carried out at specified time +/ - 2 weeks.

7. Up to 90 days after start of treatment

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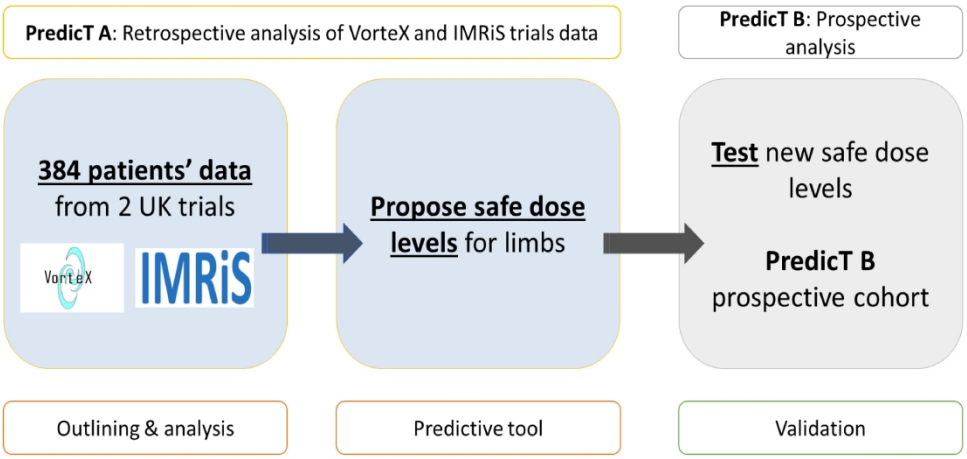


Figure 1

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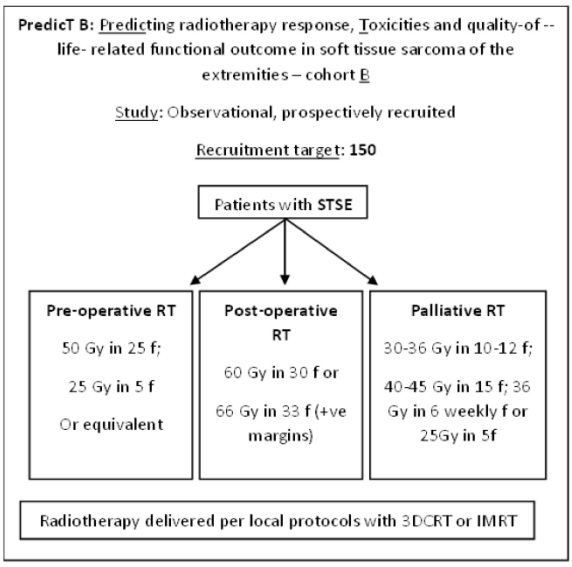


Figure 2

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# Protocol CCR 5166

**Title**: Predicting radiotherapy response, toxicities and quality of life related functional outcome in soft tissue sarcoma of the extremities: a prospective observational cohort study

**Short Title:** Predicting radiotherapy response and Toxicities in soft tissue sarcoma of the extremities – cohort B (PredicT B)

| | |
|---|---|
| Chief Investigators: | • Dr Aisha Miah - The Royal Marsden Hospital; The Institute of Cancer Research |
| Co-investigators: | • Mrs Rita Simoes - The Royal Marsden Hospital; The Institute of Cancer Research; National Radiotherapy Trials Quality Assurance (RTTQA) group; University College London Hospitals<br>• Dr Matthew Blackledge – The Institute of Cancer Research<br>• Dr Sarah Gulliford – University College London Hospitals; The Institute of Cancer Research<br>• Dr Shane Zaidi- The Royal Marsden Hospital<br>• Professor Kevin Harrington - The Institute of Cancer Research; The Royal Marsden Hospital<br>• Dr Christina Messiou - The Institute of Cancer Research; The Royal Marsden Hospital<br>• Mr Andrew Hayes - The Institute of Cancer Research; The Royal Marsden Hospital<br>• Mr Dirk Strauss - The Royal Marsden Hospital<br>• Mr Myles Smith - The Institute of Cancer Research; The Royal Marsden Hospital<br>• Dr Khin Thway - The Royal Marsden Hospital<br>• Dr Beatrice Seddon - University College London Hospitals<br>• Dr Hakim-Moulay Dehbi – University College London<br>• Mrs Elizabeth Miles - National Radiotherapy Trials Quality Assurance (RTTQA) group<br>• Dr Henry Mandeville- The Royal Marsden Hospital; The Institute of Cancer Research<br>• Professor Janet Shipley- The Institute of Cancer Research<br>• Dr Robin Jones - The Institute of Cancer Research; The Royal Marsden Hospital<br>• Dr Amy Trinh- The Royal Marsden Hospital<br>• Mr Jonathan Hannay, The Royal Marsden Hospital |

| | |
|---|---|
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| Statistician: | • Mr Kabir Mohammed – The Royal Marsden Hospital<br>• Dr Hakim-Moulay Dehbi – University College London |
| Study coordinator | • Mrs Stephanie Elston, RMH Sarcoma Study Trial Coordinator |

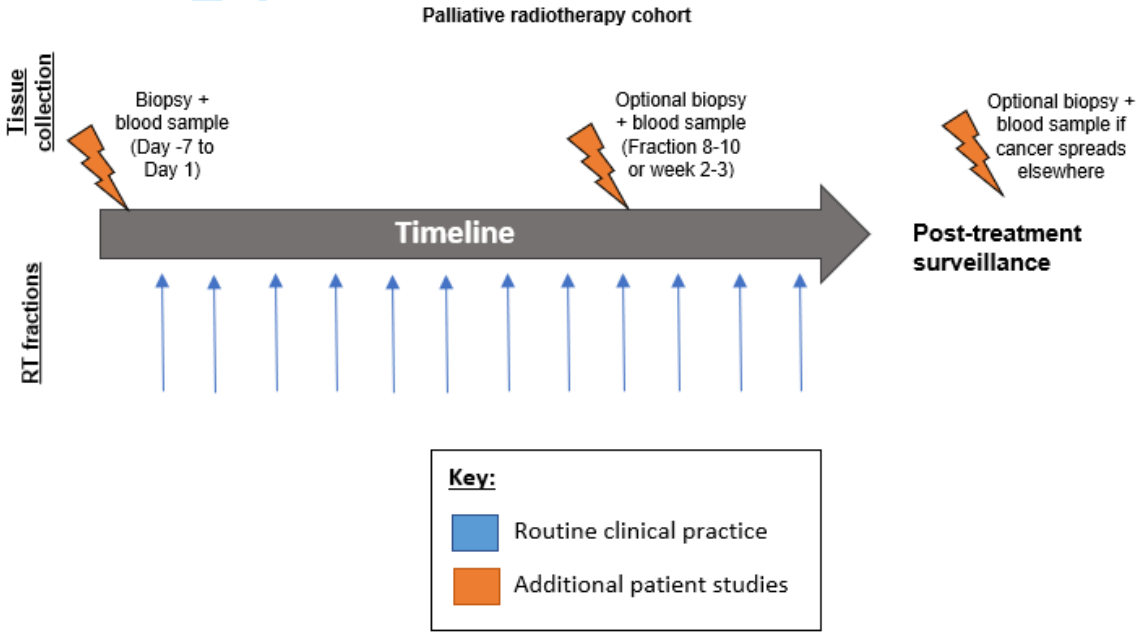
**Sponsor:** The Royal Marsden NHS Foundation Trust

**Address:** The Royal Marsden NHS Foundation Trust, Fulham Road, London SW3 6JJ

**Management:** Study team

| | |
|---|---|
| **Protocol Reference:** | CCR5166<br>IRAS ID:269876 |
| **Version Number & Date:** | V2.0 DRAFT |
| **Effective Date:** | TBA |
| **Superseded Version Number & Date (If applicable)** | V1.0 310321 |

## Table of Contents



# Summary of changes

Protocol version 2.0 <to add date>

- Study summary, rationale section 2a (page 10): added 'primary' / palliative;
- Section 4, secondary objective point 6 (page 18): added 'primary' / palliative;
- Section 5, dose volume constraints validity testing (page 18): dose prescription updated;
- Section 7, inclusion criteria (page 20): further guidance provided on the inclusion criteria for patients receiving Neoadjuvant chemotherapy;
- Section 8, radiotherapy (23): dose prescription updates in pre-operative and palliative settings;
- Section 9, pre-treatment assessments (page 25): pre-registration and follow-up assessments udated;
- Section 15 (page 35): MRI data exportation to XNAT file repository updated details;
- Appendix 1, objectives (page 43): primary and econdary objectives updated;
- Appendix 1, sub-study design (page 44): recruitement and volunteer sample size updated;
- Appendix 1, volunteer study (page 44): MR scan charateristics updated;
- Appendix 1, patient study (page 45): Details on multi-parametric MRI studies provided;
- Appendix 1, timeline (page 46): timeline updated and sample size corrected;
- Appendix 1, secondary endpoint number 3 (page 47): Bland-Altman analysis updated;
- Appendix 1, exclusion criteria (page 48): patient contraindications updated and patient sample size updated;
- Appendix 1, MR-imaging and tissue collection (pages 49-50);
- Appendix 2, Biopsies, research blood samples, tissue collection and radiotherapy dose fractionation schedules updated (page 55-60);

# Summary

| | |
|---|---|
| Title of the study | Predicting radiotherapy response, toxicities and quality of life related functional outcome in soft tissue sarcoma of the extremities: a prospective observational cohort study (PredicT B) |
| Study description | This is a multicentre prospective cohort study, primarily aimed at reporting the frequency and intensity of radiotherapy side-effects of patients with soft tissues sarcoma of the extremities (STSE).<br><br>Two sub-studies are proposed within this study:<br><br>• *MRI radiation response assessment*<br><br>Aimed at establishing whether changes in median apparent diffusion coefficients (ADC) are predictive of pre-operative STSE response measured using histopathology.<br><br>• *Biomarker development and Immune mediators associated with radiotherapy*<br><br>Aimed at establishing prognostic markers which may refine selection of cases for pre-operative, palliative or no radiotherapy.<br>Also, aimed at determining if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in anti-tumour immunity and if certain subtypes could potentially benefit from the addition of immunotherapy with radiation.<br><br>Patients participation in the sub-studies is optional. |
| Objectives | **Main study**<br><br>***Primary objective***<br><br>The primary objective is to report the frequency and intensity of radiotherapy side-effects in STSE.<br><br>***Secondary objectives*** |

| | |
|---|---|
| | 1. To test the validity of radiotherapy constraints as derived in the analysis of the IMRiS and VorteX, that predict for the incidence of grade 2+ of:<br>a. Lymphoedema<br>b. Fibrosis<br>c. Fracture<br>d. Joint stiffness<br>e. Delayed wound healing following pre-operative RT<br><br>2. To report the incidence of grade 2+ toxicities at 3, 6, 12, 18 and 24 months for:<br>a. Subcutaneous tissue fibrosis<br>b. Lymphoedema<br>c. Bone fractures<br>d. Joint stiffness<br>e. Delayed wound healing following pre-operative RT<br><br>3. To determine the time to developing early and late side-effects.<br><br>4. To determine radiological and histological response rates to radiotherapy and where applicable chemo-radiotherapy for STSE of different histological subtypes.<br><br>5. To determine quality of life-related functional outcomes and explore correlations with dose-volume parameters for patients who have received pre, post-operative or palliative radiotherapy for STSE.<br><br>6. To determine predictive and prognostic factors for local and distant recurrence and overall survival for patients receiving pre-operative and palliative RT.<br><br>**Sub-Studies**<br><br>*MRI radiation response assessment sub-study*<br><br>*Primary objective*<br><br>To establish whether baseline measurements in apparent diffusion coefficient (ADC) measured at baseline, and/or changes in ADC measured midway through fractionation (after fraction 8) |

