Newcastle University

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NHS Foundation Trust

Full Title:	**SarcoSIGHT:** A Randomised-Control Trial of Fluorescence Guided Sarcoma Surgery Versus the Standard of Care
Short Title/Acronym:	**SarcoSIGHT**
Protocol Version & Date:	**5.0 25 JUN 2024**

Statement:

This protocol has regard for the HRA guidance.

RESEARCH REFERENCE NUMBERS

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted. The Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the Research Governance Framework, Good Clinical Practice (GCP) guidelines, the relevant Standard Operating Procedures and other regulatory requirements as applicable.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

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TRIAL SUMMARY

Trial Title	**SarcoSIGHT**: A Randomised-Control Trial of Fluorescence Guided Sarcoma Surgery Versus the Standard of Care	
Acronym	**SarcoSIGHT**	
Summary of Trial Design	A prospective, 2-arm, open-label, UK multi-centre, randomised control trial comparing fluorescence-guided surgery (FGS) with indocyanine green (ICG) to standard care (no fluorescence guidance) to determine the effect on the unexpected positive margin rate (UPM) in patients with sarcoma.	
Summary of Participant Population	Patients of all ages with a histologically confirmed diagnosis of intermediate to high-grade bone or soft tissue sarcoma suitable for curative resection.	
Planned Sample Size	Total n=500 (FGS with ICG n=250, Standard Care n=250)	
Planned Number of Sites	Up to 20	
Intervention Duration	12–24-hour time period between the administration of intravenous (IV) ICG and the start of the resection.	
Follow Up Duration	Patients will be followed up for 12 months following the procedure, with the option of an additional time point at 5 years if further funding is secured.	
Planned Trial Period	58 months (7 months setup, 30 months of recruitment, 3 months surgical delay, 12 months follow up, 6 months final report/dissemination)	
	Objectives	**Outcome Measures**
Primary	To determine whether FGS using ICG reduces the UPM rate compared to the current standard of care (SoC).	The UPM rate, defined as the percentage of patients with an unexpected positive margin, will be compared between the two treatment arms.
Secondary	Complications	Rates of intra- and postoperative complications as recorded in the clinical notes.
	Length of index operation	Difference in length of operation in minutes.

	Length of inpatient stay	Difference in length of inpatient stay in days.
	Perceived impact of intervention in surgical decision making	The percentage of operations in which the surgeon stated FGS changed their planned resection.
	Local recurrence (LR) rate	The difference in rate of local recurrence at 12 months.
	Regional/distal recurrence rate	The difference in rate of regional/distal recurrence at 12 months.
	Overall survival (OS) rate	The difference in rate of overall survival at 12 months.
	Identify rates of adjuvant and neo-adjuvant therapies	Rates of adjuvant and neo-adjuvant therapies as per clinical notes.
	Quality of life (QoL) measurements	The difference in scores on EQ-5D-5L/EQ-5D-Y.
	Recovery following resection	The difference in scores on the Toronto extremity salvage score (TESS)/ Paediatric Toronto extremity salvage score (pTESS)
Exploratory Objectives	To determine the role of ICG in the pathological margin assessment of resected sarcoma specimens	Fluorescence microscopy to assess extracellular and cellular ICG spatial orientation in tumour tissue versus surrounding normal tissues
	To develop artificial intelligence algorithms to improve the interpretation of fluorescence	Fluorescence mapping will be performed on all images taken during the trial and correlated with the pathological margin assessments
Intervention	**Standard care:** the resection will be performed as per the operating surgeon's standard procedure, without fluorescence guidance. This will be based on the relevant pre-operative imaging. **FGS with ICG:** Patients will be administered 1mg/kg of ICG intravenously (IV) 12-24 hours pre-operatively. Only the Stryker SPY-PHI near-infrared camera will be used for the intervention arm.	

	Stryker will work with the SarcoSIGHT team to bring cameras to sites that do not already have one available. If camera from a different manufacturer is used this will be a protocol deviation and the participant will need to be withdrawn from the trial. The Stryker SPY-PHI camera will be used to image periodically throughout the resection; any decision to make changes to the planned operative procedure as a result of this will be at the discretion of the operating surgeon.

Contents

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
CRN	Clinical Research Network
DMC	Data Monitoring Committee
DPA	Data Protection Act
FGS	Fluorescence Guided Surgery
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
GDPR	General Data Protection Regulations
HRA	Health Research Authority
HTA	Human Tissue Authority
HTAct	Human Tissue Act
ICF	Informed Consent Form
ICG	Indocyanine Green
ID	Identification code
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trials Number
ITT	Intention to Treat
IV	Intravenous
LR	Local Recurrence
MAR	Missing at Random

NCTU	Newcastle Clinical Trials Unit
NENC	North East and North Cumbria
NIHR	National Institute for Health and Care Research
NIHR CRN	National Institute for Health and Care Research Clinical Research Network Efficacy and Mechanism Evaluation
NIHR EME	National Institute for Health and Care Research Efficacy and Mechanism Evaluation
NHS	National Health Service
NUTH	The Newcastle upon Tyne Hospitals NHS Foundation Trust (Lead site)
OS	Overall Survival
PI	Principal Investigator
PIS	Participant Information Sheet
PP	per Protocol Population
pTESS	Paediatric Toronto Extremity Salvage Score
QC	Quality Control
QoL	Quality of Life
R&D	Research & Development
RACT	Risk Assessment Categorisation Tool
RCT	Randomised Control Trial
REC	Research Ethics Committee
RSE	Related Serious Event
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SoC	Standard of Care
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics

TESS	Toronto Extremity Salvage Score
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UPM	Unexpected Positive Margin
URSE	Unexpected Related Serious Event
USB	Universal Serial Bus (*N.B. apparatus for transferring data, usually referred to as a 'USB stick')
USM	Urgent Safety Measure

1. BACKGROUND

Sarcomas are rare malignant tumours accounting for 1% of all cancers with around 5300 patients diagnosed annually in the UK.[1] Sarcomas affect a wide age range of the population with the highest incidence of some sub-types in children and the overall prevalence affecting a younger age group than other cancers. The median age of patients at diagnosis is 56 years, which is lower than most other cancer types.[2] Sarcomas are heterogenous with at least 70 different sub-types arising from soft tissues and bone, most commonly occurring in the extremities.[3] Survival is poor, with a five year survival rate of approximately 65%, reducing to 50% in higher grade sarcomas, with little improvement in survival outcomes over the last 30 years.[4] Surgery is the mainstay of treatment, with the goal being complete resection of the tumour whilst leaving as much normal tissue as possible to maximise functional outcomes. Many of these operations are complex, often requiring input from surgeons from more than one specialty and resulting in major functional compromise to the patient due to the volumes of tissue that need to be resected. Currently, surgeons use pre-operative imaging to plan the resection of the tumour and operate by eye and palpation but have no other intra-operative options to guide the surgery. This often leads to uncertainty during the operation, particularly in difficult anatomical locations, as to where the tumour ends and normal tissue begins.[5]

1.1. MARGINS

The most important prognostic factor surgically is the resection margin. Margins are assessed post-operatively by a pathologist. A clear margin, known as a negative margin, means that the resected tumour is surrounded by normal tissue, which implies there is no disease left in the patient. By contrast, a positive margin means that sarcoma cells are visible at the edge of the tumour. Positive margins can be either macroscopic or microscopic; macroscopic mean that tumour can be seen at the edge of the specimen with the naked eye, whereas microscopic means a microscope is required to visualise tumour cells at the margin. Sarcoma margins are most commonly defined by the R classification system,[6] with both positive macroscopic and microscopic margins associated with increased local recurrence (LR) and decreased overall survival (OS).[7-9] Positive margins may be classified as either planned when they are expected pre-operatively due to the preservation of a critical structure as seen on images or unexpected, when the pathologist reports the presence of tumour at the margin despite the surgeon having intended to remove it in its entirety. The latter classification, known as an unexpected positive margin (UPM) is associated with worse oncological outcomes.[8,9] The histological grade assigned to the tumour is also important, with high grade tumours not only carrying a worse prognosis, but also being associated with higher rates of positive margins. [10]

The reported positive margin rate varies widely in the literature, with quoted values between 8% and 29.9%.[7,8,10-14] The largest of these studies reported the percentage of patients who have a UPM on histopathological assessment of the resection specimen (known as UPM rate) as 9% (n=2217).[8] Analysis of 224 patients with intermediate to high grade bone and soft tissue sarcomas at the North of England Bone and Soft Tissue Tumour Service in a five-year period between 2010 and 2015 found a UPM rate of 13.8% (n=224). Figure 1 includes data from this cohort and demonstrates that positive margins significantly correlate with a higher rate of local recurrence, reduced time to local recurrence and poorer overall survival.

Given that the positive margin rate remains considerable and has such clear associations with poor oncological outcomes, there is a requirement for technologies that will assist sarcoma surgeons to achieve lower positive margins rates, thereby improving both short- and long-term outcomes for patients.