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| | or following treatment are predictive of soft-tissue sarcoma response measured using histopathology.<br><br>*Secondary objectives*<br><br>1. To develop, optimise and test clinical OE-MRI and MRE protocols for use of STSE imaging using healthy volunteer and MRI test-object studies.<br><br>2. To quantify the single-centre reproducibility of OE-MRI and MRE in STSE tumours.<br><br>3. To demonstrate that sub-regions identified using AI-segmented MRI demonstrate different biological phenotypes through molecular profiling and regional histopathology in soft-tissue sarcoma.<br><br>4. To demonstrate whether heterogeneous sub-volumes identified from AI segmentation models correlate with histological STSE response to radiotherapy.<br><br>5. To identify a correlation between (i) pre-treatment measurements, (ii) mid-RT changes and (iii) post-RT changes of tissue hypoxia (measured using OE-MRI) and tissue stiffness (measured using MRE) with post-radiotherapy changes in tumour cellularity (measured using DW-MRI)<br><br>6. To determine whether MR-imaging parameters measured within this sub-study are predictive of healthy tissue toxicity in STSE.<br><br>7. To develop AI-models for identifying texture features in X-ray CT images that correlate with MRI-derived sub-volumes.<br><br>*<u>Biomarker development sub-study and Immune mediators associated with radiotherapy sub-study</u>*<br><br>*Exploratory objectives*<br>1. Prognostic markers which may refine selection of cases for pre-operative radiotherapy versus palliative radiotherapy and no radiotherapy.<br><br>2. To determine if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in |

| | |
|---|---|
| | anti-tumour immunity, to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation. |
| Rationale | The rationale for this study is to develop a personalised approach to recommending radiation treatment for STSE. The study will follow and support the patient pathway to help define:<br><br>1. The incidence and severity of radiation related side effects following a review of dose-volume parameters generated from the radiotherapy treatment plans. VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes. It is anticipated analysis for the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side effects and describe the relationship between radiation dose delivered to these specified areas and short and long-term toxicities. The results of from the first study (PredicT A) will be tested in PredicT B.<br><br>2. The effect of radiation on specific STSE subtypes using standard and novel functional MR imaging techniques and also the pathological and molecular changes associated with pre-operative radiation. In this exploratory study, we hope to identify potential markers of radiation response and radiation sensitivity alongside biomarkers to evaluate potential prognostic markers to predict for early development of metastatic disease. These data could lead to greater personalisation in the management of sarcoma patients, by:<br>a. Prognostic markers which may refine selection of cases for pre-operative radiotherapy, primary/palliative radiotherapy and no radiotherapy<br>b. Guiding radiation dose escalation/ de-escalation strategies (with possible hypofractionation) for different histological subtypes |

| | |
|---|---|
| | c. Evaluate radiation response and whether addition of systemic therapy could enhance the therapeutic index<br>d. Determine the sequence of changes during radiotherapy, evaluating tumour perfusion and vasculature with functional MRI and histological and genomic changes.<br>e. Determine if radiotherapy stimulates the tumour microenvironment to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation. |
| Number of patients | 150 patients |
| Patients inclusion criteria | Inclusion criteria<br>• Histopathological diagnosis of soft tissue sarcoma of the upper or lower limb or limb girdle<br>• Patients receiving pre-operative (neo-adjuvant), post-operative (adjuvant) or palliative radiotherapy<br>• Patients receiving radiotherapy planned as per local protocols (neo-adjuvant chemotherapy will be allowed)<br>• WHO performance status 0-2<br>• Aged ≥16 years<br>• Patients fit enough to undergo radiotherapy treatment and willing to attend follow up visits, during two years<br>• Female patients of child-bearing potential and male patients with partners of child-bearing potential must agree to use adequate contraception methods, which must be continued for 3 months after completion of treatment<br>• Capable of giving written informed consent |
| Patients exclusion criteria | Exclusion criteria<br>• Previous radiotherapy to the same site<br>• Pregnancy<br>• Patients with concurrent or previous malignancy that could compromise assessment of primary and secondary endpoints of the trial |
| Study population | Patients with STSE undergoing to radiotherapy in the pre-operative, post-operative or palliative settings |
| Study design | Prospective observational cohort study |

| | |
|---|---|
| Study duration | Recruitment will have the duration of 18 months;<br>Patients will be followed-up during 24 months. |

# 1. Background

Soft tissue sarcomas of the extremities (STSE) are rare cancers, accounting for 1% of all malignancies arising in adults, with 3,272 cases in the UK in 2010.[1,2] Localized disease is potentially curable, with 5-year survival rates of 60% in high-grade disease.[3]

Radiotherapy (RT) is often used in the management of STSE, either in the pre-operative, post-operative or definitive settings.[4] For large, deep-seated high-grade tumours, RT is recommended as an adjunct in the pre-operative or post-operative settings to improve local control rates of >80%.[5] An international consensus has been made to deliver RT in the pre-operative setting. This allows for smaller volumes to be treated to a lower total dose, which translates into similar local control rates as post-operative RT and a lower incidence of long-term complications, but at the expense of increased acute toxicities, specifically wound complications. However, certain tumours may demonstrate an intrinsic partial radio-resistance with marginal growth during RT which may hinder optimal surgical resection. Under these circumstances, post-operative radiotherapy is recommended with the expectation that a patient may experience increased incidence of long-term side-effects.

RT delivery has improved considerably by the use of various techniques, including intensity-modulated radiotherapy (IMRT) and image-guided radiotherapy (IGRT) to minimise acute toxicities but little progress has been made in stratifying radiation treatment according to histological subtypes (as is practised in the delivery of systemic therapy). Routine and more consistent use of radiotherapy has improved local tumour control rates, but there is still scope for improving outcomes, especially with respect to predicting the response to radiation treatment and treatment-related side-effects.

**Radiotherapy related toxicities**

Acute RT side-effects are defined as an adverse effect of radiation, which can occur up to three months after treatment has completed. For STSE, these include fatigue, skin toxicity (inflammation, erythema and desquamation), and increased risk of surgical wound complications. Late radiotherapy side-effects occur beyond three months, and include soft tissue fibrosis of irradiated normal tissues, limb oedema, joint stiffness and bone fractures. Acute and late side-effects are dependent on anatomical location, tumour size, treated volume, radiation dose and fractionation, as well as the radiosensitivity of the normal tissue surrounding the tumour.[5] Adjuvant radiotherapy leads to better tumour control when compared to limb-sparing surgery alone.[6] However, it may also worsen limb function, with an increased risk of clinically significant oedema and of poorer limb motion range resulting in a negative impact on quality-of-life of sarcoma survivors.[1]

High-grade fibrosis of normal tissues surrounding the tumour is an important side-effect, manifesting as soft tissue contracture, pain in the treated area, limb weakness, affected gait or dexterity problems, with resultant difficulty in undertaking normal activities of daily living. It can occur months or years after treatment and may worsen over time. A previous randomised phase III trial comparing pre-operative and post-operative radiotherapy in STSE has provided important information on late toxicity. High-grade toxicities at two years following treatment are described below:[5]

- Fibrosis in 48.2% and 31.5% of patients, who received post-operative and pre-operative radiotherapy, respectively;
- Joint stiffness in 23.2% and 17.8% of the patients receiving post-operative and pre-operative radiotherapy;
- Lymphoedema (swelling of the limbs) in 23.2% and 15.5% of patients receiving post-operative and preoperative radiotherapy, respectively.

The UK multicentre phase III VorteX trial compared standard post-operative radiotherapy for STSE against an experimental arm using reduced target volumes, aiming to reduce treatment-related side-effects while maintaining local tumour control. High-grade toxicities reported were subcutaneous (47% and 41% in the standard and experimental arms respectively), bone (11% and 15%) and joint (18% for both arms) toxicities.[7]

**Normal Tissue dose-volume constraints**

For some normal tissues, specific dose-volume constraints have been associated with the development of clinically significant toxicities. For example, xerostomia (dry mouth) can be avoided if one parotid gland is spared to a mean dose of less than 20Gy or if both parotids receive a mean dose below 25Gy.[8] Clinicians often use cut-offs in the volume of normal tissues receiving specific doses to avoid or minimise intolerable side-effects after radiotherapy.

There is a knowledge gap in predicting side-effects of radiotherapy for STSE, compared to other tumours. As mentioned above, evidence-based dose-volume constraints can be used by clinicians to predict patients at risk of toxicity and strategies to minimize side-effects can then be implemented during treatment planning.

The most relevant resources relating to radiotherapy toxicity and dosimetric constraints are the Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) papers, and the work by Emami and colleagues.[9,10] The latter was drawn from a consensus of expert experience and limited historical toxicity data from local clinics.[10] However, it did not define dose-volume constraints for STSE. The QUANTEC effort, which summarised all available evidence on relationships between radiation dose and normal tissue response, identified relevant dose-volume constraints for specific healthy tissues, but again, did not include information on STSE.[9] However, there is some limited information available. Dickie and colleagues[11] studied a retrospective cohort of 21 and 53 patients with and

without fractures, respectively, who had received radiotherapy for STSE. They defined that treated bones should not receive a mean dose higher than 40Gy and that volume of bone receiving 40Gy should be kept below 64% to reduce radiotherapy-related fractures.[11] The RTOG protocol 063020, a phase II trial investigating the role of pre-operative image-guided radiotherapy for STSE in 98 patients, stated that no more than 50% of a longitudinal strip of skin and subcutaneous tissue, arbitrarily defined by the clinician, should receive more than 20Gy.[12] However, this was a protocol recommendation and was not evidence-based.

The standard radiotherapy technique for STSE uses multi-field 3D-conformal radiotherapy (3DCRT). 3DCRT techniques rely on conformal treatment plans, which use several radiation beams that are shaped to conform to the target volume.[13,14] Intensity-modulated Radiotherapy (IMRT) has built upon this paradigm to deliver increasingly conformal radiotherapy by modulating the radiotherapy dose across the treatment volume, allowing better conformality than 3DCRT. Therefore, IMRT has the potential to allow higher doses to be delivered to the target volume while sparing normal tissues. Improvements in conformality achieved by IMRT may lead to a reduction in side-effects, but there is also a higher volume of normal tissues treated to lower radiation doses. For STSE, the result is that the entire circumference of the limb may receive significant radiation doses, and currently there is not a good understanding of consequences in terms of long-term toxicities. It is therefore important to determine dose-volume constraints to inform radiotherapy planning.

**Predict A**

The VorteX trial investigated whether a reduced volume of post-operative radiotherapy improved limb function without compromising local control.[7] Two hundred and sixteen patients were recruited between 2008 and 2013. The majority were treated with modern 3DCRT; the standard radiotherapy technique for STSE and still widely employed clinically. Approximately 11% of patients in VorteX were treated with IMRT. High-quality radiotherapy planning data and long-term toxicity information were collected. Primary endpoints were limb function (measured with the patient-reported Toronto Extremity Salvage Score (TESS)) and time to local recurrence. Secondary endpoints included the evaluation of soft tissue and bone toxicity (RTOG clinician-reported scoring system), overall level of disability (measured with two general questions in TESS), as well as disease-free and overall survival.

The UK IMRiS phase II clinical trial tested IMRT in three sarcoma cohorts, namely one STSE and two bone sarcoma cohorts. The STSE cohort recruited 168 patients in total, with 56 and 112 receiving post-operative and pre-operative radiotherapy respectively. Recruitment was completed in July 2017. The primary endpoint is the rate of high-grade fibrosis at 2 years. Secondary endpoints are the incidence of other high-grade toxicities, patient-reported limb function and quality-of-life (measured with the TESS score), time to local recurrence, disease-free and overall survival.

VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Although 3DCRT is the standard technique for treating STSE, IMRT is expected to become standard of care in the future. Moreover, VorteX only included post-operative radiotherapy, whereas most patients treated in IMRiS received pre-operative treatment. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes.

It is anticipated that the analysis of the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side-effects and the relationship between radiation dose delivered to these specified areas and short and long-term toxicities.

The results of PredicT A will derive potential dose-volume constraints which could be used in radiotherapy planning in order to reduce radiotherapy-related toxicities. The validity of dose-volume constraints generated in PredicT A will be tested in the observational cohort of patients recruited prospectively in this study (PredicT B).

*This study will be conducted in compliance with the protocol, standard operating procedures, policies, local R&D management guidance, Good Clinical Practice including the Research Governance Framework 2005 (2nd edition) and other applicable regulatory requirement(s) including but not limited to the Human Tissue Act 2004, Human Tissue (Quality and Safety for Human Application) Regulations 2007, the Medical Devices Regulations 2002, Ionising Radiation (Medical Exposures) Regulations 2000 as amended from time to time.*

## 2. Rationale

The rationale for this study is to develop a personalised approach to recommending radiation treatment for STSE. The study will follow and support the patient pathway to help define:

1. The incidence and severity of radiation related side-effects following review on dose-volume parameters generated from a radiotherapy treatment plan. VorteX and IMRiS combined datasets provide a unique complementary resource. VorteX includes mainly patients treated with 3DCRT (85%), whereas IMRiS only includes patients treated with IMRT. Combining the two datasets will provide a range of treated volumes and a range of radiation doses to different volumes which can then be correlated to clinician and patient reported outcomes. It is anticipated analysis for the 2 datasets described above will provide clear definitions of anatomical regions of interest which relate to the development of treatment related side effects and describe the relationship between radiation dose

delivered to these specified areas and short and long-term toxicities. The results of the first study (PredicT A) will be tested in PredicT B.

## 3. Hypothesis

- Patients whose radiotherapy treatment plans indicate that the dose to OAR of the extremities fail the dose constraints derived in PredicT A will have higher incidence of toxicity than patients whose treatment plan met the constraints.

## 4. Study objectives

***Primary objective***

The primary objective is to report the frequency and intensity of radiotherapy side-effects in STSE.

***Secondary objectives***

1. To test the validity of radiotherapy constraints as derived in the analysis of the IMRiS and VorteX, that predict for the incidence of grade 2+ of:
    a. Lymphoedema
    b. Fibrosis
    c. Fracture
    d. Joint stiffness
    e. Delayed wound healing following pre-operative RT

2. To report the incidence of grade 2+ toxicities at 3, 6, 12, 18 and 24 months for:
    a. Subcutaneous tissue fibrosis
    b. Lymphoedema
    c. Bone fractures
    d. Joint stiffness
    e. Delayed wound healing following pre-operative RT

3. To determine the time to developing early and late side-effects.

4. To determine radiological and histological response rates to radiotherapy and where applicable chemo-radiotherapy for STSE of different histological subtypes.

5. To determine quality of life-related functional outcomes and explore correlations with dose-volume parameters for patients who have received pre, post-operative or palliative radiotherapy for STSE.

6. To determine predictive and prognostic factors for local and distant recurrence and overall survival for patients receiving pre-operative and primary/palliative RT.

## 5. Study design

This is a multicentre prospective cohort study, primarily aimed at validating the dose-volume parameters identified in the analyses of the VorteX and IMRiS trials datasets.

- **Delineation of healthy tissues**

  Pre-defined outlining guidelines of normal tissues as bones, muscle compartments, joints, lymph drainage basins and subcutaneous tissue from Predict A will be delineated in radiotherapy planning computed tomography (CT) images. All cases will be delineated by a single observer (Rita Simoes). Verification of all outlines will be carried out by Dr Aisha Miah (clinical supervisor).

- **Dose-volume constraints validity testing**

  Patients will be treated as per local protocol treatment technique.
  Radiotherapy, clinical and toxicities data will be collected, with no new intervention on the treatment. Patients enrolled will receive standard radiation prescription doses as described below:
  - Pre-operative radiotherapy- 50 Gy in 25 fractions equivalent (pre-operative radiotherapy). Where appropriate hypo-fractionated schedules as per institutional guidelines can considered: eg. 25 Gy/ 5 daily fractions. In myxoid liposarcomas, 36 Gy in 18 fractions can be considered where suitable;
  - Post-operative radiotherapy- 60 Gy in 30 fractions or 66 Gy in 33 fractions (positive resection margins); alternative hypo-fractionated schedules as per institutional guidelines can be considered;
  - Palliative radiotherapy- 30-36 Gy in 10-12 daily fractions, 40-45 Gy in 15 fractions, 30-36 Gy in 5-6 once weekly fractions or 25 Gy in 5 daily fractions.

  Toxicity will be assessed with the TESS and RTOG scoring instruments and Stern score for lymphoedema. Patients enrolled in the study will fill in a specific quality-of-life questionnaire to assess quality of life related functional outcomes following treatment for STSE. This questionnaire is based on validated questions for assessing quality-of-life. Patients will be followed up at 3, 6, 12, 18, and 24 months post-radiotherapy.

  The recruitment of the 150 patients is expected to occur during a period of 18 months (as detailed in the sample size section below).

The dose-volume constraints generated in PredicT A will be tested in the validation cohort using odds ratios (OR) as previously reported by Gulliford et al.[15] Patients will be divided into those meeting or not meeting constraints and comparisons of toxicity levels between both groups will be carried out. In parallel, a similar MVA will be performed in this cohort to assess if dose-volume and toxicity relationships are similar in the validation cohort when compared to the IMRiS and VorteX dataset.

## 6. Endpoints

***Primary endpoint***

The incidence of any RTOG grade ≥2 toxicities following treatment for STSE at 24 months.

***Secondary endpoints***

1. The ability of the fitted model from dose-volume constraints from PredicT A (IMRiS and Vortex analysis) to correctly predict the incidence of grade 2+ among PredicT B patients.

2. The incidence of radiotherapy-induced late toxicities at 3, 6, 12, 18 and 24 months for:
   a. Subcutaneous tissue fibrosis
   b. Lymphoedema
   c. Bone fractures
   d. Joint stiffness
   e. Delayed wound healing (following pre-operative RT)

3. Time elapsed between day 1 of radiotherapy and the day to develop early and late side-effects. Early side effects recorded up to 3 months and late side effects recorded from 3 months to 24 months.

4. Exploration of factors which may be related to tumour changes (as defined by comparison of tumour volume between planning CT and CBCTs captured during RT) for pre-operative patients as follows:

   4.1. Tumour volume shrinkage after patients have received a total dose of 16 Gy (fraction number 8) and 32 Gy (fraction number 16).
   4.2. Tumour volume enlargement after patients have received a total dose of 16 Gy (fraction number 8) and 32 Gy (fraction number 16).
   4.3. Tumour volume shrinkage after patients have received a total dose of 50 Gy (fraction number 25).

4.4. Tumour volume enlargement after patients have received a total dose of 50 Gy (fraction number 25).

5. To identify if specific dose-volume parameters correlate with poor quality life reported outcomes for patients who have received pre, post-operative or palliative radiotherapy for STSE.

6. Patient-related and tumour related factors associated with response and distant recurrence and overall survival as determined by univariate and multivariate models as appropriate.
   The following will be reported:
   Local control rates at 2 years
   Metastatic disease-free survival rates at 2 years
   Overall survival at 2 years

## 7. Study population

The study population to be enrolled in this observational study will have had been diagnosed with STSE and referred to receive a course of radiotherapy either in the primary, palliative, pre-operative or post-operative settings at the two study centres; The Royal Marsden Hospital and University College London Hospitals.

**Inclusion criteria**

- Histopathological diagnosis of soft tissue sarcoma of the upper or lower limb or limb girdle;
- Patients receiving pre-operative (neo-adjuvant), post-operative (adjuvant) or palliative radiotherapy;
- Patients receiving radiotherapy planned as per local protocols (neoadjuvant chemotherapy will be allowed). Neoadjuvant chemotherapy patients may be approached as they commence chemotherapy;
- WHO performance status 0-2;
- Aged ≥16 years;
- Patients fit enough to undergo radiotherapy treatment and willing to attend follow up visits, during two years;
- Female patients of child-bearing potential and male patients with partners of child-bearing potential must agree to use adequate contraception methods, which must be continued for 3 months after completion of treatment;
- Capable of giving written informed consent.

**Exclusion criteria**

- Previous radiotherapy to the same site;
- Pregnancy;

- Patients with concurrent or previous malignancy that could compromise assessment of primary and secondary endpoints of the trial.

**Subject withdrawal criteria**

- Patients expressing a wish to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with procedures for reporting study withdrawal. Withdrawal data instigated by the investigator or the patient will be collected and reasons for withdrawal will be recorded during this study;
- The investigators will try to replace patients who are withdrawn whilst study recruitment is open, in order to allow for a two year follow-up. After recruitment completion, patients that are withdrawn will not be replaced by other individuals in the trial;
- As this is an observational study, patients will not experience a change in their regular standard of medical care if there is a study withdrawal.

# 8. Methodology

An observational cohort of 150 patients with STSE will be recruited over 18 months. This main study is a non-interventional study, therefore there will be no change to patient's standard treatment. Patients will be treated either with radiotherapy, in the pre-operative, post-operative or palliative settings as per local protocols.

**Patient screening**

Patients meeting the inclusion criteria will be identified during routine multidisciplinary team (MDT) meetings for STSE. No public methods for recruitment (e.g. through leaflets or websites) will be adopted in this study. Any patient who meets any of the exclusion criteria will not be invited to participate in this study. Once identified, the named clinical team members of the study or research nurses will discuss options for the patient to join this imaging study. Consent will be taken by named clinical team members including appropriately trained research nurses and radiographers nominated by the CI and local PIs.

**Informed Consent**

Co-Investigators, or, where delegated by the co-investigator, other appropriately trained site staff, are required to provide a full explanation of the study prior to trial entry. During these discussions, the current approved trial patient information should be discussed with the patient. A **minimum of twenty-four (24)** hours should be allowed for the patient to consider and discuss participation in the study. However, in order to prevent unnecessary return visits patients may consent on the same day as being given the information sheet,

provided the member of staff taking consent is satisfied that the patient understands the study.

All suitable patients will be informed on the rationale behind the study, the additional imaging required, and any additional hazards to which they may be exposed. They will be assured that all data retained for the purposes of the study will be kept strictly confidential, and only used by the co-investigators for the purposes of this study. It will be conveyed to patients that this study will involve the use and storage of (i) toxicity, clinical, radiotherapy and follow-up imaging acquired data (ii) data acquired from imaging sub-study to be run at RMH only, and (iii) tissue obtained following resection of their tumours following radiotherapy, as part of the recruitment in the sub-study (RMH patients only). In addition, they will be made aware that this study will have no impact on their subsequent healthcare, and a conventional clinical pathway will be appropriated. Patients will be supplied with a patient information sheet (PIS) for the study, and patients agreeing to participate will be asked to sign and date an informed consent form prior to recruitment (in accordance with GCP guidelines).

Patients will be informed that participation in the sub-studies is optional and that will be given the option to participate in PredicT B if the participation in the sub-studies is declined.

Patients will be informed that data acquired from the sub-study may be used in future retrospective studies, exploring potential imaging biomarkers and correlates with histopathological analyses. In addition, patients will be made aware that they may request transportation funds for the additional imaging time-points explored in this study.

A further discussion of the study will be held when patients return to the hospital to confirm treatment and at least 24 hours to review PIS to consent to study and/or sub-study. Patients will not be expected to make extra visits to the hospital for participation in the main study as all data collection points will be incorporated into their scheduled clinical follow-up appointments.

Centres recruiting patients are responsible for assessing a patient's capacity to give informed consent.

Sites must ensure that all patients have been given the current approved version of the patient information sheet, are fully informed about the trial and have confirmed their willingness to take part in the trial by signing the current approved consent form.

Sites must assess a patient's ability to understand verbal and written information in English and whether or not an interpreter would be required to ensure fully informed consent. If a patient requires an interpreter and none is available, the patient should not be considered for the study.

**Radiotherapy**

Radiotherapy should aim to start within 4 weeks of registration, and no longer than 12 weeks after surgery. For adjuvant RT patients, if wound healing delays start of radiotherapy, this will be permissible and must be discussed co-chief investigators. Any radiotherapy planning technique will be permitted as per local protocols. The radiation dose will be given as follows:

- Pre-operative RT: 50 Gy in 25 daily fractions biologically equivalent dose, delivered Monday to Friday over 5 weeks, 25 Gy/5 daily fractions; 36 Gy in 18 fractions for myxoid liposarcomas. The suitable fractionation is as per clinician guidance and institutional guidelines;
- Post-operative RT: 60 Gy in 30 daily fractions to the high dose planning target volume (PTV) (PTV_6000) and 52.2 Gy in 30 daily fractions to the low dose PTV (PTV_5220) treated concurrently, or 2 phase technique 50 Gy in 25 daily fractions then 10-16 Gy in 5-8 fractions delivered Monday to Friday over 6 weeks;
- Post-operative RT with positive resection margins: 66 Gy in 33 daily fractions to the high dose PTV (PTV_6600), and 53.46Gy in 33 fractions to the low dose PTV (PTV_5346) treated concurrently, delivered Monday to Friday over 6 1⁄2 weeks
- Palliative radiotherapy schedules: 30-36 Gy in 10-12 daily fractions, 40 Gy in 15 daily fractions, 30-36 Gy in 5-6 fractions delivered once a week, 25 Gy in 5 daily fractions. Applying $\alpha/\beta$= 3 Gy.

Radiotherapy planning CT scan and immobilisation should follow recruiting centres local protocols.
Toxicity will be assessed with the TESS, Stern and RTOG scoring instruments. Quality of life will be reported following specific EORTC quality of life validated questionnaires and measures. Patients will be followed up at 3, 6, 12, 18, and 24 months post- radiotherapy.

Radiotherapy CT scan, structures, dose plan, re-plan, cone-beam CT and diagnostic imaging (where applicable) be will be anonymised and uploaded in the National Radiotherapy Quality Assurance (RTTQA) upload platform or in the RTTQA secure file transfer. From the RTTQA system, the RT data will be exported to an excel spreadsheet. This spreadsheet will then be merged with the toxicity MACRO database including the following per patient entries: patient identification, dose-volume histogram parameters, treatment technique, side-effect measures (clinician-reported RTOG and patient-reported TESS toxicity scores), patient characteristics and relevant co-morbidities (e.g. diabetes, hypertension, smoking habits).

For further information please refer to the radiotherapy uploading guidelines.

A data transfer agreement will be completed following the Trust policies.

Patients treated within PredicT B with a pre-operative intention will be asked to take part in the sub-study.

**Patient withdrawal**

Patients wishing to withdraw from the study will be asked if data up to that point can be used and they will be withdrawn in line with centres procedures for reporting study withdrawal. Withdrawal data instigated by the investigator or the patient itself will be collected and reasons for withdrawal will be recorded during this study.

**Study completion**

This main study is part of a doctoral project which is to be completed in March 2024. The sub-study will be including patients for pre-operative radiotherapy only with interventions occurring during treatment and follow-up complete 2 years after treatment.

**End of study definition**

The definition of the end of this study is the date of completion of any follow-up monitoring and data collection described earlier in the protocol. Any change to this definition will be notified as a substantial amendment. Final analysis of the data (following 'lock' of the study database) and report writing will occur after formal declaration of the end of the study.

## 9. Assessments and data acquisition

**Pre-registration evaluation**

Patients must give written informed consent **before** any trial specific screening investigations may be carried out. The following assessments or procedures are required to evaluate the suitability of patients prior to entry into the trial:

- Histological confirmation of disease;
- Diagnostic MRI and/or CT (if there is a contraindication to MRI) of the primary tumour site;
- Patients receiving post-operative radiotherapy the MRI/ CT should ideally have been performed within 1 month prior to surgery;
- Patients receiving pre-operative radiotherapy, the MRI/CT should ideally be performed within 1 month of starting radiotherapy, although decisions on repeating scans older than 1 month will be made at the treating clinician's discretion;
- Chest imaging (CT or chest x-ray) within 3 months of registration, as per routine practice.

<u>Within 14 days prior to registration:</u>

- Clinical review of relevant medical history;
- Completion of the assessment of adverse events (AEs) using CTCAE v5.0;

- Assessment of WHO performance status;
- Pregnancy test (urine or blood) in females of child bearing potential;
- Measurement of height & weight, assessment of smoking status, diabetic status and limb function or mobility.

**Pre-treatment assessments**

Within 28 days prior to starting treatment:

- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- EORTC QLQ-C30 quality of life questionnaire*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Toronto Extremity Salvage Score (TESS) questionnaire*;
- Musculoskeletal Tumour Society Rating Scale;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).

*Patients having palliative RT may opt out the QoL assessments if they wish to.

The following pre-registration assessments do not need to be repeated if done within 28 days prior to starting treatment:

- Clinical review;
- Pre-treatment routine blood tests: FBC, U&Es, LFTs, CRP, LDH and clotting (for pre-operative or palliative radiotherapy patients or as per institutional guidelines);
- Assessment of AEs using CTCAE v5.0;
- Assessment of WHO performance status;

**Post-surgery assessment of wound complications up to 120 days after surgery**

Patients should be assessed for wound complications during assessment visits occurring from surgery and up to 120 days after surgery. Post-Surgery Wound Assessment wound complications are defined as

- 2nd operation under general or regional anaesthesia for wound repair (debridement, operative drainage, unplanned secondary wound closure using free muscle flaps or skin grafts);

- Wound management without 2nd operation (invasive procedure without general or regional anaesthesia, e.g. aspiration of seroma, readmission for wound care such as intravenous antibiotics, persistent deep wound packing for ≥120 days).