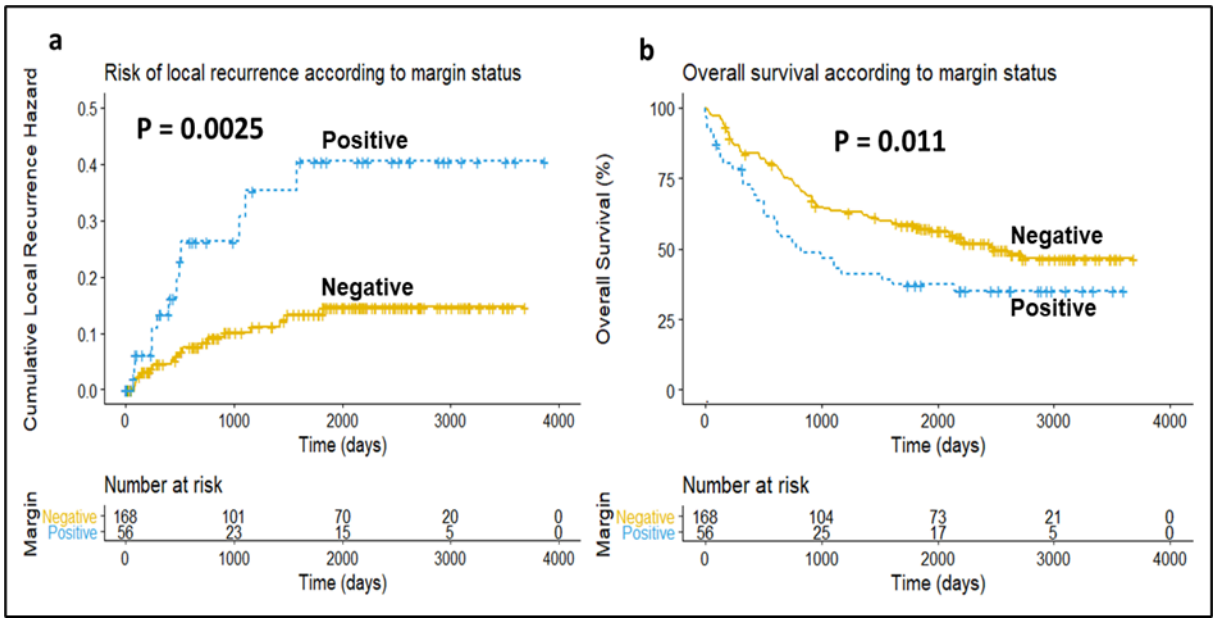


Figure 1. a) Kaplan Meier plot of cumulative risk of local recurrence according to margin status. b) Kaplan Meier plot of overall survival

For the purposes of this trial, we will be renaming one of the margin statuses from that usually described in the literature. Whilst the terms 'negative' and 'unexpected positive' margins will be kept unchanged, 'planned positive' margins in the context of preserving a critical structure will instead be changed to 'acceptable close' or 'acceptable positive' margins, depending on the final histology. We felt the term 'planned positive' could be misleading to trial participants and stakeholders and could erroneously imply that patients were being included in the trial unnecessarily. See Section 2 for a complete outline of the margin terminology that will be used in this trial.

1.2. FLUORESCENCE GUIDED SURGERY

The most promising technology to reduce the positive margin rate in sarcoma surgery is fluorescence guided surgery (FGS). FGS is an established method, which involves the administration of fluorescent dye followed by visualisation of the fluorescence intensity with a near-infrared (NIR) camera intra-operatively.

Indocyanine green (ICG) is a widely used fluorescent dye, which has been used for decades to assess cardiac output[15] and hepatic function.[16] ICG is approved by the European Medicines Agency (EMA). It is considered safe and has rarely been linked to adverse events in other settings.[17,18] More recently it has been used for tissue perfusion assessments intra-operatively in other surgical fields, allowing surgeons to more accurately identify healthy tissue from tissue that is diseased or poorly vascularised.[19,20]

The most recent application of FGS using ICG is for tumour margin identification of solid cancers.[21-25] ICG is administered intravenously prior to the operation, allowing time for uptake of the dye into the tumour and clearance from the peritumoral tissue. The half-life of ICG in the circulation is short at approximately three minutes[26] but is long enough to allow for tumour uptake. The first reports of FGS using ICG for tumour margin identification are from liver cancer resection, demonstrating efficacy for tumour margin identification following administration of an intravenous ICG dose of 0.5mg/kg at least one day prior to surgery.[27] In 2019 surgeons and researchers at the North of England Bone and Soft Tissue Tumour Service, conducted and published the world's first case series demonstrating that most intermediate- to high-grade sarcomas fluoresce, and in some procedures this fluorescence was used to guide surgery.[28] During the same time period, an early phase trial assessing the feasibility of FGS using ICG for tumour margin identification in all types of paediatric solid cancers was conducted at St Jude Children's Research Hospital in Tennessee USA.[29] The methodology for this trial was intravenous injection of ICG at 1.5mg/kg one day prior to surgery. The results demonstrated that from the 65 cases recruited, 29 were sarcomas, of which 27 demonstrated fluorescence with high sensitivity (88%) for tumour identification, although specificity was lower (77%). This trial complements our case series findings that tumour margin identification using FGS could have the potential to greatly improve patient outcomes but requires further investigation. It also validates our proposed methodology of ICG dosage, timing, and route of administration. There were no adverse events related to the ICG administration indicating it is a safe and valid technique.[28,29]

1.3. Indocyanine Green

The Newcastle Sarcoma Research Group has undertaken extensive preclinical and histopathological studies to investigate the mechanisms of uptake of ICG in sarcoma cell lines and patient tumour samples.[30] We have demonstrated that a key mechanism of ICG cellular uptake is clathrin mediated endocytosis (Figure 2). Figure 2 illustrates how ICG uptake is diminished following treatment with the inhibitor. We have also demonstrated that cellular proliferation rate correlates with fluorescence intensity, indicating that cancer cells with the shortest doubling time take up more ICG. Furthermore, we have been successful at visualising ICG at the cellular level at the margins of sarcoma specimens (Figure 3). There are two factors involved in ICG uptake in sarcoma, 1) is the enhanced permeability and retention effect in the tumour, which allows the ICG to accumulate in areas of haemorrhage and necrosis within in the tumour and 2) is the sarcoma cell uptake via the endocytosis mechanism. This has explained our case series finding that higher grade sarcomas take up more ICG[28] because of the combination of increased cellular activity and the presence of areas of haemorrhage and necrosis in these tumours.

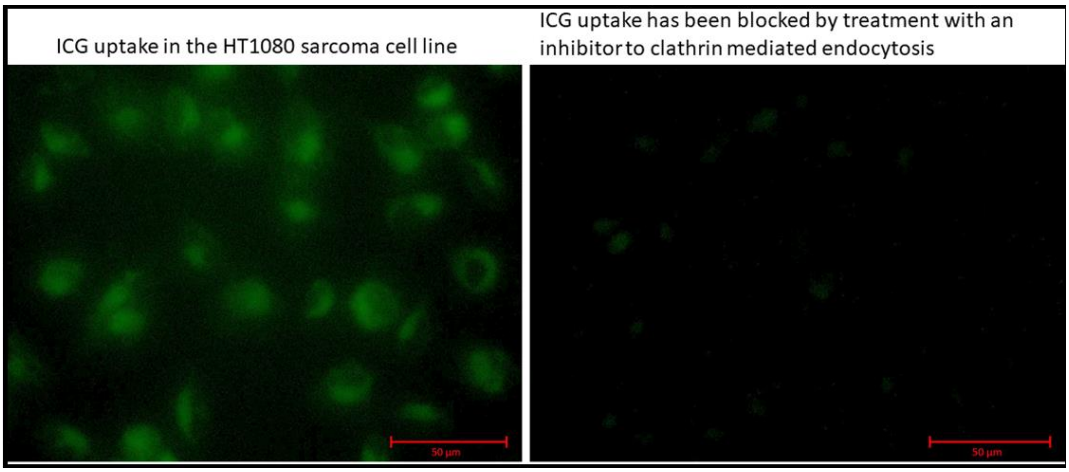


Figure 2. *In vitro* Fluorescence microscopy images of ICG uptake in HT1080 sarcoma cells with and without the presence of an inhibitor of clathrin mediated endocytosis. The image on the right demonstrates that ICG uptake is diminished following treatment with the inhibitor.

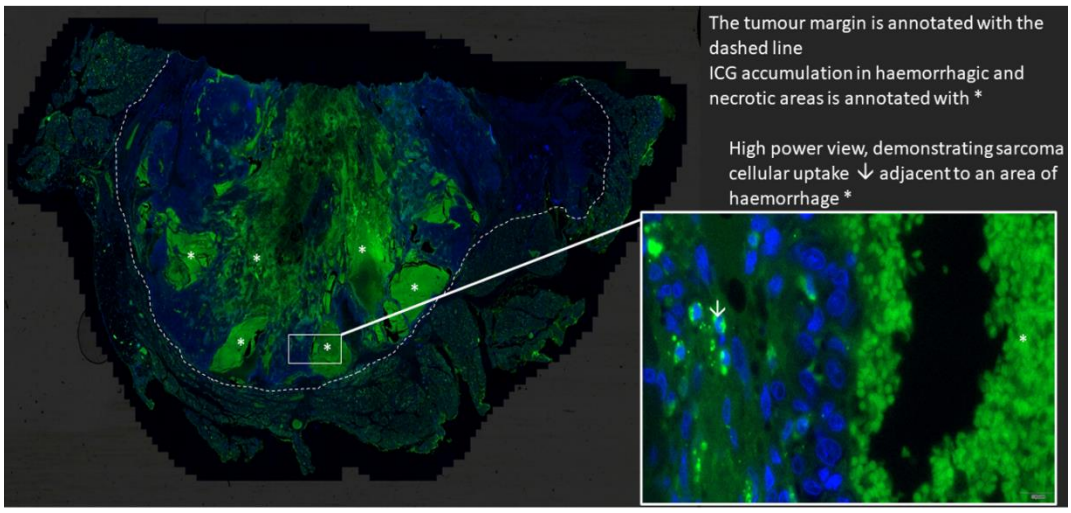


Figure 3. Histopathology slide imaged using an NIR microscope from an osteosarcoma patient who received ICG for FGS. ICG accumulation is demonstrated in areas of haemorrhage and there is uptake into osteosarcoma cells. A white dashed line has been marked, which demonstrates the margin.

2. RATIONALE

During evidence synthesis for this trial, we found some variability in reported negative versus positive margins rates from centres around the UK. Furthermore, the definitions of margin status can be misleading. Statements in the literature regarding 'planned positive margins' are not helpful. Expert sarcoma surgeons understand the concept that a 'planned positive margin' is usually acceptable in terms of not undertaking a further resection because the margin is at a critical structure such as a major nerve or blood vessel. However, a 'planned positive margin' is not always positive- it may be negative, albeit close to the tumour. Therefore, for the purposes of this trial the terminology for margins post-surgery based on the pathology report of the resected specimen will be as follows:

- Negative margin: with the closest distance to the tumour measured in millimetres
- Unexpected positive macroscopic margin
- Unexpected positive microscopic margin
- Acceptable positive macroscopic margin
- Acceptable positive microscopic margin

In November 2020, the National Cancer Research Institute Sarcoma Research Group (UK) reached a consensus that trials in surgery are a priority to assess new technologies that may improve surgical performance.[31] To date there have been no randomised sarcoma trials with a surgical intervention. Recently published case series data suggested that the use of FGS using ICG for high grade sarcoma resection may reduce the UPM rate.[32] As such, undertaking this randomised control trial of FGS in sarcoma is well-timed. This trial will be exclusive to sarcoma patients, but inclusive of all ages and subtypes except low grade sarcomas because they do not fluoresce. Delivery of this trial will provide the sarcoma community, nationally and internationally, with crucial insights into performing more effective surgery, the opportunity to develop improvements to the current standard surgical and histopathological techniques, answers to questions on functional outcomes and the optimal timing of adjuvant therapies. With UPMs clearly linked to inferior oncological outcomes in the short term (e.g., decreased time to local recurrence) and longer term (e.g., poorer overall survival), this randomised surgical trial represents an important step towards improving these outcomes for sarcoma patients.

2.1. Risk Assessment

The NCTU Risk Assessment Categorisation Tool was completed signed off by the relevant authority. The Newcastle University Data Protection Impact Assessment was completed and signed off by the relevant authority.

The trial is categorised as low risk.

3. OBJECTIVES AND OUTCOME MEASURES

3.1. Objectives

3.1.1. Primary Objectives

To determine whether there is a reduction in the unexpected positive margin rate (UPM) in patients receiving fluorescence-guided surgery (FGS) using indocyanine green (ICG) compared to standard of care (SoC).

3.1.2. Secondary Objective(s)

To determine the effect of FGS using ICG compared to the SoC over a 12-month period on the following areas:

- Complications
- Length of index operation
- Length of inpatient stay

- Perceived impact of the intervention on surgical decision and change in surgeon preference throughout the trial
- Local recurrence
- Regional/distal recurrence
- Rates of adjuvant and neo-adjuvant therapies.
- Overall survival
- Quality of life (QoL)
- Recovery following resection

3.1.3. Exploratory Objectives

There are two exploratory objectives: 1) to determine the role of ICG in the pathological margin assessment of resected sarcoma specimens whilst improving understanding of the cellular mechanisms of FGS using ICG and 2) to develop fluorescence mapping algorithms to improve interpretation of fluorescence.

3.2. Outcome Measures

3.2.1. Primary outcome measure

The primary outcome measure is the UPM rate. The margin status of each tumour will be taken from the pathology report for each patient enrolled in the trial. This will be recorded at six months post-surgery and will be classified according to the R classification system.[6] Positive margins will be classified as the visualisation of tumour cells at the inked margin and will then be classified as acceptable or unexpected. The UPM rate for each arm will be defined by calculating the percentage of patients in that arm with a UPM on histopathological assessment of the resection specimen. If a negative margin is recorded, the size of the closest margin should be recorded in millimetres.

In this trial, the estimand aims to answer the research question: does fluorescence-guided surgery using indocyanine green (ICG) reduce the UPM compared to the standard of care (SoC)?

Table 1. Primary estimand attributes.

Estimand attribute	Description
Treatment	Fluorescence guided surgery (FGS) using indocyanine green (ICG) compared to standard of care (SoC) surgery
Population	Patients of all ages with a histologically confirmed diagnosis of intermediate to high grade bone or soft tissue sarcoma suitable for curative resection
Variable (outcome)	Unexpected positive margin (UPM)
Intercurrent events	There are no anticipated intercurrent events with respect to the observation of the primary outcome
Population-level summary measure	The difference in the UPM rates between the FGS group and SoC group

3.2.2. Secondary outcome measures

Complications
Complications may be split into both intraoperative and postoperative. Intraoperative complications could include:

- Blood loss requiring transfusion
- Inadvertent damage to nerves
- Inadvertent damage to tendons/ligaments
- Inadvertent damage to bony structures
- Myocardial infarction
- Stroke
- Other events determined by the PI to be a complication of the surgery

Postoperative complications could include:

- Wound infection
- Wound dehiscence
- Seroma
- Flap complications
- Deep vein thrombosis
- Pulmonary embolism
- Myocardial infarction
- Stroke
- Other events determined by the PI to be a complication of the surgery

Post operative complications will be graded by the PI at site using the Calvien Dindo Classifications of Surgical Complications [33]. The scale indicates the severity of the complication and the therapy needed to correct the complication. The scale consists of 7 grades of severity (I, II, IIIa, IIIb, IVa, IVb, V). See Section 16.4, Appendix 4 for a table of the Calvien Dindo Classifications of Surgical Complications.

Length of index operation
Length of index operation and length of inpatient stay will be measured in minutes and days, respectively.

Recurrence
Local/regional/distal recurrence is defined as the recurrence of sarcoma at the site of primary resection (local) or at a site other than that of the primary tumour, including distal metastasis. Investigations must be tailored to circumstances but where possible should include histological confirmation.

Therapies
Adjuvant and neo-adjuvant therapy rates will include radiation therapy and/or chemotherapy. The type and frequency of therapy will be recorded.

Survival Status

Overall survival (OS) is defined as death due to any cause. Where possible, the specific cause of death should be documented, allowing the calculation of disease specific survival.

Quality of Life

The QoL will be assessed using the EQ-5D-5L questionnaire that assesses quality of life in participants, evaluating the following five domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression.[34] The questionnaire is two pages in length and the '5L' refers to the five levels of severity on which responses to questions can be scored, ranging from 'no problems' to 'extreme problems'. This is used to produce a score to rate the participant's health related QoL. The EQ-5D-5L is not appropriate for participants younger than 16 years old because they may struggle to understand some of the questions. As such, the EQ-5D-Y questionnaire will be used for participants 8 to 15 years of age. This is based upon the same principles, but with the questions and answers phrased in a more child-friendly manner.[35] For participants 4 to 7 years old a proxy version of the EQ-5D-Y questionnaire will be completed by the participant's parent or main carer. For participants who may struggle to complete the EQ-5D-5L/EQ-5D-Y/proxy EQ-5D-Y on their own the questionnaire can be completed with the assistance of a research nurse in person or via telephone.

Recovery Following Resection

After undergoing surgical resection for sarcomas of the extremity, participants are often left with an element of functional impairment in the affected limb due to the extent of the resection required. There are separate questionnaires called the Toronto Extremity Salvage Score (TESS) for adult participants undergoing surgery on the upper and lower extremities. In these questionnaires participants are asked to rate their ability to perform several tasks, rating them from 'impossible' to 'not at all difficult', as well as overall rating of how disabled they feel they are. There is a paediatric version of the TESS (pTESS) which will be used for participants aged 7 to 17.9 years.

Surgical Impact and Decision Making

Immediately following the surgical procedure, a surgical case report form (eCRF/CRF) will be completed by the surgeon who performed the surgery. This eCRF/CRF will record if there was the potential for an acceptable close/positive margin due to the preservation of a critical structure, the length of the operation and any intra-operative complications. If the participant was randomised to the intervention arm, the eCRF/CRF will also record whether the surgeon felt this influenced their operative decision making.

3.2.3. Exploratory Outcome Measures

Fluorescence microscopy

Samples will be imaged using fluorescence microscopy and ICG cellular locality assessed. Assessment of the margin with fluorescence microscopy will be compared to assessment of the margin with standard H&E staining and fluorescence intensity correlated to the margin reports.