**Assessments during radiotherapy**

During treatment patients should be seen weekly or as per local protocol (in an appropriate on-treatment review clinic, which may be run by a doctor, radiotherapy nurse or therapy radiographer). The following assessments will be completed:

Weekly (or equivalent as per local practice)

- Clinical review mid and at end of RT;
- Cone Beam CT imaging review of treatment volume where applicable;
- Additional imaging review where applicable.

**Assessments on completion of radiotherapy**

The following should be carried out at least **28 days (and up to 35 days)** after the last fraction of radiotherapy:

- Clinical review;
- Assessment of ARs using CTCAE v5.0;
- Assessment of acute radiation morbidity using the RTOG Acute Radiation Morbidity Scoring Criteria;
- WHO performance status;
- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- Toronto Extremity Salvage Score (TESS) questionnaire (if applicable)*;
- Radiological response assessment (approximately 4-6 weeks after pre-op RT);
- Pathology response assessment (after resection, approximately 8-10 weeks after pre-op RT);
- EORTC QLQ-C30 quality of life questionnaire*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).

- *Patients having palliative RT may opt out the QoL assessments if they wish to.

In addition to the assessments listed above, we will collect each patient:

- Diagnostic scans which may have been acquired before treatment commences;
- Radiotherapy planning scans;
- Structures, Plans and dose cubes;
- Treatment imaging review, including CBCT which may have been acquired as per imaging verification protocols.

**Follow-up assessments after completion of radiotherapy**

Patients will be followed approximately 4 weeks after pre-op RT or 6 weeks after post-op RT, then at 3 months after completion of radiotherapy, then 3-monthly for up to 2 years after date of registration. All visits should be carried out at the specified time +/- 2 weeks.

N.B. For pre-operative RT patients, following their last fraction of RT, it may be necessary to omit a follow up visit immediately after surgery, as it may be difficult for the patient to attend clinic.

Patients should have the following assessments at each visit unless stated otherwise:

- Clinical review;
- WHO performance status;
- Assessment of radiation morbidity:
  - RTOG Acute Radiation Morbidity Scoring Criteria up to day 90 after start of treatment;
  - RTOG/EORTC Late Radiation Morbidity Scoring Criteria skin, subcutaneous tissue fibrosis, joint stiffness, bone) from day 91 after start of treatment;
  - Stern's scale for oedema from day 91 after start of treatment
- Clinical assessment of local tumour control at primary site at each 3-monthly visit;
- Assessment of wound related clinical findings up to 120 days after surgery (if applicable);
- Chest x-ray/ CT imaging (as per local institutional guidelines at each 3-monthly follow up visit;
- TESS questionnaire at 1 year and 2 years after registration*;
- Musculoskeletal Tumour Society Rating Scale at 1 year and 2 years after registration;
- EORTC QLQ-C30 quality of life questionnaire at 1 year and 2 years after registration*;
- EORTC QLQ-FA12 fatigue questionnaire*;
- Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location).
- Site of relapse if applicable and subsequent treatment details.
-
- *Patients having palliative RT may opt out the QoL assessments if they wish to.

**Assessments after disease recurrence**

If a patient progresses within 2 years from the date of registration, they should continue to be followed up if possible, fitting in with their routine oncological care. Investigators

should use their judgement on a case-by-case basis to perform follow up on patients according to their circumstances and what is clinically reasonable.

Where possible the following assessments should be performed:

- Clinical review;
- WHO performance status;
- Assessment of radiation morbidity:
  - RTOG Acute Radiation Morbidity Scoring Criteria up to day 90 after start of treatment;
  - RTOG/EORTC Late Radiation Morbidity Scoring Criteria (skin, subcutaneous tissue fibrosis, joint stiffness, bone) from day 91 after start of treatment;
  - Stern's scale for oedema from day 91 after start of treatment (Appendix);
- Clinical assessment of local tumour control at primary site;
- TESS questionnaire at 1 year and 2 years after registration*;
- Musculoskeletal Tumour Society Rating Scale at 1 year and 2 years after registration (Appendix);
- EORTC QLQ-C30 quality of life questionnaire at 1 year and 2 years after registration After the 2 year follow up visit patients should continue to be followed up on a regular basis as per standard oncological care*;
- EORTC QLQ-FA12 fatigue questionnaire*;
  Measurement of circumference of the limb (The measurement of the limb and contralateral limb diameters should be taken at approximately 10 cm below groin, 5cm below knee or 5 cm below armpit depending on the tumour location). *Patients having palliative RT may opt out the QoL assessments if they wish to.

## 10. Data Analysis

*Delineation of normal tissues*

All patients will have GTV, CTV, PTV and OAR outlined in the planning CT scan. Further bones, muscle compartments, joints, lymph drainage basins and subcutaneous tissue will be delineated in radiotherapy planning computed tomography (CT) by a single observer (Rita Simoes). Verification of all outlines will be carried out by Dr Aisha Miah (clinical supervisor) and reviewed with a specialist radiology team at ICR/RMH. A planned exercise to repeat delineation of the normal tissue structures will be performed after 2 months to account for intra-observer and inter-observer variability.

*Radiotherapy database*

A specific database including dose-volume parameters for each pre-defined normal tissue structure d will be merged with a toxicity database including the following per patient entries: patient identification, dose-volume histogram parameters, treatment technique, side-effect measures (clinician-reported RTOG and patient-reported TESS toxicity scores), patient characteristics and relevant co-morbidities (e.g. diabetes, hypertension, smoking habits).

The dose-volume constraints generated in PredicT A will be tested in the validation cohort using odds ratios (OR) as previously reported by Gulliford et al.[15] Patients will be divided into those meeting or not meeting constraints and comparisons of toxicity levels between both groups will be carried out. In parallel, multivariate analysis will be performed in this cohort to assess if the development of radiation-induced side-effects is related with clinical co-morbidities.

Patients will be followed up for two years and the planned timing for analysis will last 6 months from the completion of the last patient follow up appointment.

## 11. Study organisation/ trial monitoring and management strategy

The following study-related responsibilities have been defined:

**Responsibilities**

- The Royal Marsden NHS Foundation Trust is the Sponsor of this study and has responsibility for ensuring appropriate ethics committee opinion and authorisations are obtained.

- The Chief Investigator, Dr Miah, will have overall responsibility for the study and will conduct it in accordance with the UK Policy Framework for Health and Social Care Research and the principles of Good Clinical Practice (GCP).

- Monitoring of study progress will be the responsibility of the CI of this study (Dr Miah). Monitoring of the sub-study progress will also be the responsibility of Dr Matt Blackledge. This role will be supported by co-investigators, trial statistician and additional members of the trial management group. In addition, dedicated PPI representatives will be invited to all quarterly ITC meetings to ensure that all research falls within the remit of patient benefit.

- All participants within the sub-study will complete an MRI safety questionnaire with the research radiographers prior to being scanned. The imaging protocols will be maintained by the research radiographers and Dr Blackledge. The routine sequences of the MRI scans will be reviewed and reported by radiologists and made available to the clinical units in the standard way.

- Statistician, Mr Mohammed, will input into protocol, CRF and database design and will work with the investigators to ensure the quality and completeness of the data.

- Data Analysis will be performed by: Mrs Rita Simoes, Dr Miah, Dr Blackledge, Ms Thrussell, Dr Zaidi and Mr Hayes.

- The trial co-ordinator will be responsible for study administration including progress reporting, amendments and notification of study closure in accordance with ethical and R&D approval procedures.

Following review and notification in writing to the Chief Investigator of approval by the Committee for Clinical Research /Research & Development (CCR/R&D) and by the Health Research Authority (HRA), the study will be activated on the Hospital Information System and the Principal Investigator and trial coordinator notified that the study is open for recruitment. This will be classified as the start date for the R&D database. The study will be deemed to have reached completion at 24 months after the last patient has been recruited. The final questionnaires are due at 24 months and an extra three months has been added to allow for scheduling delays and completion of the investigations.

There will be a quarterly progress meeting for all stakeholders in the study to discuss all aspects of the study's progress and any issues encountered. There will be monthly progress meetings run by the Chief Investigator to review the day to day running and progress of the study. All study personnel will have attended a Good Clinical Practice (GCP) course within the previous three years. The Data Manager will maintain a Study Site File in which all critical study documentation including study protocol, correspondence with Local Research and Ethics Committee (LREC) and the RMH CCR, study amendments, copies of Adverse Event Forms, CVs and GCP certificates for all study personnel will be held.

## 12. Safety reporting

The following definitions have been adapted from Directive 2001/20/EC, ICH E2A "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" and ICH GCP E6:

**Adverse Event (AE)**

Any untoward medical occurrence in a patient treated on a trial protocol, which does not necessarily have a causal relationship with radiotherapy. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of radiotherapy, whether or not related.

**Adverse Reaction (AR)**

All untoward and unintended responses to radiotherapy treatment related to any dose administered. A causal relationship between radiotherapy and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

**Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)**

A SAE or SAR that at any dose:

- Results in death
- Is life threatening (the term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalisation or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is otherwise medically significant (e.g. important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above)

**Related and Unexpected Serious Adverse Reaction**

An adverse reaction meeting the following criteria:

- Serious – meets one or more of the serious criteria above
- Related – assessed by the local investigator or sponsor as causally related to one or more elements of the trial treatment
- Unexpected – the event is not consistent with the applicable reference safety information (RSI)

It is anticipated that Serious Adverse Events will not occur since this is a non-Clinical Trial (non-CTIMP) study. However, all adverse events defined as those resulting in death, as being life-threatening, requiring hospitalisation or prolongation of hospitalisation, resulting in persistent or significant disability or incapacity to a study participant will be reported in line with Trust's Generic SOP for Adverse Events Reporting for Non-CTIMP Trials sponsored and hosted by RMH/ICR (gSOP-03-03)(gSOP-03-03).

## 13. Statistical analysis

**Sample size**

The primary endpoint is descriptive of toxicity rates as observed in patients recruited in the study. Published studies[6,7] reported toxicity rates of around 40%, the rate in our patient sample n=126 is expected to be within two sided 95% confidence interval +/- 8.6%. The study has also therefore been powered for the secondary endpoint number 1 (The ability of the fitted model from IMRiS and Vortex analysis to correctly predict the incidence of grade 2+ among PredicT B patients in whom dose-volume constraints were exceeded compared to the patients in whom dose/volume constraints were met).

It has been assumed that number of patients developing RTOG grade≥ 2 subcutaneous tissue fibrosis would be greater for patients whose radiotherapy treatment plan did not meet the radiotherapy dose-volume constraints compared to those patients whose radiotherapy treatment plan met the dose-volume constraints. We therefore aim to find differences in toxicity levels between two groups of patients: (1) patients not exceeding the previously defined dose-volume constraint and (2) patients exceeding the previously defined dose-volume constraints.

Dose-volume constraints of interest will be defined in the Predict A study, using previously available data of two clinical trials of patients undergoing radiotherapy for STSE (VorteX and IMRiS).

To detect a difference in toxicity rate of 30% between patients treated with a dose below and above a specific constraint (assuming 70% probability of grade 2+ toxicity in patients above constraint and 40% for patients below), using the 126 patients sample size with assumed ratio of 2:1 between patients below (84 patients) and above the constraint (42 patients). This secondary endpoint will have 90% power to detect difference in the toxicity rate between the two groups at 2 sided 5% significance level.
The difference in toxicity rate between groups (30%) has been assumed by using a preliminary review of available VorteX trial toxicity outcome reports. Assuming 19% drop-out rate during the 2 years follow-up, recruitment will continue until 150 (additional 24) patients are recruited.

***Primary endpoint analysis***

Incidence of any grade ≥2 toxicities at 24 months will be calculated using descriptive methods in the form of number and proportion of patients with grade ≥ 2.

***Secondary endpoints***

Endpoint no 1 - To report the differences in experienced by patients with STSE for whom dose-volume constraints were below or exceeded. Binary logistic regression will be used to quantify the differences using odds ratios (OR) from the fitted models. Univariate logistic regression models will be fitted to predict for the parameters relevant to toxicity grades in the RTOG, Stern's and TESS scales. ORs for each of the parameters will be used to judge for significant differences between the groups.

Endpoint no 2 – Frequencies and proportions of radiotherapy-induced late toxicities at the specified at 3, 6, 12, 18 and 24 months will be reported descriptively at each time point for the overall events and by types of events:

a. Subcutaneous tissue fibrosis
b. Lymphoedema
c. Bone fractures
d. Joint stiffness
e. Delayed wound healing (following pre-operative RT)

Endpoint no 3 – Time elapsed between day 1 of radiotherapy and the day to develop early and late side-effects using Kaplan-Meier methods. Median time to early side-effects with 95% confidence interval will be reported. Patients without any side-effects (on day 90 from end of RT) will be censored. Similarly, median time to late side-effects with 95% confidence interval will be reported. Patients without an event will be censored at the 24 months assessment visit or at last follow-up date known to be on the study without any event.

Endpoint no 4 – Differences in the tumour volumes (in cc and %) will be calculated from CT and CBCT images at fractions 8, 16 and 25 for patients receiving pre-operative RT. Changes in target volumes during radiotherapy will be assessed by computing dissimilarity indices (e.g., Simpson's dissimilarity index) in sequential CBCT images captured during treatment compared to radiotherapy planning CT. Tumour factors are tumour histotype, staging and tumour size.

Endpoint no 5 – Correlations between functional outcomes (expressed in TESS and EORT-QLQ- C30 and QLQ-FA12 fatigue questionnaire scores) with dose-volume parameters (expressed in Gy/volume, where volume will be defined in % of total volume and cc) will be calculated using Spearman's correlation method. Patients will be categorised according to dose volume constraints and compare the groups using t-test or Mann-Whitney non-parametric test as appropriate.

Endpoint no 6 – Percentage of patients responding to treatment will be calculated, this will be reported in the overall patients and for dose-volume constraints groups. Differences in the response rates will be assessed according to: histopathological semi-quantitative scores (pathological: % viable cells). Radiological: % volume change (RECIST), % cystic sold component). Tumour factors are: tumour subtype, staging and size. Local control rates, disease-free survival rates and overall survival at 2 years will be calculated using Kaplan-Meier methods. Disease free survival events are local or distance disease recurrence and death from disease related causes. Overall survival events are death from any causes.

# 14. Regulatory & Ethics Committee Approval

**Ethical Considerations**

The study will be carried out in accordance with the Declaration of Helsinki (2013), and local R&D and Ethics Committee approval will be sought. The study will also be reviewed and approved by HRA. It is the responsibility of the Chief Investigator to obtain a favourable ethical opinion prior to recruiting patients and to conduct the study in accordance with the conditions of ethical approval.

**Informed Consent**

Written informed consent will be obtained from each patient by clinical members of the study team or named research nurses/radiographers with appropriate training and nominated by the CI. It is the responsibility of the Chief Investigator (or designated representative) to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. Sufficient time will be allowed for the patient to decide on study entry, and they will be informed about their right to withdraw from the trial at any time. The patient information sheet, which will be given to each patient before enrolment, will be an approved patient information sheet according to national guidelines, with support from our dedicated PPI representatives.
All delegated staff consenting patients will have had appropriate consent training.

**Data Protection and Patient Confidentiality**

Patient confidentiality will be maintained in accordance with the Data Protection Act 1998 and local confidentiality code of practice and data protection policy and procedure.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

Participants will be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

## 15. Data Handling and Record Keeping

Patients and volunteers will be assigned a study number, record of this and personal patient data will be anonymized and stored within the Royal Marsden Hospital on a secure server, supported by the NIHR imaging clinical research facility team. Imaging studies will be anonymized and archived at the RTTQA platform and on our secure imaging repository at the Royal Marsden Hospital (XNAT).

MRI data will be exported to XNAT, which is an image file repository located behind the RMH firewall. All data will be anonymised with a study number prior to analysis to ensure patient confidentiality. Anonymised MRI data will be transferred to another XNAT system (designed for anonymised images, and located behind the ICR firewall) for analysis.

The investigators will make source data and documents available for the purposes of trial-related monitoring, audits, IRB/IEC review and regulatory inspections as required, without compromising patient confidentiality.
The chief investigator carries overall responsibility for ensuring that data handling and record keeping for this study is in accordance with the Data Protection Act and Caldicott principles. Only the named investigators will have access to study data.

## 16. Financing, Indemnity & Insurance

Main study primary, secondary endpoint analyses will be conducted as part of a doctoral fellowship programme, funded by Health Education England and the National Institute for Health Research (HEE/NIHR ICA Programme Clinical Doctoral Research Fellowship, Mrs Ana Rita L Simoes dos Reis Ferreira, known as Rita Simoes, ICA-CDRF-2018-04-ST2-004).

MRI sub-study endpoints analyses will be conducted by Dr Blackledge. Additional MRI scans required for the endpoint number sub-study analyses are funded by Sarcoma UK, along with travel costs for patients participating in these scans. Funding from the ICR will support the PhD programme of Ms Thrussell.
Clinical trial infrastructure is supported by the NIHR imaging clinical research facility at the RMH.

The NHS Litigation Authority will cover standard clinical negligence by employees, staff and health professionals employed by the Royal Marsden NHS Foundation Trust. For more information visit the following website: http://www.nhsla.com/Claims/Pages/Clinical.aspx

There is unlimited liability and no excess. Insurance is provided under the Clinical Negligence Scheme for Trusts and there is no cover for non-negligence claims.

For all notification of claims please contact the Board Secretariat.

## 17. Publication Policy

All results will be submitted for peer-review at 2-3 international scientific meetings (funding already obtained from NIHR/HEE clinical doctoral programme for Mrs Simoes and from the ICR for Dr Blackledge). Incorporating feedback from these meetings, research articles will be published in open-access journals in relevant fields, to be led by Dr Miah (main study), Dr Blackledge ((MRI radiation response assessment sub-study,

Appendix A) and Dr Zaidi and Mr Hayes (Biomarker development and immune mediators associated with radiotherapy sub-study (BIODATA) Appendix B) . Publications will be shared amongst our STSE working group, who will meet on a quarterly basis, along with our nominated PPI representatives.

We will identify opportunities for writing guidelines and delivering a STSE software tool that could be used for clinical purposes within the health sector. The research conducted throughout this programme of work will involve development of new computer algorithms, for which patent protection and potential commercial exploitation will be considered. In addition, copyright protection will exist in material prepared for presentations, and when scientific articles are published in peer-review journals. The Enterprise Unit (EU) of the Institute of Cancer Research will work with the investigators of the study (A. Miah and M. Blackledge) to review outputs and identify any potential commercial or copyright materials. The EU representative (Dr Alan Stuttle) will manage IP for both the ICR and RMH, where the clinical studies will be performed.

The Biomedical Research Council (BRC) will be acknowledged in all the publications arising from this study.



## 18. Abbreviations

| | |
|---|---|
| 3DCRT | 3D-Conformal Radiotherapy |
| ADC | Apparent Diffusion Coefficient (from DWI) |
| AE | Adverse Event |
| AI | Artificial Intelligence |
| AR | Adverse Reaction |
| CBCT | Cone-Beam Computed Tomography |
| CI | Chief Investigator |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CT | Computed tomography |
| CTV | Clinical Target Volume |
| DCE-MRI | Dynamic Contrast Enhanced MRI |
| DWI | Diffusion-Weighted Imaging (MRI) |
| EF | Enhancement Fraction |
| EORTC | European Organisation for Research and Treatment of Cancer |
| EoT | End of Treatment |
| GCP | Good Clinical Practice |
| GIST | Gastrointestinal Stromal Tumour |
| GTV | Gross Tumour Volume |
| Gy | Gray (radiation unit) |
| ICR | Institute of Cancer Research |
| IGRT | Image-Guided Radiotherapy |
| IMRT | Intensity Modulated Radiotherapy |
| MRE | Magnetic Resonance Elastography |
| MRI | Magnetic Resonance Imaging |
| NIHR | National Institute for Health Research |
| OE-MRI | Oxygen Enhanced MRI |
| OR | Odds Ratio |
| PE | Phase-Encode MRI gradient direction |
| PI | Principal Investigator |
| PD | Proton Density MRI scan |
| PPI | Patient and Public Involvement |
| PTV | Planning Target Volume |
| QLQ | Quality of Life Questionnaire |
| $R_1$ | Longitudinal Tissue Relaxivity |
| $R_2$* | Transverse Tissue Relaxivity |
| RMH | Royal Marsden Hospital |
| RO | Readout MRI gradient direction |
| RT | Radiotherapy |
| RTOG | Radiation Therapy Oncology Group |
| SAE | Serious Adverse Event |
| SAR | Serious Adverse Reaction |
| SPAIR | SPectral Attenuated Inversion Recovery |
| SS | Slice-Select direction |
| STS(E) | Soft-Tissue Sarcoma (of the Extremities) |

| | |
|---|---|
| TESS | Toronto Extremity Salvage Score |
| WHO | World Health Organization |

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# Appendix 1.

## Sub-Study: Artificial Intelligence for automated assessment of multi-parametric MRI in soft-tissue Sarcoma – application to neoadjuvant RadioTherapy (AIMS-RT). (Lead: Dr Matthew Blackledge – The Institute of Cancer Research)

**Background**

Histological changes of STSE following pre-operative radiotherapy have been previously reported in a retrospective series, which also demonstrated that partial MRI response (>50% reduction in tumour volume) was predictive of pathological response (percentage of treatment-related necrosis).[16] The gross tumour volume (GTV) was delineated according to T1-weighted magnetic resonance imaging (MRI) including gadolinium enhancement, and the clinical target volume (CTV) encompassed margins for microscopic disease and peri-tumoral oedema, as visualised on T2-weighted MRI sequences. However, there is still a lack of concordance or understanding of the histological changes occurring within STSE tumours following radiotherapy that lead to the imaging phenotypes observed with T1-weighted or T2-weighted MRI. Moreover, STSE tumours are highly heterogeneous; imaging tools may allow for dose-escalation to resistant tumour sub-regions if the biological underpinning of imaging signatures in STSE could be better understood.

Accurate delineation of the GTV combined with IMRT and IGRT allows for accurate RT dose delivery and has the potential to minimise radiation dose to adjacent normal tissue structures. This could consequently translate to a lower incidence of acute and long-term toxicities. Furthermore, tumours are typically heterogeneous and imaging tools may allow for dose-escalation or de-escalation based on biological information.

Quantitative MRI is highly applicable to monitoring response of STSE, due to its excellent soft-tissue contrast and the lack of ionising radiation. Recent advances in scanner technologies now allow the exploitation of quantitative MRI techniques in clinical trials, as they enable (i) non-invasive investigation of the entire tumour volume and (ii) can provide information about the biological properties of tumours through functional measurements. For example, maps of apparent diffusion coefficient (ADC) derived from diffusion-weighted MRI inform on tissue cellularity, with lower ADC values observed in highly cellular or more aggressive regions within tumour.[17] Using contrast enhanced MRI, the signal enhancement after intravenous injection of a gadolinium-based contrast agent provides information about tumour vascular perfusion and permeability (DCE-MRI)[18], and by using so-called Dixon MRI, the presence of fat in sarcomas can also be measured and quantified.[19] In our previous work, we have demonstrated a negative correlation between ADC and tissue cellularity in STSE, and a positive correlation between MR-derived estimates of fat-fraction with histopathology-derived measurements of fat

content.[20] Furthermore, this study revealed significant increases in median ADC measurements following pre-operative radiotherapy in 14 patients. These imaging methods are now part of the EORTC-STBSG guidelines for radiological examination and reporting of STSE.[21] More recent additions to the collection of functional MRI techniques include Oxygen-Enhanced MRI (OE-MRI), which provides an indirect measure of tissue oxygenation[22], and magnetic resonance elastography (MRE), which measures tissue stiffness by imaging the effects of acoustic shear waves as they transverse through the tissue.[23] The former of these approaches is particularly attractive in the setting of radiotherapy, as previous studies have demonstrated an inverse relationship between tissue hypoxia and radiotherapy effectiveness.[24,25] The ability to quantify and map the hypoxic status within the tumour could provide very powerful predictive biomarkers of radiotherapy response, and allow clinicians to target radiotherapy doses to regions of the tumour that are not expected to respond well. Tissue stiffness as measured by MRE could also provide biomarkers of (i) tumour response to radiotherapy, and (ii) healthy tissue toxicity following irradiation exposure by monitoring the onset of fibrosis and oedema.

With the development of more powerful MR-imaging hardware, it is now possible to acquire all of these quantitative mapping techniques in a single radiological study. By combining these approaches, we can non-invasively gather information about the biology of STSE and how they respond to treatment, allowing oncologists to make informed judgments on personalized, adaptive RT plans. However, radiological evaluation of such multi-parametric information is confounded by the large quantity of data that is delivered by the scanner. This could be particularly important for any future studies of neoadjuvant STSE radiotherapy that involve the MR-linac[26], as functional imaging could be acquired at each treatment fraction, resulting in up to 20-30 imaging studies per patient. It is therefore vital that new image analysis techniques be developed that can capture relevant information from multi-parametric MRI in STS. With the advent of new methods in artificial intelligence (AI) in image analysis, the time for exploiting these techniques when analyzing multi-parametric MRI in STSE is ripe.

This sub-study provides the opportunity to standardise the radiological and histological response criteria used in assessing response to radiation. Although percentage necrosis is a well-established criterion of response to pre-operative chemotherapy in osteosarcoma, currently there are no standardized histological response criteria for pre-operative therapy in soft tissue sarcoma. A consensus has been sought to utilise percentage viable tumour cells as a marker of pathological response. Applying post-treatment imaging to guide the analysis of representative sections of the tumour specimen may provide a methodology in consistency in reporting response. In addition, this study will perform early biopsy and imaging of the tumour to determine if predictive changes can aid adaptation of the RT dose delivered entirely or partially to the tumour.

Our current practice has demonstrated that there are variations in RT response with different histological subtypes, shown radiologically and histologically and no clear standardised response assessment. There may be also the potential to consider pre-

operative chemotherapy in selected histological subtypes which may influence the timing and/or necessity for RT.

**Rationale**

The effect of radiation on specific STSE subtypes using standard and novel functional MR imaging techniques and also the pathological and molecular changes associated with pre-operative radiation. These data could lead to greater personalisation in the management of sarcoma patients, by determining the sequence of changes during radiotherapy, evaluating tumour perfusion and vasculature with functional MRI and histological and genomic changes.

**Hypotheses**

- MR-imaging changes measured within soft-tissue sarcomas observed during radiotherapy fractionation are predictive of final response to radiotherapy.
- Imaging phenotypes of STSE measured using multi-parametric MRI correlate with distinct molecular and histopathological alterations; regions of molecular/histopathological heterogeneity in STSE can be successfully identified using AI-based image analyses of multi-parametric MRI.

**Objectives**

***Primary***

1. To establish whether baseline measurements in apparent diffusion coefficient (ADC) measured at baseline, and/or changes in ADC measured midway through fractionation (after fraction 7-11 inclusive) or following treatment are predictive of soft-tissue sarcoma response measured using histopathology (% necrosis).