Fluorescence mapping

Intra-operative fluorescence mapping images will be analysed and fluorescence intensity of areas of interest measured. The fluorescence intensity will be correlated to the histopathology reports to create fluorescence mapping algorithms to improve the interpretation of the fluorescence intra-operatively in subsequent studies.

3.3. Outcomes

3.3.1. Primary Outcome

The pathology report of the resected tumour will provide a margin status. The percentage of patients with unexpected positive margins will be calculated for each group to give the unexpected positive margin rate. This will then be compared between the two groups as described in section 10.2.1.

3.3.2. Secondary Outcomes

The length of index operation and type and frequency of adjuvant and neo-adjuvant therapies will be compared between FGS and SoC groups on the day of operation. Local/regional/distal recurrence and difference in overall survival will be compared between the two groups at the 12-month time point. The number of complications and length of hospital inpatient stay will be compared between the two groups at day of operation and at 1-, 6- and 12-month follow-up time points. EQ-5D-5L/EQ-5D-Y scores and TESS/pTESS scores we be compared between the two groups at every follow up time point.

3.3.3. Exploratory Outcome

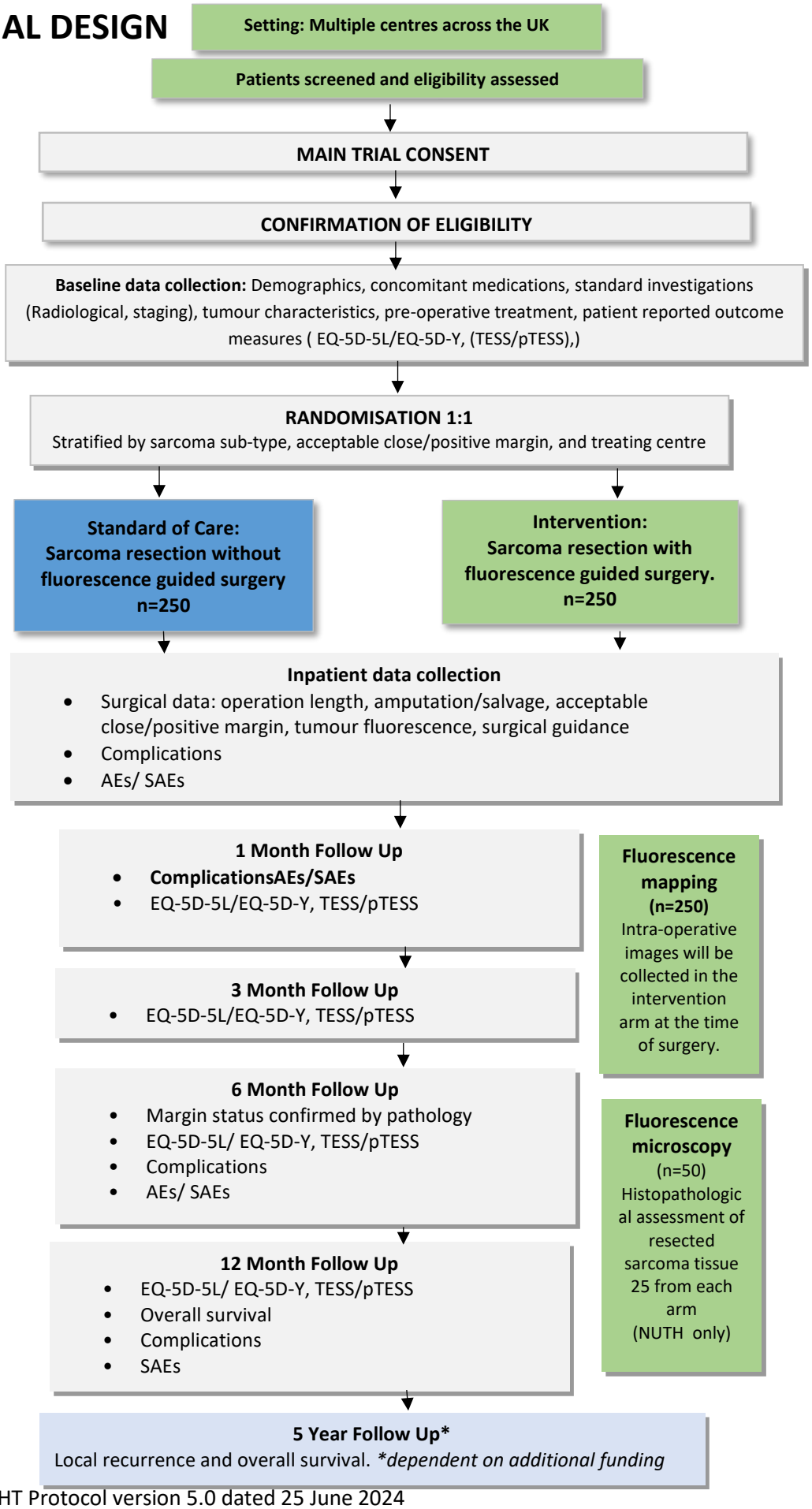
Fluorescence microscopy

The outcome will be the identification of ICG cellular locality within resected sarcoma specimens, as well as preliminary evaluation of the role of fluorescence microscopy in the pathological assessment of sarcoma specimens in identifying the tumour margin.

Fluorescence mapping

The outcome will be the production of fluorescence mapping algorithms to aid surgeons with the assessment of fluorescence intra-operatively.

4. TRIAL DESIGN



4.1. Main Trial

SarcoSIGHT is a prospective, two-arm, open-label, UK multi-centre randomised control trial comparing FGS using ICG to the standard of care (no fluorescence guidance) to determine the effect on the UPM rate. Surgical decision-making and interpretation of fluorescence guidance will be at the discretion of the operating surgeon.

Five hundred patients will be randomised on a 1:1 basis to either FGS using ICG or standard of care. Randomisation will be stratified by acceptable close/positive margin, sarcoma subtype and treating centre. Patients and the operating surgeons and senior statistician will not be blinded, but histopathology staff and the senior trial statistician will be blinded, allowing blinded measurement and analysis of the primary outcome.

Patients will be followed up for a total of 12 months, with clinic visits as per the standard of care at the treating institution post-operatively, with eCRF/CRFs completed at baseline, day of resection, 1-, 6- and 12-months. EQ-5D-5L/ EQ-5D-Y questionnaires and, if appropriate, TESS/pTESS questionnaires will be completed preoperatively and then at 1-, 3-, 6- and 12-months post-operatively by the patients.

4.2. Fluorescence Microscopy

As part of the exploratory analysis, a sub-sample of participants recruited at the lead centre in Newcastle will be selected to have their tissue sample further analysed at the NovoPath Newcastle MRC Node. This sub-sample will include 25 tissue samples from participants who received ICG, along with 25 tissue samples from participants who did not receive ICG acting as controls. Fluorescence microscopy will be used to assess cellular ICG locality versus standard haematoxylin and eosin staining and against our validated sarcoma specific marker matrix metalloproteinase-1 (MT1-MMP), for determining margin depths and distances.

4.3. Fluorescence Mapping

As part of the exploratory analysis a sub-sample of still images from the Stryker SPY-PHI camera used to guide surgery in the FGS arm will be collected from all 250 patients and sent to our collaborators at University College Dublin. Our collaborators will use the images to perform fluorescence mapping and will correlate fluorescence intensity to histopathological margin reports. This information will be used to develop artificial intelligence algorithms with the goal of improving the interpretation of fluorescence intra-operatively.

5. TRIAL SETTING

This trial will be conducted in up to 20 specialist sarcoma centres in the UK. These are:

- The Newcastle upon Tyne Hospitals NHS Foundation Trust (lead centre)
- Manchester University Hospitals NHS Foundation Trust
- The Royal Orthopaedic Hospital NHS Foundation Trust
- The Royal National Orthopaedic Hospital NHS Trust
- Oxford University Hospitals NHS Foundation Trust
- The Leeds Teaching Hospitals NHS Trust
- Sheffield Teaching Hospitals NHS Foundation Trust

- Nottingham University Hospitals NHS Trust
- North Bristol NHS Trust
- University Hospitals Plymouth NHS Trust
- Royal Devon University Healthcare NHS Foundation Trust
- The Royal Marsden NHS Foundation Trust
- The Christie NHS Foundation Trust
- Swansea Bay University Health Board
- Lancashire Teaching Hospitals NHS Foundation Trust
- NHS Grampian (Aberdeen)
- NHS Lothian (Edinburgh)
- NHS Greater Glasgow and Clyde (Glasgow)
- Belfast Health and Social Care Trust
- South Eastern Health and Social Care Trust (Belfast)

6. ELIGIBILITY CRITERIA

Patients of all ages with a histological diagnosis of intermediate- to high-grade sarcoma will be eligible for the trial. A patient's eligibility for the trial will be initially discussed during local hospital multidisciplinary team (MDT) meetings. Those patients who are interested in taking part in the trial will have their eligibility status assessed by a clinician who has been formally delegated the responsibility by the Principal Investigator (PI). Eligibility assessment and outcome will be documented in the patient's clinical notes. Please note eligibility assessment is not the same as confirmation of eligibility. Confirmation of eligibility for the trial can only take place after consent to take part in the trial has been given by the patient or the patient's parent or carer.

To be eligible for the trial, **all the following inclusion and exclusion criteria must apply**.

6.1. Inclusion Criteria

1. Patients of any age
2. Capacity to provide written, informed consent (or legal guardian if <16 years of age)
3. Histologically confirmed diagnosis of intermediate to high grade sarcoma
4. Amenable to surgical resection as a part of curative intent for the patient

6.2. Exclusion Criteria

1. Due for surgery with palliative intent
2. Recurrent tumours
3. Intracranial, retroperitoneal, and visceral anatomical locations
4. A woman of child bearing potential* who is currently pregnant (as confirmed by urine pregnancy test)
5. A woman who is currently breastfeeding
6. Known allergy to ICG, iodine, iodine dyes or shellfish.
7. Unable to provide written, informed consent
8. Patients with hyper-thyroidism or autonomic thyroid adenomas
9. Premature infants/neonates with exchange transfusion indication due to hyperbilirubinemia

*For the purposes of this trial a woman is considered of child-bearing potential (i.e., fertile) following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

6.3. Other Clinical Considerations

- There is an increased risk of adverse reactions to ICG in patients with increased renal insufficiency. As such ICG should only be administered after a careful risk/ benefit assessment
- The Iodine content of ICG can interfere with thyroid tests performed before or after administration of the dye. Therefore, radio-active iodine uptake studies should not be performed at least one week following ICG injection. Clinicians should discuss this with their patients
- Although, anaphylactoid and anaphylactic reactions are very rare with ICG (<1/10,000), a clinician should still be available to respond in an emergency.

NB: Enrolling a patient onto the trial who does not meet the inclusion/exclusion criteria is considered a protocol waiver and is in breach of Regulation 29 (SI 2004/1031) of the Medicines for Human Use (Clinical Trials) Regulations 2004**. PROTOCOL WAIVERS ARE NOT PERMITTED as is UK legislation.**

7. TRIAL PROCEDURES

7.1. Recruitment

7.1.1. Patient Identification

Most patients treated for sarcoma in the UK are referred to one of the 20 specialist centres in the UK; this may occur either before or after diagnosis, but before definitive surgery. Suitable patients should therefore be identified at a multidisciplinary team (MDT) meeting as soon as a histological diagnosis is made and prior to definitive surgery.

7.1.2. Screening

All patients discussed at the MDT with a new histologically confirmed diagnosis of intermediate to high grade sarcoma should be screened for eligibility. Because the time between diagnosis and surgery tends to be relatively short (i.e., a couple of weeks), it is important that screening is achieved in a timely manner so that patients can be approached, given time to consider the trial and then consented to the trial prior to their surgery.

An electronic screening log on the Sealed Envelope Red Pill database will capture pseudonymised screening data including the following:

- Date of screening

- Age of patient at screening
- Sex of patient at birth
- Histologically confirmed diagnosis of intermediate to high grade sarcoma
- Tumour Amenable to curative surgical resection
- Patient interest in taking part (Yes/ No)
    - If no, reason given
- Patient information sheet (PIS)given
- Date PIS given

7.1.3. Initial Approach

Following diagnosis, the treating clinician, ideally along with a member of the department research team, should make the initial approach to the patient during the clinic appointment. The clinician will explain that the patient may be eligible for the trial, as well as the basic premise of the trial. If the patient is interested in knowing more about the trial, they will be given the patient information sheet (PIS) to take away and advised to read and discuss with family members, friends or their GP before making a decision. If it is not possible to approach the patient during a clinic appointment the appropriate PIS(s) can be sent in the post. However, this approach to patient should only take place after a patient has been told of their diagnosis by their clinician. Patients will be given the opportunity to ask more questions and discuss the trial further at their pre-operative visit, prior to consenting. Patients must be given a minimum of 24 hours between the initial approach and consenting to allow sufficient time for them to consider whether the participating in the trial is right for them.

7.2. Consent

After allowing a minimum of 24 hours to consider their involvement in the trial, the trial can be rediscussed with the patient. If the patient is willing to participate and their eligibility criteria have not changed, written informed consent for the trial may be given. Consent will be taken by the PI, or, where delegated by the PI, other appropriately trained (clinical and non-clinical) site staff. A surgical consent form, separate from the trial, will also be completed by the patient as part of standard of care procedures.

The original signed consent form must be filed in the investigator site file (ISF), a copy must also be filed in the patient's medical records, and another copy given to the patient for their records. Copies of the version of PIS given to the patient must be filed in the ISF and the patient's medical notes. Patients should be asked at every follow up assessment if they would like to continue in the trial. This discussion and response should be recorded in the patient's clinical records.

In the case of protocol amendments or if information becomes available which may affect participants' willingness to continue in the trial, it may be necessary to re-consent participants on an updated consent form after necessary regulatory approvals are obtained. If more than 28 days lapses between consent and surgery, patients will need to be reconsented and eligibility reconfirmed. If a child who was consented into the trial by a parent/carer turns 16 during their time in the trial, they will need to be reconsented using the 16+ consent form.

7.3. Randomisation

Confirmation of eligibility will be taken by a clinician delegated to the trial. Confirmation of eligibility must be documented in the patient's medical records. Only after eligibility is confirmed can the patient be randomised into one of the trial arms. If the patient has not met all the eligibility criteria, they cannot continue in the trial and will be entered as 'withdrawn' on the database.

Eligible participants will be randomised in a 1:1 ratio to standard surgery vs FGS using ICG, stratified by acceptable close/positive margin, sarcoma sub-type and treating centre. Randomisation will be conducted by a delegated and trained member of the research team at each site using the Sealed Envelope system, which is a central, secure, 24-hour web-based randomisation system with concealed allocation. Randomisation should take place as soon as possible after consent and no more than one week after a participant has consented. The allocation sequence will be computer-generated, using a permuted block design. Block size will not be disclosed, to ensure concealment.

For those patients who have been randomised to having FGS using ICG, clinicians should document in the medical notes the dosage requirement of ICG as 1mg/kg. This will ensure the prescribing doctor on the day of ICG administration, who may not be delegated to the trial, prescribes the correct dosage. Under dosing a patient is likely to happen if dosage is done using the SmPC as a guide. **Under dosing a patient could mean the tumour does not adequately fluoresce.**

Randomisation system URL: https://www.sealedenvelope.com/access/

This system is available 24 hours a day, seven days a week. If the online system is not accessible at the site, NCTU can liaise with Sealed Envelope Support to investigate the cause. Site staff should contact NCTU Database Support during normal working hours:

Email: nctu.database.support@newcastle.ac.uk
Telephone: 0191 208 8211

7.4. Blinding

Participants and operating team will not be blinded to the trial intervention due to the requirement for the administration of ICG to the participant and the use of extra technology during the procedure by the operating team. The histopathology staff and senior trial statistician will be blinded and unaware of the surgery type to allow for blinded measurement and analysis of primary outcome. The trial statistician will not be blinded.

7.5. Baseline Assessments & Data

Pre-operative investigations will be as per the treating institution's standard practice but must include biopsy for histological confirmation prior to confirmation of eligibility for the trial. Typically, baseline assessments will also involve local magnetic resonance imaging (MRI) of the primary tumour, as well as computed tomography (CT) scanning of the chest, abdomen, and pelvis to stage the patient. An assessment of fitness for surgery pre-operatively would also be pertinent.

Baseline information will include:

- Age
- Sex at birth
- Treating centre
- Stage of tumour
- Histological subtype
- Tumour size
- Tumour grade
- Tumour depth relative to fascia
- Tumour anatomical location
- Planned operation (limb salvage vs. amputation)
- Concomitant medications - specifically tyrosine kinase inhibitors and immunotherapy
- Height and weight
- Ethnicity
- Smoking status
- Diagnosis of diabetes
- Postcode
- EQ-5D-5L/EQ-5D-Y questionnaire
- TESS/pTESS questionnaire

7.6. Trial Assessments

Length of index operation and perceived impact of the intervention on surgical decision making will be collected immediately post-surgery. Follow up visits at 1, 3, 6 and 12 months will follow standard of care follow up clinic visits and will be conducted as per standard of care at the treating centre. These visits will include the completion of questionnaires (EQ-5D-5L/EQ-5D-Y and if applicable TESS/pTESS). At the day of surgery, 1-, 6- and 12-months visits serious adverse events and complications, including extra clinic visits and hospital admissions since previous assessment, will be collected. At day of surgery, 1- and 6 month visits adverse events will be collected. At the 6- month visits, information will be collected on margin status, local/regional/distal recurrence, length of inpatient stay, adverse events, and serious adverse events. At the 12-month visit overall survival will be collected. Surgeon preference for the different methods of surgery will be collected throughout the trial.