***Secondary***

2. To develop, optimise and evaluate clinical OE-MRI and MRE protocols for use of STSE imaging using healthy volunteer and MRI test-object studies.

3. To quantify the single-centre reproducibility of OE-MRI, MRE, T1, T2, magnetisation transfer (MT), DCE-MRI, and dixon imaging in STSE tumours, including all derived quantitative biomarkers.

4. To determine whether sub-regions identified using AI-segmented MRI demonstrate different biological phenotypes through molecular profiling and regional histopathology in soft-tissue sarcoma.

5. To assess whether heterogeneous sub-volumes identified from AI segmentation models correlate with histological STSE response to radiotherapy.

6. To assess the correlation between (i) pre-treatment measurements, (ii) mid-RT changes and (iii) post-RT changes of tissue hypoxia (measured using OE-MRI) and

tissue stiffness (measured using MRE) with post-radiotherapy changes in tumour cellularity (measured using DW-MRI)

7. To determine whether MR-imaging parameters measured within this sub-study are predictive of healthy tissue toxicity in STSE.

8. To develop AI-models for identifying texture features in X-ray CT images that correlate with MRI-derived sub-volumes.

**Sub-study design**

This sub-study is aimed at establishing whether changes in median ADC are predictive of pre-operative STSE radiotherapy response measured using histopathology. This sub-study will involve up to 65 patients treated with pre-operative RT and will only run at the Royal Marsden Hospital.

Recruitment will involve STSE patients receiving pre-operative radiotherapy. Patients (up to N=65) will be invited to have an MRI scan before treatment, following fraction 7-11 of radiotherapy and 4 weeks before surgical resection. Additionally, molecular and pathological assessment will be undertaken on surgical resection specimen. Whole genome sequencing has become standard of care so further correlative analysis with clinical outcome will be performed. On board weekly CBCT imaging will be reviewed to determine changes occurring during treatment.
A set of patients (N=15) will be recruited to volunteer for an repeat imaging baseline study (> 6 days after first baseline before treatment) to perform a repeatability study of the MR-imaging biomarkers. We will collect the ADC measurements of these patients measured at their normal clinical imaging time points. Additionally, molecular and pathological assessment will be undertaken on surgical resection specimen.
Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both. The total number of patients that will be recruited will range 50-65 depending on how many patients take part in both sections.

**Volunteer Study**

In the initial phase, OE-MRI and MRE protocols will be optimized prospectively on a cohort of 20 healthy volunteers at the RMH. Volunteers will undergo conventional clinical scans for STSE in the abdomen, pelvis and extremities (diffusion-weighted, Dixon, MT, Dixon, T1, T2, and DCE-MR imaging; the latter without the use of any contrast agent). For the same fields-of-view, OE-MRI and MRE sequences will be acquired; spatial resolution and geometric distortion of these new protocols will be measured by comparison to clinical imaging protocols. Initial developments of these techniques on MRI test-objects are already underway at the RMH.
All volunteer studies will be performed according to RM healthy volunteer guidelines; volunteer details will be entered into the institutional EPR system, imaging data will be

uploaded onto the RMH PACS system and anonymously stored on a secure research imaging archive hosted within the RMH; all scans will be reported for incidental findings. Anonymised data for analysis will be transferred to another XNAT system, behind the ICR firewall.

**Patient Study**

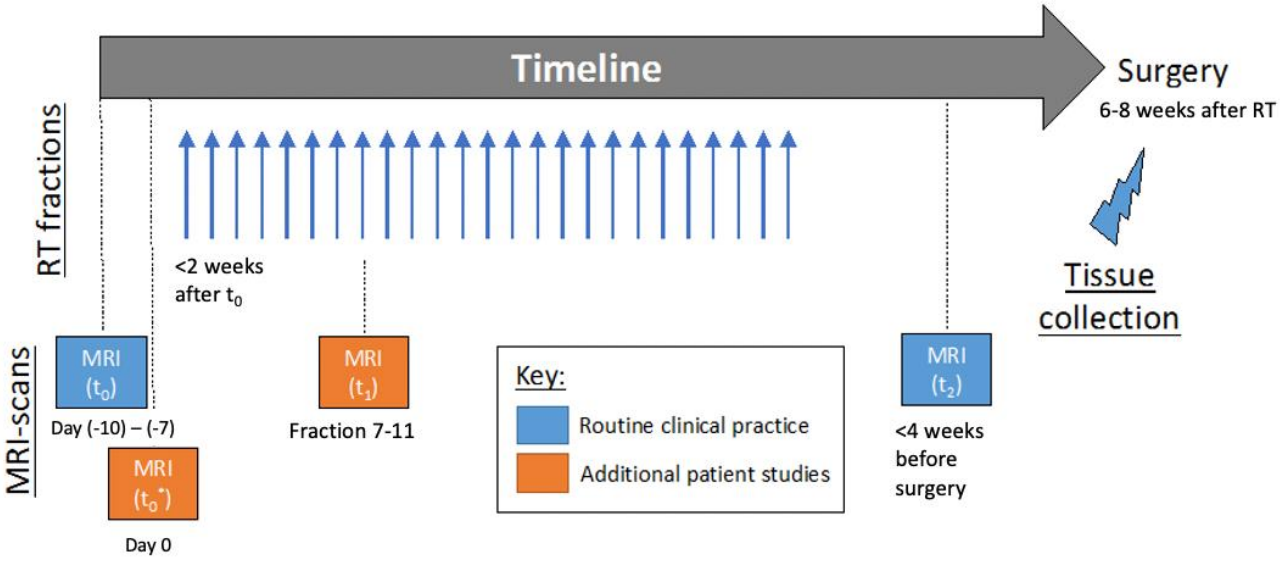
This prospective, observational imaging sub-study aims to recruit adult STSE patients receiving neoadjuvant RT as part of routine healthcare at the Royal Marsden Hospital (single-centre).

Up to 65 patients will receive multi-parametric MRI studies performed within one week prior to RT ($\mathbf{t_0}$), following 7-11 RT fractions inclusive ($\mathbf{t_1}$), and within 4 weeks prior to surgical resection ($\mathbf{t_2}$).

15 patients will receive the multi-parametric MRI study performed prior to RT ($\mathbf{t_0}$), and again 7 days after the first ($\mathbf{t_0}$*) but before treatment begins.

Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both.

An illustration of our proposed patient pathway in shown in the figure below:




In addition to the MR-imaging study, we will collect diagnostic baseline and follow-up X-ray CT data from the same 65 patients recruited on the sub-study, along with radiotherapy planning structures. These data will be used to explore surrogate measures of heterogeneous response using X-ray CT, with developed methodologies for multi-parametric MR acting as the gold-standard (objective 8). A U-NET deep-learning model will be developed and adapted to train a new AI network for this purpose.

**Specific endpoints**

***Primary***

1. ADC measurements at baseline ($t_0$), and ADC changes at mid-fractionation ($t_1$), and following RT conclusion ($t_2$) will be correlated with tumoural response, measured as % necrosis from histopathological analysis of post-surgical specimens, using a linear model.

***Secondary***

2. Clinical feasibility of OE-MRI and MRE protocols for imaging if STSE within the RMH (image quality evaluated by a consultant radiologist, and acquisition time < 20 minutes for both protocols).

3. Bland-Altman analysis of the repeated MR techniques (OE, DCE, MRE, T1, T2, MT and dixon) prior to start of RT. Intraclass-correlation coefficient (ICC) and coefficient of variance (CoV) will be measured.

4. Our system of deriving sub-volumes from STSE tumours using AI will be used to identify regions of interest for immunohistochemistry from tissue samples. Measures of (i) % viable tumour, (ii) % dedifferentiated tumour, (iii) % necrosis, (iv) % fat, (v) % ki67 uptake, and (vi) % fibrosis/hyalinisation will be recorded within these regions.

5. Changes in AI-derived measurements of STSE tumour sub-volumes at mid-fractionation and following conclusion of RT will be correlated with histopathological response.

6. Measurements at baseline, and changes at mid-fractionation, and following RT conclusion in tissue stiffness and tissue hypoxia will be correlated with tumoural response, measured as % necrosis from histopathological analysis of post-surgical specimens, using a linear model.

7. Time-to-report of tissue fibrosis, wound-healing complication and lymphoedema as measured on a 3-monthly interval following surgery (follow-up period of two years).

8. Accuracy of heterogeneous tumour sub-volumes segmented from X-ray CT images compared to our MRI approach (gold standard).

**Sub-study inclusion and exclusion criteria**

Patients receiving pre-operative radiotherapy at the Royal Marsden Hospital will be invited to participate in AIMS-RT sub-study. The specific inclusion and exclusion criteria for this sub-study are listed below.

***Inclusion criteria***

i. Patients with soft tissue sarcomas planned for surgical resection and preoperative radiotherapy at the Royal Marsden Hospital
ii. Patients due to receive pre-RT MR imaging studies as part of routine clinical care.

***Exclusion criteria***

i. Patients with contraindications to MRI (e.g. MR Unsafe implant)
ii. Claustrophobia
iii. Patients with renal failure or problems with IV access will not receive intravenous contrast but may still be recruited to undergo the non-contrast enhanced components of the imaging protocol.
iv. Patients who do not tolerate the use of an Oxygen administration mask or have Chronic Obstructive Pulmonary Disease (COPD) will not undergo OE-MRI
v. Patients who would find it difficult/uncomfortable to position the tissue of interest within the central section of the scanner

***Subject withdrawal criteria***

All patients will require MR-imaging to be acquired pre-/post-radiotherapy as part of routine care, prior to tumour removal. Patients may be withdrawn from the study if there are any reason MR-imaging becomes contra-indicated during their treatment (e.g. new implantation of an MR-unsafe implant). Should at any time they feel unable to participate in a third MR-imaging time-point, they would also be withdrawn from the study.

Up to 65 pre-operative patients will be recruited for the exploratory study from patients enrolled in the main study at RMH. However, as pre-operative patients participating in the main study may decline participating in the secondary study, this number may not be accrued during the planned 18 months of accrual of the main study. In that case, specific recruitment of pre-operative patients for the sub-study only (i.e., who would not participate in the main study) will be extended by a maximum of 12 months.

***Sub-study participation***

Patients treated within PredicT B with a pre-operative intention will be asked to take part in the sub-study.
This sub-study can be summarised in two parts: i) a

50 patients will be recruited for this sub-study which will involve administration of one additional dose of MRI contrast agent (Gadolinium-chelate), midway through radiotherapy fractionation (standard dosage of 0.1 mmol/kg). Subject compliance to our additional imaging protocols will be recorded through the use of MRI checklists, to be completed by the research radiographer during each MR-acquisition time-point.

15 patients recruited for the repeatability study will be asked to volunteer for an additional imaging baseline study (> 6 days following first baseline but prior to radiotherapy) to perform a repeatability study of the MR-imaging biomarkers explored in this cohort. This will include another MR contrast injection (0.1 mmol/kg). Once the required numbers for this secondary objective have been recruited (N = 15), we will not pursue further recruitment for repeatability assessment.

Patients can be recruited for the mid-treatment MR scan, the repeat MR scan or both.

**Data-acquisition**

***MR-Imaging***

Imaging studies at all time-points (including the repeatability sub-set), will include the imaging sequences listed in **Table A1**. Each imaging study will not last longer then 1-hour, including patient set-up, positioning and removal from the scanner couch. All sequences will be acquired in free-breathing, with the patient lying in a supine position; in all patients, a cannula will be used to administer the MRI contrast agent required for DCE sequences. In addition, patients will be required to wear an oxygen mask for pure O2 breathing during OE-MRI sequences, and 3-4 small (~ 3 x 5 $cm^2$) applicators will be placed on the surface of the STSE to emit shear waves required for MRE imaging. Our MRE equipment is not CE-marked for medical use. We have approached the medical devices team at the Medicines and Healthcare products Regulatory Agency (MHRA) regarding its use within this trial, and they have informed us that official application to the MHRA is not required in this instance as we will be using the equipment for research purposes only. All sequence parameters will be collated into an imaging manual for the study, to ensure consistency of imaging results across all patients and to provide research radiographers with detailed instructions on the timing of all imaging sequences. In addition, radiographers will be requested to complete an imaging check sheet, which will be archived to as a record of all completed scans.

| Sequence Name | Quantitative Imaging Biomarker | Exogenous Contrast Required? | Approximate Time (minutes) |
|---|---|---|---|
| **1) Localizer** | N/A: Used for positioning of subsequent sequences | - | 1 |
| **2) Dixon imaging** | Fat fraction (%) | - | 2 |
| **3) MT imaging** | Magnetic Transfer Ratio (MTR) | - | 2 |
| **4) Diffusion-weighted imaging** | ADC: Surrogate marker of tissue cellularity ($mm^2/s$) | - | 10 |
| **5) T1/T2 Mapping** | T1/T2 | - | 10 |

| | | | |
|---|---|---|---|
| **6) 3D gradient echo (PD mapping)** | N/A: Provides an estimate map of water density | - | 1 |
| **7) Dynamic contrast-enhanced imaging** | Enhancement Fraction: Surrogate marker of vascularity (%) | Gadolinium-chelate | 10 |
| **8) Magnetic Resonance Elastography** | Speed of sound: Surrogate marker for tissue stiffness (m/s) | - | 10 |
| **9) Oxygen Enhanced Imaging** | $\Delta R_1$, $\Delta R_2$*. Map of tissue hypoxia ($ms^{-1}$) | Pure $O_2$ breathing | 10 |

**Table A1.** Approximate timings for the MRI protocol investigated in this study. Including 20 minutes set-up time, a 1-hour scanning slot will be necessary for each imaging time-point (approximately 15 minutes longer than conventional MRI protocols for STS).

***Tissue Collection***

Tumours will be surgically removed following radiotherapy (approximately 6-8 weeks after RT) and orientation of tumour matched to orientation of MRI by the surgical team in consultation with the radiology team. Tissue blocks will be extracted from excised tumours and sectioned in the same plane as for MRI-acquired images. We will acquire a number of representative blocks, covering the axial field-of-view of the tumour in two concurrent sections, and for each record (i) % viable tumour, (ii) % dedifferentiated tumour, (iii) % necrosis, (iv) % fat, (v) % ki67 uptake, and (vi) % fibrosis/hyalinisation and any further histopathological and immunohistochemical measurements. Following AI-based segmentation of tumour sub-regions from MRI, we will manually locate spatially similar regions on histology samples consulting both modalities simultaneously (1 location per tumour sub-type identified). Within $1cm^2$ regions of these samples, we will measure cell/stroma ratio and stromal morphology (fibrous/hyalinised, myxoid or fibromyxoid) and perform molecular profiling using RNA-Seq/proteomics.

The management of tissue samples related to this aspect will be conducted by Mrs Emma Perkins from the sarcoma research team at the Royal Marsden. All persons involved in the collection, transportation and handling of human tissue will be adequately trained for their involvement in this work. Formalin-fixed paraffin embedded (FFPE) tumours blocks will be held in the secure storage facility in the Molecular and Systems Oncology lab, ICR. In addition, frozen surgical specimens will be stored at the appropriate temperature in freezers at the ICR. Both the RMH and ICR are Human Tissue Authority (HTA) licensed premises, with transfer of significant material between sites covered by overarching Material Transfer Agreement (MTA). All human tissue received into the lab and covered under the auspices of the HTA will be prospectively logged onto FreezerPro (Brookes Automation), a web-based lab management system to ensure HTA compliance and assist with sample tracking. To test our tissue processing pathway for this sub-study we will

leverage existing excess tissue blocks, stored within the RMH tissue archive, that was obtained as standard of care and as part previous prospective trials for which consent has been given.

**Sub-study endpoint analysis**

***Endpoint 1***

We will generate two linear models of response for predicting changes in tumoural response (measured semi-quantitatively using pathology) using (i) changes in median ADC at our mid-fractionation imaging time point ($\Delta ADC_1$), and (ii) change in median ADC following completion of RT ($\Delta ADC_2$). The accuracy of both models for predicting RT response, measured as % necrosis from histopathological analysis of post-surgical specimens, will be compared to identify whether changes occurring mid-fractionation provide substantive evidence for final treatment outcome. 50 patients would suffice for such a study; there are no conventional methods for powering linear model studies.

***Endpoint 2***

A radiologist with experience in STSE imaging will review the quality of all new imaging techniques and assess them for contrast-to-noise, presence of imaging artefacts and spatial resolution. Imaging will be acquired in the thigh of each volunteer to obtain a similar field-of-view to that required for STSE patients. A time-cap of 20 minutes will be required for these imaging techniques, but where possible this will be reduced.

***Endpoint 3***

Bland-Altman analysis will be used to test the repeatability of the metrics derived from MRE and OE-MRI (tissue stiffness and ΔR2* respectively). We will calculate the median of both parameters within the tumoural extent (regions of interest outlined by I. Thrussell, and confirmed by a radiologist), and compare the change repeat measurements with the average to obtain a coefficient of variance (CoV) measure. In addition, we will compute the intra-class correlation (ICC: measure of 1 representing ideal repeatability).

***Endpoint 4***

Our AI methodology will be developed on all patients split into a training and test datasets on a 4:1 ratio. Using the first 40 patients, we will train state-of-the-art deep-learning techniques (e.g. U-Net) to automatically segment heterogeneous sub-regions within STSE tumours (using quantitative measures from all available MRI modalities). Radiologist defined regions will provide a gold-standard for these sub-regions, using a technique we have previous developed. The cross-validation accuracy of this new methodology will be compared with our existing approach. The remaining 10 patient datasets will be used to provide the final accuracy of the trained models. We will also explore the use of AI for automatic delineation of the entire tumour extent using U-Net models or equivalent deep-learning technology as it becomes available. It should be noted that for each patient, we expect to generate data from at least 4-5 different heterogeneous regions within the tumour (both in terms of imaging and histopathology), thus increasing the effective sample size in this endpoint.

Regional histopathology and molecular profiling will be used to validate the classifications made to each tumour sub-type identified through imaging acquired prior to surgery ($\mathbf{t_2}$).

***Endpoint 5***

Volumes will be calculated for each tumour sub-region detected using our AI approach. Changes in these volumes at $t_1$ and $t_2$, denoted $\Delta V_1$ and $\Delta V_2$ respectively, will be correlated with histopathological response.

***Endpoint 6***

Linear models in the same vein as for the primary endpoint of the study will be explored for MRE, OE-MRI and DCE-MRI measures of tissue stiffness, hypoxia and tissue vascularity. Models will include ADC measures, to identify which parameters are most predictive of response, and whether baseline measures or changes at t1 provide the highest predictive power.

***Endpoint 7***

Cox proportional hazard models will be developed for changes in tissue stiffness and ADC with time to toxicity related outcomes following surgery (tissue fibrosis, wound-healing complication and lymphoedema). We will focus on the utility of simple statistics from MR-derived parameters within the entire tumour volume for this endpoint (i.e. median MRI measures).

***Endpoint 8***

We will investigate the use of U-Net models and Generative Adversarial Networks (GANs) for developing models of segmentation of heterogeneous STSE regions using X-ray CT. Our MRI methodology (endpoints 4 and 5), will act as gold-standard for this technology; we will compare volumes of measured sub-regions derived from both CT and MRI.

# Appendix 2. Biomarker development and immune mediators associated with radiotherapy sub-study (BIODATA). (Leads: Dr Shane Zaidi and Mr Andrew Hayes)

## Background

### Biomarker Development

Recent technological advances in radiotherapy planning and delivery may lead to more individualised radiotherapy by improving the therapeutic ratio based on tumour anatomy.[27] Adapting radiotherapy by applying tumour biology also has the potential to contribute to improving the therapeutic gain.

Soft-tissue sarcomas are characterised by considerable genetic and epigenetic heterogeneity driven by genomic instability.[28] Patients with the same histological subtype demonstrate a wide range of natural progression of disease and response to treatments, including radiotherapy. A major goal of personalised cancer medicine includes the use of biomarkers to tailor treatment to individual patients. Prognostic biomarkers provide information regarding disease outcome regardless of treatment, and predictive biomarkers determine which patients will derive benefit from a specific treatment.

There are few predictive biomarkers that have made the transition to clinical use in soft-tissue sarcomas. Gastrointestinal stromal tumours (GIST) harbor specific mutations in *KIT* and *PDGFRA* correlating with clinical response to the oral tyrosine kinase inhibitor imatinib.[29] Although there are other examples of predictive biomarkers for directing systemic therapy in soft-tissue sarcomas, there is an urgent need to develop markers predicting outcome to radiotherapy.

Many factors are known to influence tumour response to radiation including total dose, fractionation, hypoxia and intrinsic radiosensitivity. Pre-clinical tests to determine tumour hypoxia and intrinsic radiosensitivity may correlate with clinical outcome but are not available for routine use. Tumour response to radiation includes activation of the DNA damage response pathways providing additional potential candidate markers for evaluating radiosensitivity.[30]

Development of new molecular profiling techniques now allows us to identify specific tumour gene expression signatures and attempts have been made to develop prognostic models. Techniques include analysis of circulating tumour cells, gene expression from tumour samples, analysis of common somatic mutations and rearrangements in specific genes and analysis of blood serum proteins. Although several candidate markers have been identified there is a need to validate these prospectively in the clinical setting.

### Immune mediators associated with radiotherapy

Radiotherapy has been used to treat cancers based on the ability to cause DNA-damage leading to cell death. Recent preclinical and clinical data suggest radiation may also mediate an anti-tumour immune response. Although we need to further investigate the underlying mechanisms, radiation has an effect on the immune response to antigens including up-regulating MHC- class I antigen presentation, creating neo-antigens and activating cytotoxic T cells.[31] Analysis of paired soft tissue sarcoma tumour samples pre- and post-radiotherapy have demonstrated changes in tumour microenvironment, triggering immunogenic cell death, enhances immune-related signatures suggesting scope for combining radiotherapy with immunotherapy. Clinical studies are now combining immunotherapy with radiation in patients with soft-tissue sarcoma. There is a need for refined focus on the use of radiotherapy as an immune-modulator in patients with soft tissue sarcoma. Biomarker analysis of candidate markers may identify which patients will benefit from personalized care combing radiation with modern immunotherapy agents including checkpoint inhibitors.

**Rationale**

In this exploratory study, we hope to identify potential markers of radiation response and radiation sensitivity alongside biomarkers to evaluate potential prognostic markers to predict for early development of metastatic disease, which may

a. Refine selection of cases for pre-operative radiotherapy, palliative radiotherapy and no radiotherapy
b. Guide radiation dose escalation/ de-escalation strategies (with possible hypofractionation) for different histological subtypes
c. Evaluate radiation response and whether addition of systemic therapy could enhance the therapeutic index
d. Determine if radiotherapy stimulates the tumour microenvironment to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation (see laboratory manual).

Participation in this sub-study will be offered to patients receiving pre-operative and palliative radiotherapy.

**Hypothesis**

Biological markers will provide predictive information for tumour response for specific histological subtypes following radiotherapy and scope for combining radiation with immunotherapy.

**Exploratory objectives**

1. Determine if radiotherapy stimulates the tumour microenvironment, resulting in measurable change in anti-tumour immunity, to determine if certain subtypes could potentially benefit from the addition of immunotherapy with radiation.

a. Quantify the change in infiltration of immune cell populations.
b. Measure activation and exhaustion of therapeutically relevant populations.
c. Profile expression of therapeutically tractable immune checkpoint markers.
d. Investigate TCR clonality, neoantigens and immunoediting due to radiotherapy.

2. Prognostic markers which may refine selection of cases for pre-operative radiotherapy, palliative radiotherapy or no radiotherapy.

**Sub-study design**

This prospective sub-study is aimed at developing biomarkers that are predictive of response following radiotherapy delivered in the pre-operative / palliative setting.

This single centre sub-study (Royal Marsden Hospital, London) will aim to recruit 50 patients treated with pre-operative and 10 patients treated with palliative radiotherapy.

Recruitment will involve patients receiving pre-operative or palliative radiotherapy (minimum n=5/group) classified into the following groups:

1. Undifferentiated pleomorphic sarcoma (minimum number n =20),
2. Synovial sarcoma
3. Myxofibrosarcoma
4. Myxoid liposarcoma
5. Malignant peripheral nerve sheath tumour
6. Leiomyosarcoma
7. Other

**Biopsies**

Patients receiving pre-operative radiotherapy:

Freehand (surgical) tumour biopsies will be performed at baseline (during screening between day -7 and day 1) and fraction 8-10 (optional). Representative samples of the final resection specimen will also be collected for analysis. If patients are no longer scheduled for surgery (due to development of new or progressive metastatic disease or other factors) will collect a freehand (surgical) tumour biopsy (optional) following the post- radiotherapy MRI scan (after Day 40-50), ideally day 54-64. There is a separate consent form for this biopsy.

Patients receiving primary (palliative) radiotherapy:
Freehand (surgical) tumour biopsies will be performed at baseline (during screening) with an additional optional biopsy performed during treatment (see table 1 below).

Patients entering the BIODATA sub-study who develop local recurrence and/or metastatic progression during routine post-operative surveillance will be asked to consent for collection of a standalone biopsy. Tumours will be easily and safely amenable to freehand or image guided biopsy. There is a separate consent form for this biopsy. If they have surgery including metastasectomy to resect metastatic disease, patients will be asked if they consent for samples to be taken from the resected tissue for research purposes.

**Research blood samples**

Blood samples will be used to store PBMC and plasma: a total of 60 mL blood will be collected at each time-point.

Patients receiving pre-operative radiotherapy:
Blood samples will be collected at baseline (between day -7 and day 1), fraction 8-10 (optional) and at surgery to enable biomarker analyses.
If patients are no longer scheduled for surgery (due to development of new or progressive metastatic disease or other factors), will collect optional blood samples following the post- radiotherapy MRI scan (after Day 40-50), ideally day 54-64.

Patients receiving palliative radiotherapy:
Blood samples will be collected at baseline (during screening prior to surgery) and selected time-points (optional) to enable biomarker analyses (see table 1).

Patients entering the BIODATA sub-study who develop local recurrence and/or metastatic progression during routine post-operative surveillance will be asked to consent for collection of a standalone set of blood samples.