7.7. Schedule of Events

☐**Procedure/Assessment**	**Screening**	**Baseline** Up to 28 days before resection	**12-24 hours before start of resection**	**Day of Resection**	**1 month** @ + / – 7 days	**3 months**@ + / – 7 days	**6 months** + / – 14 days	**12 months** + / – 14 days
Initial eligibility assessment	X							
Informed consent		X						
Urine pregnancy test£		X						
Eligibility confirmation^		X						
Demographics&		X						
Staging information		X						
Tumour subtype/size/location		X						
Concomitant medications		X						
EQ-5D #		X			X@	X@	X@	X@
TESS $		X			X@	X@	X@	X@
Randomisation		X						
ICG intravenous injection (*If randomised to intervention arm)			X*					
Resection				X				
Surgeon eCRF %				X				
Margin status							X	
Complications				X	X		X	X
Adverse Events (see section 9.2)				X	X		X	
SAE Check				X	X		X	X
Survival status								X
Cause of death if applicable								X

£ A woman is considered of child-bearing potential (i.e., fertile) following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

^Eligibility confirmation must be documented in the medical notes by a clinician delegated to the trial. This can be done one of three ways by the clinician delegated to the trial: 1) as a written entry in the patient's medical notes which includes confirmation that all criteria have been met and references the current version and date of the protocol, 2) by completing the eligibility checklist on paper and filing it in the patient's medical notes or 3) by completing the eligibility checklist on the Sealed Envelope database.
&Demographic data will be collected at screening and baseline and will include age, sex at birth, height, weight, ethnicity, smoking status, diagnosis of diabetes and postcode.
@ To be conducted remotely via ePRO
# - EQ-5D -Age on day of baseline assessments – continue using the same version of the questionnaire throughout the trial.
FOR 16+: EQ-5D-5L **FOR 8–15-year-olds**: EQ-5D-Y **FOR 4-7-year-olds:** EQ-5D-Y Proxy version 1 completed by a parent or carer.
$ TESS – Only to be completed for participants undergoing extremity limb salvation resections. There is a separate **upper** and **lower** extremity questionnaire, please use the correct one for the participant. **FOR 7–17-year-olds**: there is a paediatric version of each questionnaire to be used.
% Can be completed directly on database by surgeon or can be completed on paper and transcribed.

7.8. Withdrawal Criteria

Participants have the right to withdraw from the trial at any time without giving a reason. Investigator sites should try to ascertain the reason for withdrawal and document this reason within the eCRF and participants' medical notes.

The Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Symptomatic deterioration
- Participant withdrawal of consent
- Significant protocol deviation or non-compliance
- Investigator's discretion that it is in the best interest of the participant to withdraw
- An adverse event that requires discontinuation of the trial intervention or renders the participant unable to continue in the trial
- Termination of the clinical trial by the sponsor

Participants who withdraw from the trial prior to intervention will be replaced, whilst those withdrawing following the procedure will not.

7.9. Storage and Analysis of Samples

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the Data Protection Act (DPA). Biological samples collected from participants as part of this trial will be transported, stored, accessed, and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and 2006 Human Tissue (Scotland) Act.

7.10. End of Trial

The end of the trial will be defined as completion of data collection after LPLV or completion of the transfer and analysis of samples and near infrared images associated with exploratory objectives, whichever comes latest.

8. TRIAL INTERVENTION

8.1. Name and Description of Interventions

8.1.1. Control arm

Patients randomised to standard of care will undergo surgical resection without pre-operative ICG administration or intra-operative fluorescence guidance. This will be performed as per the preferences of the operating surgeon but will be planned based on pre-operative imaging of the tumour, with appropriate skin mark-up.

8.1.2. Intervention arm

The protocol for FGS using ICG is summarised in Section 16.3, Appendix 3. Prior to being able to recruit patients to the trial, surgeons must have completed training in FGS using ICG (see Section 8.4. Surgeon Training) Patients randomised to the FGS arm will be administered 1mg/kg ICG intravenously 12-24 hours prior to the procedure. Reconstitution of ICG will be done in line with the manufacturer's patient information leaflet provided in the pack. However, a dosing reference table document will be provided to site to aid staff in reconstitution of the drug. The skin will be marked based on pre-operative imaging as per standard of care and prepped using chlorhexidine to reduce background fluorescence from iodine-based solutions. The surgeon should then proceed with their planned resection as per standard of care. As they proceed with the resection they must use the Stryker SPY-PHI camera to image areas of interest; interpretation of images and any influence on operative decision making is at the discretion of the operating surgeon. If fluorescence changes the procedure at any time, further images (in white light, overlay mode, SPY fluorescence mode and colour segmented overlay mode) should be taken as appropriate. Following the resection, both the resected specimen and the wound bed should be imaged (in all modes as above), and a decision made regarding the requirement for the removal of any further tissue. Following reconstruction and closure, the operating surgeon must complete the trial Surgery CRF, stating whether, and if so at what point and how, fluorescence guided the procedure and changed decision making, as well as documenting the images taken. These pseudonymised images will be removed from the camera at site immediately after surgery by a member of the research team. Access to images will be restricted to delegated members of staff only and the location of the images will be monitored via a tracking log. It is essential that no personal identifiable data is included with the images on the camera stack or while in transfer. The images will be sent to the database team at NCTU and later transferred to collaborators at University College Dublin for exploratory analysis.

8.1.3. Margins

Following surgeries in the control arm and the intervention arm, the surgeon must confirm on the CRF and in the participant's clinical notes whether they believe the full tumour was resected or whether they are expecting an acceptable close/positive margin. Resected specimens should then be sent to the pathology laboratory to undergo trimming and margin assessment by a histopathologist as per standard practice. Once the pathology report is available and discussed at the MDT, the surgical team will complete a pathology specific trial report form, documenting the margin status. In the case of a UPM, plans for re-excision should also be documented, and the results of this also added to the 6- month follow up eCRF. It is important to note that a single case may have both acceptable and unexpected positive margins if a positive margin is present in an area not adjacent to the preserved structure; in this case it should be recorded as a UPM.

8.2. Schedule & Modifications

Patients will be discussed in the MDT and operated on at a time deemed appropriate by the treating team. No modification to the imaging protocol described in section 8.1.2. is allowed, although the interpretation of this imaging, and any decisions made as such, is at the discretion of the operating surgeon.

8.2.1. Known Risks of ICG

- Anaphylaxis (especially in those with iodine or shellfish allergies)
- Urticaria
- Cough
- Nausea

- Itch
- Rash

8.2.2. Known Risks of Surgery

- Pain
- Infection
- Bleeding
- Scar
- Stiffness
- Deep Vein Thrombosis/ Pulmonary Embolism
- Damage to nerves/vessels
- Recurrence
- Need for further procedure(s)
- Further risks specific to the planned procedure

8.3. Concomitant Medications & Therapies

Participation in another interventional clinical trial in the 30 days preceding surgery **are** permitted.

All neo-adjuvant treatment regimens **are** permitted.

The class of concomitant medications that need to be reported are:

- Tyrosine kinase inhibitors
- Immunotherapy

8.4. Surgeon Training

Surgeons taking part in this trial will have different levels of experience in performing surgeries with FGS using ICG. As such there is a flexible approach to training surgeons which will include the following elements. To complete their training each surgeon will, at a minimum, have:

1. Participated in at least one FGS using ICG alongside a trained surgeon.
2. Watched the training video for FGS using ICG which includes a step-by-step recording of the procedure.
3. Completed a questionnaire to confirm they feel competent to perform FGS using ICG.

Surgeons who do not feel competent in the procedure can repeat steps 1 and 2 until they are satisfied, they can effectively perform FGS using ICG.

9. SAFETY REPORTING

9.1. Definitions

Table 2. Terms and definitions used in safety reporting for a non-CTIMP trial.

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant, including occurrences which are not necessarily caused by or related to the intervention under study.
Adverse Reaction (AR)	An untoward or unintended response in a participant to which is related to the intervention under study i.e., that a causal relationship between the trial intervention and an AE is at least a reasonable possibility and the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial intervention qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening* • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect • Other important medical events that jeopardise the participant or require intervention to prevent one of the above consequences * - life-threatening refers to an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Related Serious Event (RSE)	Any serious event that is classed in nature as serious, where there is evidence to suggest there is a causal relationship between the event and the trial procedures/intervention.
Unexpected Related Serious Event (URSE)	Any SAE that is classed in nature as serious, where there is evidence to suggest there is a causal relationship between the event and the trial procedures/intervention, where the event is unexpected.

9.2. Recording AEs

AEs will be recorded for surgery and ICG intravenous injection separately.

AEs related to surgery will not be collected directly via an AE form but instead via the complications eCRF. Complications will be recorded from day of surgery, at the 1-, 6- and 12-month follow-up visit. Any complications that are collected that also meet the criteria for an SAE, the site will complete an SAE form. Complications will be graded using the Calvien-Dindo Classifications of Surgical Complications system [33] (see Section 16.4, Appendix 4).

Only AEs related or possibly related to the ICG intravenous injection (that take place within 72 hours of ICG intravenous injection) will be recorded in the AE eCRF. The research team will check the records on the day of resection, 1- and 6-month follow-up to check these have been reported if they have occurred. If the AE meets the criteria for an SAE, the site will complete an SAE form.

All SAEs will be recorded in the database.

9.3. Recording and Reporting SAEs

For each SAE the following information will be collected:

- Full details in medical terms and case description
- Event duration (start and end dates, if applicable)
- Severity of event
- Narrative of event
- Seriousness criteria
- Intervention information
- Causality in the opinion of the investigator
- Whether the event is considered expected or unexpected in accordance with the approved Reference Safety Information if a causal relationship is suspected. Rationale for expectedness assessment
- Action taken
- Outcome

9.3.1. SAE form criteria

All SAEs that occur from the administration of the ICG to patient (for intervention arm patients) or from surgery (for control and intervention arm patients) up until the 12 month follow up visit must be documented by research staff at site. SAEs should be documented on an SAE form and emailed to the NCTU (i.e., **nctu.sarcosight.sae@nhs.net**) via secure email or other secure email as per site policies as soon as it is available or at least within 24 hours of the information becoming available. SAEs will be logged by the NCTU team on the trial safety database and will be given a unique SAE number. The reporting site will be sent confirmation of receipt of the SAE from NCTU. Events will be followed up until the event has resolved or a final outcome has been reached.

See Section 16.1, Appendix 1 for the Safety Reporting Diagram.

9.4. Recording and Reporting Related Serious Events (RSE)

All RSEs occurring as determined using the SAE Report Diagram (see Section 16.1, Appendix 1) occurring from the administration of the ICG to patient up until the 12 month follow up must be reported to the NCTU on an SAE form.

The assessment of expectedness as indicated on the SAE form will be reviewed and confirmed by the CI against the details of the SmPC for ICG.

Unexpected Related Serious Events (URSE) must be reported no later than 15 calendar days after the NCTU has first knowledge of the event to the NHS REC. Any relevant follow-up information should be sought and reported as soon as possible after the initial report.

As soon as a site suspects that a RSE may be a URSE they must contact the CI, sponsor representative and the trial manager immediately. The reporting timeframe starts at day 0 when the NCTU is in receipt of a minimum set of information:

- Sponsor trial reference and trial name (sponsor reference)
- Patient trial number and date of birth
- Name of intervention
- Date of notification of the event
- Medical description of the event
- Date and time of the onset of the event (including event end date if applicable)
- Causality assessment
- Seriousness of the event, particularly if life threatening or fatal
- An identifiable reporter (e.g., Principal Investigator)

This information must be provided via secure email (e.g., NHS.net). The site is expected to fully cooperate with the NCTU in order that a full and detailed report can be submitted to the NHS REC within the required timelines.

PIs will be informed of all URSEs by the NCTU.

9.5. Responsibilities

9.5.1. Principal Investigator (or delegated person at site)

- Checking for relevant AEs and complications at four time points: day of resection,1-, 6- and, in the case of complications only 12- month follow up visits.
- Completing complications eCRF at four time points: day of resection and 1-, 6- and 12- month follow up visits.
- Using medical judgement in assigning seriousness and causality and providing an opinion on expectedness of events.
- Ensuring that all SAEs and RSEs, including URSEs, are recorded and reported to the NCTU within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
- Ensuring that relevant AEs and complications are recorded in line with the requirements of the protocol.

9.5.2. Chief Investigator

- Clinical oversight of the safety of trial participants, including an ongoing review of the risk/benefit.
- Using medical judgement in assigning seriousness, causality, and expectedness of SAEs where it has not been possible to obtain local medical assessment.
- Using medical judgement in assigning expectedness to RSEs.
- Immediate review of all URSEs.
- Review of reported SAEs in accordance with the trial risk assessment and protocol.

9.5.3. NCTU

- Assessment of expectedness of any URSEs
- Expedited reporting of URSEs to the Research Ethics Committee (REC) within required timelines
- Notification of all investigator sites of any URSE that occurs
- Responsibility to perform delegated duties as documents in the Safety Reporting Plan with regards to recording, notifying, and reporting safety information.

9.5.4. Trial Steering Committee

The Trial Steering Committee (TSC) will oversee and supervise the progress of the trial to ensure it is being conducted in accordance with applicable guidance and regulations. The TSC will provide overall supervision of the trial on behalf of the Sponsor and Funder. The TSC will help to design the trial and discuss and endorse substantial amendments as appropriate. The TSC will advise on trial progress and ensure regulatory approvals are obtained in line with protocol requirements to maximise the chance of completing the trial in the proposed timescale.

The TSC will consist of an independent Chair and one other clinician with research experience. It will also include the trial CI, trial statistician and lay representatives. Sponsor, Funder and Trial Management Group (TMG) representatives can attend TSCs but do not have voting rights. Other observers may be asked to join TSC meetings for specific discussions where their expertise could be useful.

9.5.5. Data Monitoring Committee

The Data Monitoring Committee (DMC) is independent of the Sponsor and the trial team and is composed of independent members. Although members of these groups can attend DMC meetings. The DMC provides oversight of ethical and safety considerations of patients during the trial.

The DMC has authority to request un-blinded comparative data by treatment group including accumulating safety and outcome data. It is the responsibility of the DMC to ensure participants are not exposed to any excess risk by taking part in the trial. The DMC will report recommendations directly to the TSC and or TMG.

The DMC will consist of at least three independent experienced specialists including at least one clinician who is experienced in the clinical area and at least one statistician.

9.6. Notification of Deaths

Death of a participant taking part in the trial is classified as an SAE and as such will follow the reporting procedures outlined above in section 9.3. All deaths will be reported to the DMC.

9.7. Reporting Urgent Safety Measures

An Urgent Safety Measure (USM) is an action that the Sponsor or an Investigator may take to protect the subjects of a trial against any immediate hazard to their health or safety. An USM may also be used when one arm of a trial shows a clear efficacy over other arm(s) of a trial, and it would be unethical for patients in other arm(s) of the trial to continue on the protocol. If a site identifies an USM they must report this to the NCTU within 24 hours of becoming aware of the USM. This will be done by completing the required sections of the USM Reporting From and emailing it via secure email to the trial manager at the NCTU. The NCTU must inform the NHS REC within 24 hours of the USM being reported and follow up with the REC in writing within three days further outlining the USM and plan of action in accordance with the NCTU's standard operating procedures.

10. STATISTICAL CONSIDERATIONS

10.1. Analysis Population

Patients of all ages with a histologically confirmed diagnosis of intermediate- to high-grade bone or soft tissue sarcoma suitable for curative resection who meet the inclusion and exclusion criteria.

10.2. Statistical Analyses

10.2.1. Analysis of the Primary Outcome Measure

Once margin data has been collected for 250 patients, an interim analysis will be conducted using a Barnard test to calculate a one-sided p-value, with non-binding stopping rules for both overwhelming efficacy and futility with regards to the primary outcome. The final analysis will then occur either after the interim analysis (if the trial stops) with all participants that have been recruited by the time the interim decision is made, or after the full target enrolment is reached (if the trial does not stop). All participants will be followed up for all outcomes, with final data cleaning and database lock occurring after that time. The primary analysis will be based on the Intention-to-treat (ITT) population with all participants included in the group they were randomised to. We do not anticipate a significant number of noncompliance and cross-over issues. If we encounter any protocol deviations or violations related to the treatment allocation, we will also define a per-protocol (PP) population for secondary analyses, in which participants for whom the allocated strategy was not followed will be excluded. All analyses will be described in a Statistical Analysis Plan (SAP) that will be finalised prior to trial statisticians receiving any unblinded data. For the analysis of the primary outcome, UPM rate, we will use a logistic regression that adjusts for key covariates including stratification factors used in the randomisation. A Wald test and a 95% confidence interval will be extracted from this model. A two-sided p-value will be found from the Wald test, although ICG will only be recommended as efficacious if the one-sided p-value for superiority of ICG is <0.0227.
Secondary analyses for the primary outcome will include the per-protocol population and a method that can account for the learning effect such as the hierarchical model approach proposed by Cook

et al [36]. Since the UPM rate is measured quickly after randomisation, we anticipate very low missing outcome data. Nevertheless, if this rate is higher than 5%, we will investigate applying a suitable missing at random (MAR) approach such as multiple imputation.

10.2.2. Analysis of Secondary Outcome Measures

Descriptive analysis will be performed to analyse the nature of the data. Secondary outcomes with binary/multiple measures will be analysed with logistic regression. Survival analysis like the Kaplan-Meier statistic will be used to find the overall survival in each arm. For variables with continuous measure, appropriate regression models will be used. Appropriate Statistical tests depending on the nature of the variables (whether qualitative or quantitative) will be used for comparison between the two treatment arms.

10.2.3. Interim Analyses and Criteria for the Premature Termination of the Trial

A non-binding interim analysis will be conducted after margin data for 50% of the target sample size has been achieved. The trial can be terminated if the unexpected positive margin rate is larger in the ICG arm.

10.2.4. Subgroup Analyses

Subgroup analysis will be performed and will be stratified by planned positive margin, sarcoma sub type and treating centre.

10.3. Sample Size Calculations

The reported positive margin rate varies widely in the literature, with quoted values between 8% and 29.9%.[7,8,10-14] The largest scale paper to have reported UPM rates reported a rate of 9% (n=2217),[8] although this also included patients with low grade sarcomas, which have lower rates of positive margins,[10] as well as including patients from as far back as 1989, limiting its relevance. Analysis of all patients meeting the trial inclusion criteria between 2010 and 2015 from the North of England Bone and Soft Tissue Tumour Service found an UPM rate of 13.8% (n=224). Data received from two other centres in Europe reported a UPM of 16.3% (n=306). As 13.8% lies between these values, and contains only patients eligible for the trial, this was used for the power calculation. The only published margin rates for patients with intermediate to high grade sarcoma undergoing FGS with ICG is our recent case series, which reported a UPM of 5.1%.[32]
As such, figures of 13.8% for the SoC group and 5.1% for the ICG were used for the power calculation. This calculated a sample size of 480 to demonstrate a reduction in the UPM from 13.8% to 5%, at a 1-sided 5% level of significance, with a power of 90%. The sample size of 480 was then inflated to 500 to allow for a modest dropout rate, given the early assessment of the primary outcome.