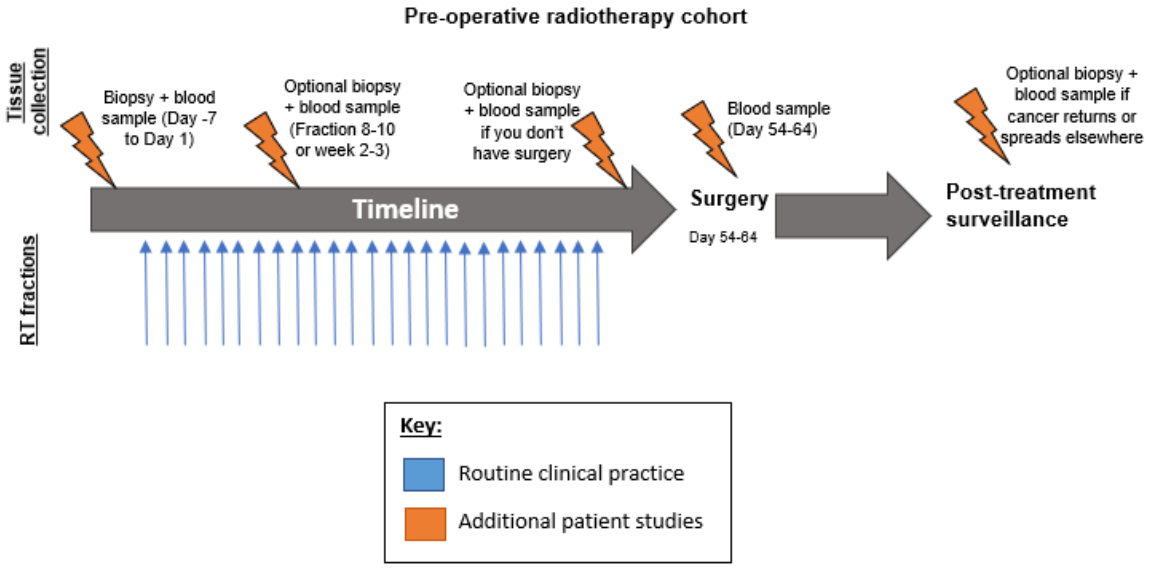



**Figure 1.** Illustration of proposed biomarker pre-operative radiotherapy STSE study.

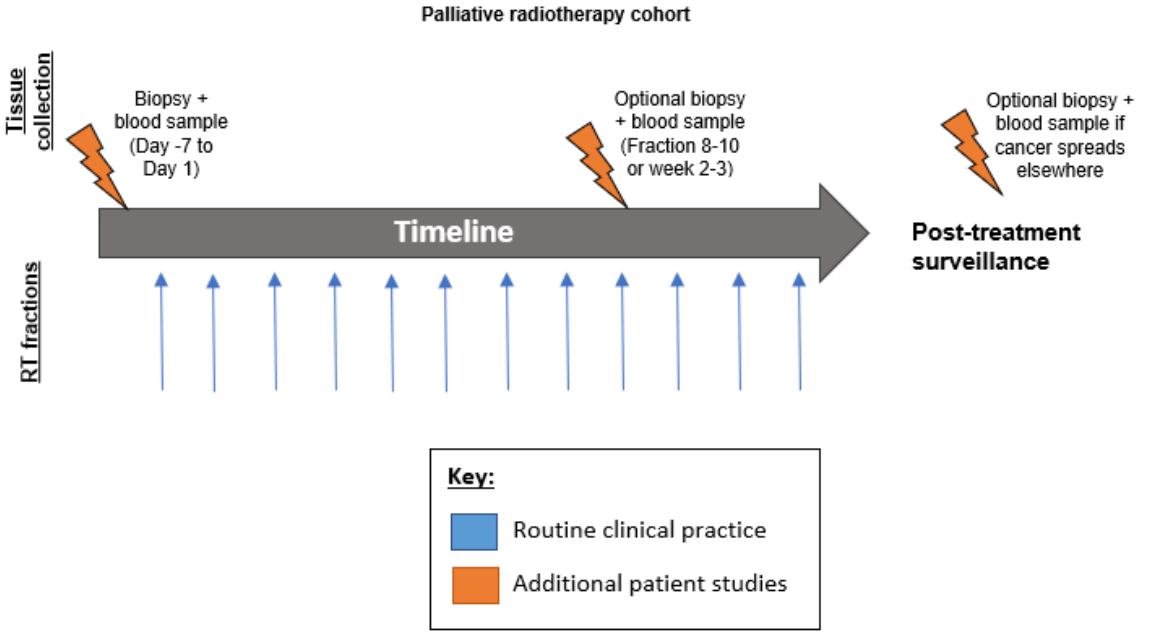



**Figure 2.** Illustration of proposed biomarker palliative radiotherapy STSE study.

| Dose-fractionation | RT delivery | Baseline research biopsy (during screening) | Second (optional) research biopsy | Baseline research bloods | Second (optional) research bloods | Third Research bloods |
|---|---|---|---|---|---|---|
| **Pre-operative radiotherapy cohort (tumour samples collected from resected specimen)** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50 Gy/25# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | At surgery 6-8 weeks after completing RT |
| 36 Gy/18# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | At surgery 6-8 weeks after completing RT |
| **Palliative radiotherapy cohort** | | | | | | |
| 40-45 Gy/15# | Daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 36 Gy/12# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 30 Gy/10# | daily | Between D-7 and D=0 | #8-10 | Between D-7 and D=0 | After #8 | NONE |
| 30 Gy/5# | Once Weekly | Between D-7 and D=0 | Just before #3 | Between D-7 and D=0 | Just before #3 | NONE |
| 36 Gy/6# | Once weekly | Between D-7 and D=0 | Just before #3 | Between D-7 and D=0 | Just before #3 | NONE |
| 25 Gy/5# | daily | Between D-7 and D=0 | After #5 | Between D-7 and D=0 | After #5 | NONE |

**Table 1:** RT dose fractionation schedules and timing of tests.

### *Tissue Collection*

Tissue collection will be coordinated by Eniola Ayeni, and Emma Perkins in consultation with the Sarcoma Unit of the RMH, and the research cancer nurse specialists who will be acquiring blood samples. Tissue samples will be stored in The Royal Marsden and The Institute of Cancer Research in accordance with the Human Tissue Act 2004. We have already tested our tissue processing pathway for this sub-study as part of the RMH sponsored CCR4640 APPLE Study (REC Ref 18/LO/0240).

### Specific endpoints

1. To establish whether pre-operative or palliative radiotherapy results in a measureable change in tumour microenvironment

    a. Analyse PD-L1 and PD-L2 expression at baseline and after treatment using IF.

    b. Predict changes to immune cell populations and radiation induced "neoantigen" expression from whole exome sequencing and RNA sequencing on frozen tumour samples

c. To analyse the impact of treatment on phenotype and function of immune cell infiltrates (baseline, during and surgery tumour specimens)

i. Phenotype and activation status by multi-IF for T cells (including but not limited to CD3, CD8, GzmB), B cells (CD20, IgG), Treg (FOXP3) and TAM (CD163)

ii. T cell activation/proliferation through multi-IF for CD8, Foxp3 and KI67 (or costimulatory molecules), and expression of other immune check point receptors.

d. To analyse the impact of treatment on plasma cytokines. The levels of circulating growth factors and T cell cytokines analyzed by Luminex or Elisa technology.

2. Biomarkers identified through laboratory analysis will be correlated with development of local recurrence, Metastatic disease-free survival and overall survival at 24 months.

***Inclusion criteria***

1. Patients with extremity soft-tissue sarcomas who are either due preoperative radiotherapy followed by surgical resection, or palliative radiotherapy, at the Royal Marsden Hospital.
2. Include but not limited to the following soft-tissue sarcoma histological sub-types: leiomyosarcoma, myxoid liposarcoma, liposarcoma, undifferentiated pleomorphic sarcoma, fibrosarcoma, epithelioid, clear cell, and synovial sarcoma.
3. Able and willing to undergo tumour biopsies.
4. Written informed consent.

***Exclusion criteria***

1. Patients on anti-coagulation who are unable to have a safe tissue biopsy performed (to discuss with Chief Investigator on an individual basis). Patients on anti-platelet medication can be entered into the study.
2. Previous radiotherapy within the treatment area.

**Data analysis**

Time course data on immune infiltration, mutations and neoantigens as a result of radiotherapy in sarcoma do not currently exist. This study involves consenting 60 patients with the aim of collecting a set of 3 serial tissue samples per patient (Day 8 biopsy is optional). A complete set of samples which pass quality control for sequencing and imaging is anticipated to be in the region of 20-25. This is a descriptive study with numbers anticipated to be too low for formal statistical tests. Data generated as a result of this

study will be used to guide both the design and the appropriate statistical power calculations for a number of follow-on studies.

Genomic analysis (DNA whole exome sequencing, DNA methylation, RNAseq) and protein analysis (multiplex immunohistochemistry/IF) will be used where necessary to address the following analysis questions. Genomic and protein analysis results will also be correlated with clinical endpoints (Local recurrence, metastatic disease-free survival, and overall survival).

1. DNA analysis will be used to identify defects in DNA damage response and repair pathway genes.
2. IHC/multiplex ImmunohistochemistryIF will be used to assess markers of DNA damage and DNA repair competence.
3. RNAseq and multiplex IHCIF will be used to first identify radiation induced changes to immune populations. This will involve established and extensively validated methods to predict immune populations from RNAseq data (CIBERSORT immune deconvolution). Predicted populations will be validated by multispectralmultiplex IHCIF.
4. Immune expression of clinically tractable immune-checkpoint targets will be determined. Changes on RNAseq will be used to validate immune checkpoint expression on therapeutically important cell populations by multiplex IHCIF.
5. The ability of radiation to alter the expression of mutated proteins which are subsequently predicted to be processed and presented to the immune system (referred to as tumour “neoantigens”) will be assessed from whole exome sequencing and RNAseq data.
6. Peripheral blood can be used to test if circulating immune populations recognise predicted antigens in point 45, and if this recognition has been altered by radiotherapy.
7. Clinical endpoints will be collected (local recurrence, metastatic disease-free survival , and overall survival at 24 months).

**Radiotherapy dose fractionation schedules**

The following radiotherapy dose fractionation schedules are permitted for primary (palliative) radiotherapy.

40-45Gy in 15 fractions, delivered daily, Monday-Friday;
36 Gy in 12 fractions, delivered daily, Monday- Friday;
30 Gy in 10 fractions, delivered daily, Monday- Friday;
36 Gy in 6 fractions, delivered once weekly;
30 Gy in 5 fractions, delivered once weekly;25 Gy in 5 fractions, delivered daily over one week.

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# Appendix 3. Schedule of assessments for main study

| Investigations | pre-registration/ post consent[4] | Within 14 days prior to registration | Within 28 days prior to starting treatment | From surgery and up to 120 days after surgery | During Radiotherapy | 28 days (and up to 35 days) after the last fraction of radiotherapy[5] | Month 1 post RT[6] | Month 2 Post RT | Month 3 Post RT | Month 6 post RT | Month 9 Post RT | Month 12 Post RT | Month 15 Post RT | Month 18 post RT | 2 years post registration (EOT) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Histological confirmation of disease | X | | | | | | | | | | | | | | |
| Diagnostic MRI and/or CT (if there is a contraindication to MRI) of the primary tumour site[1, 2] | X | | | | | | | | X | X | X | X | | X | |
| Chest imaging (CT or chest x-ray) within 3 months of registration, as per routine practice | X | | | | | | | | X | X | X | X | | X | |
| Clinical review of relevant medical history | | X | | | | | | | | | | | | | |
| Completion of the assessment of adverse events (AEs) using CTCAE v4.03 | | X | | | | X | | | | | | | | | |
| Assessment of WHO performance status | | X | | | | X | X | X | X | X | X | X | | X | X |
| Pregnancy test (urine or blood) in females of child bearing potential | | X | | | | | | | | | | | | | |
| Medical History ( smoking, diabetic status) | | X | | | | | | | | | | | | | |
| Measurement of height & weight | | X | | | | | X | X | X | X | X | X | | X | X |
| Physical Exam (limb function/ mobility)[3] | | X | X | | X | X | X | X | X | X | X | X | | X | X |
| EORTC QLQ-C30 quality of life questionnaire | | | X | | | X | | | | | | X | | | X |
| EORTC QLQ-FA12 fatigue questionnaire | | | X | | | X | | | | | | X | | | X |
| Toronto Extremity Salvage Score (TESS) questionnaire (1 and 2 years post registration) | | | X | | | X | | | | | | X | | | X |
| Musculoskeletal Tumour Society Rating Scale | | | X | | | | | | | | | X | | | X |
| Post-Surgery Wound Assessment | | | | X | | | | | | | | | | | |
| Cone Beam CT imaging review of treatment volume where applicable (additional imaging review where applicable) | | | | | X | | | | | | | | | | |
| RTOG Acute Radiation Morbidity Scoring Criteria[7] | | | | | | X | X | X | X | | | | | | |
| RTOG/ EORTC Late Radiation Morbidity Scoring Criteria from day 91 after start of treatment | | | | | | | | | X | X | X | X | X | X | X |
| Stern's scale for oedema from day 91 after start of treatment | | | | | | | | | X | X | X | X | X | X | X |
| Measurement of circumference of the limb | | | X | X | | X | | | X | X | X | X | X | X | X |
| Clinical assessment of local tumour control | | | | | | | | | X | X | X | X | X | X | X |
| Radiological response assessment (approximately 4-6 weeks after pre-op RT) | | | | | | X | | | | | | | | | |
| Pathology response assessment (after resection, approximately 8-10 weeks after pre-op RT) | | | | | | X | | | | | | | | | |
| Assessment of wound related clinical findings up to 120 days after surgery | | | | X | | | | | | | | | | | |

1. Patients receiving post-operative radiotherapy the MRI/ CT should ideally have been performed within 1 month prior to surgery

2. Patients receiving pre-operative radiotherapy, the MRI/ CT should ideally be performed within 1 month of starting radiotherapy, although decisions on repeating scans older than 1 month will be made at the treating clinician's discretion

3. Assessment of wound related clinical findings up to 120 days after surgery (if applicable within 28 days prior to starting treatment)

4. The following pre-registration assessments do not need to be repeated if within 28 days prior to starting treatment: clinical review, pre-treatment routine bloods (FBC, U&Es, LFTs, CRP, LDH and clotting), Assessment of Aes using CTCAEv4.03, WHO performance s

5. we will collect each patient: Diagnostic scans which may have been acquired before treatment commences, Radiotherapy planning scans, Structures, Plans and dose cubes, Treatment imaging review, including CBCT which may have been acquired as per imaging ve

6. All visits should be carried out at specified time +/ - 2 weeks.

7. Up to 90 days after start of treatment

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