11. DATA HANDLING

11.1. Data Collection Tools and Source Document Identification

Data will be collected using Case Report Forms (CRFs), electronic Case Report Forms (eCRFs) self-report questionnaires, histopathology reports and information taken from participant clinical notes. These will be considered the source documents containing the source data. Data will be transcribed by site staff with delegated responsibility from these source documents onto the eCRFs onto the clinical data management software package, Sealed Envelope Red Pill. Data transferred from site to the secure validated database by remote access will be secure and encrypted.

11.2. Data Handling and Record Keeping

Data will be handled, stored, and transferred in accordance with the UK General Data Protection Regulations (GDPR) 2018.

Some patient personal identifiable data will be stored for screening and recruitment tracking purposes. These files will be stored at the recruiting NHS site in a shared trial folder on a secure server. The files will be password protected. Paper copies which include personal identifiable data, such as consent forms and enrolment logs, will be stored in locked rooms and in participants clinical notes. Access to this information will be strictly limited to members of the research team who have been delegated the responsibility.

All participants will be given a unique trial identification (ID) code. All participant trial related data will be connected to this unique ID. Trial data will be collected on paper CRFs and eCRFs. A copy of data collected on paper CRFs will be filed in the participant's clinical notes. Data contained in paper CRFs, data relevant to the trial that has been recorded in participant's clinical notes will be transferred to the secure encrypted password protected trial clinical data management system, Sealed Envelope Red Pill. Pseudonymised image data from the Stryker SPY-PHI camera used during surgery for those participants in the intervention arm will be, where possible, stored at site in a shared trial folder on the NHS server. Pseudonymised images from the Stryker SPY-PHI camera will be uploaded by NHS sites to Newcastle University Secure File Drop Off Service to be retrieved by Database Managers at NCTU. If NHS sites are not able to store pseudonymised images on a shared trial folder on the NHS server or are not able transfer the images to the NTCU via a secure file drop off service, NHS sites can send an encrypted USB stick with the pseudonymised images to NCTU via tracked and recorded delivery. The pseudonymised images will be stored in secure research folders on the Newcastle University server.

11.3. Access to Data

Staff involved in the conduct of the trial, including PIs, recruiting site's delegated trial team, TMG members will have access to trial site files. Access to a participant's personal identifiable data will be strictly limited to delegated members of the research team at the recruiting NHS site. Access to the pseudonymised trial data will be limited to the relevant members of the recruiting site as well as the Sponsor, NCTU, and trial oversight committees such as the DMC and TSC.

Access to Sealed Envelope Red Pill database will be password protected and restricted to a user's particular role and will be limited to a site's PI and their delegated research team members. NCTU's trial management team will have monitor role access to the trial's Red Pill database for all sites for monitoring purposes.

Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor or its designee, regulatory authorities, the DMC or the TSC.

The PI and trial staff involved in this trial may not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial.

Written agreement from the Sponsor or designee must be obtained before the disclosure of any confidential information to other parties.

Until publication of the trial results, access to the full dataset will be restricted to the Trial Management Group and to authors of the publication.

11.4. Archiving

Data will be archived in accordance with the sponsor archiving guidelines and any relevant UK regulations as applicable. All trial related documents will be archived in line with the Sponsor's archiving Standard Operating Procedures (SOPs) after the Research Ethics Committee (REC) has received the end of trial notification. The trial documents will be archived after authorisation from the Sponsor. Authorisation will be requested from the Sponsor to destroy the trial documents at the end of the archiving period.

12. MONITORING, AUDIT & INSPECTION

Monitoring will be undertaken to ensure the trial is being conducted, recorded, and reported in accordance with the protocol, standard operating procedures, GCP and regulatory requirements.

The nature and extent of monitoring, as outlined in the trial monitoring plan, was based on the trial risk assessment categorisation tool (RACT). The monitoring plan was agreed with the Sponsor. The monitoring plan will include off-site, central, on-site monitoring.

The trial may be subject to audit by representatives of the Sponsor or inspection by the Human Tissue Authority (HTA). Each investigator site will permit trial-related monitoring, audits and regulatory inspection including access to all essential and source data relating to the trial.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1. Research Ethics Committee Review and Reports

The NCTU, on behalf of the sponsor, will obtain a favourable ethical opinion from an NHS Research Ethics Committee (REC) prior to the start of the trial. All parties will conduct the trial in accordance with this ethical opinion.

The NCTU will notify the REC of all required substantial amendments to the trial and those non-substantial amendments that result in a change to trial documentation. (e.g., protocol or patient information sheet). Substantial amendments that require a REC favourable opinion will not be implemented until this REC favourable opinion is obtained. The NCTU will notify the REC of any serious breaches of GCP or the protocol, urgent safety measures or URSEs that occur during the trial.

The NCTU will notify the REC of the early termination or end of trial in accordance with the required timelines.

13.2. Peer Review

The trial has been through peer review during the NIHR Efficacy and Mechanism Evaluation (EME) Programme application process. At both stage 1 and stage 2 of the application, the trial was

reviewed in depth by a board of clinical academics from the funding committee. Following the decision to fund, several additional queries were raised which have since been answered.

13.3. Public and Patient Involvement

The SarcoSIGHT trial team has liaised with a representative and founder of the charity Sarcoma UK, who is a sarcoma patient and who is a member of the National Cancer Research Institute Sarcoma Research Group. The representative has extensive links to patient groups, including in Europe. Throughout the design phase the PPI representative attended the trial design meetings, offering valuable insight and ensuring public and patient involvement aspects were not overlooked. The CI has formed a patient group and during the design phase of the trial, multiple patient and public involvement sessions were conducted. During these, the concept of the trial was explained to the patients, and they were given time to ask any questions and ensure that they understood. The concept was well received and feedback from these sessions were incorporated this into the trial design.

13.4. Regulatory Compliance

The trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research. Before any site can enrol patients into the trial, that site must be in receipt of Health Research Authority (HRA) Approval, have issued capacity and capability confirmation and been issued the greenlight to recruitment by Sponsor.

13.5. Protocol Compliance

It is the responsibility of the CI to ensure the trial is run in accordance with the protocol. This task will be delegated to the trial management team in the NCTU, but the CI will retain overall responsibility.

Protocol deviations, violations or breaches are departures from the approved protocol. Deviations from the protocol occur in trials and the majority of these are not considered serious breaches. A deviation is a change or departure from the trial protocol, GCP and or other applicable regulations that does not result in harm to the participants or significantly affect the scientific value of the reported results of the trial. Deviations should be documented on the Deviation Tracking Log (which is provided as part of the Investigator Site File). NCTU will ask sites to provide copies of their Deviation Tracking Log at intervals throughout the trial and before any monitoring visits. If no deviations have been identified during a particular interval, sites are required to send an email to the NCTU to confirm this.

Some deviations may be considered a violation. A violation is a consistent variation in practice from the trial protocol, GCP and or other applicable regulations that could potentially impact on participants' rights/safety or affect the scientific value or outcome of the trial. Systematic deviation from the trial protocol and or GCP/applicable regulations is also considered a violation.

Sites must notify the NCTU trial team within three working days of becoming aware of the violation.

13.5.1. Notification of Serious Breaches to GCP and/or the Protocol

A serious breach is a violation that has been judged to likely affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial

The sponsor must be notified immediately of any incident that may be classified as a serious breach. The NCTU will notify the NHS REC within 7 calendar days of determining that a serious breach has occurred in accordance with the NCTU SOP.

13.6. Data Protection and Patient Confidentiality

All investigators and trial staff must comply with the requirements of the UK General Data Protection Regulation (GDPR) 2018, with regards to the collection, storage, processing and disclosure of personal information and access to data will be limited to the minimum number of individuals necessary for quality control audit and analysis.

13.7. Indemnity

The sponsor will provide indemnity in the event trial participants suffer negligent harm due to the management of the trial. The indemnity will be provided under the NHS and Newcastle University Indemnity schemes.

The sponsor will provide indemnity in the event trial participants suffer negligent harm due to the design of the trial. The indemnity will be provided under the NHS indemnity scheme.

The trial sites will provide indemnity if a trial participant suffers negligent harm due to the conduct of the trial at their site under the NHS indemnity arrangements for clinical negligence claims in the NHS. NHS Organisations must ensure that site staff without substantive NHS contracts hold honorary contracts to ensure they can access patients and are covered under the NHS indemnity arrangements.

The substantial employers of the protocol authors will provide indemnity if trial participants suffer negligent harm due to the design of the trial.

This is a non-commercial trial and there are no arrangements for non-negligent compensation.

13.8. Amendments

It is the responsibility of the Sponsor to determine if an amendment is substantial or not and trial procedures must not be changed without the mutual agreement of the CI, Sponsor, Trial Management Group and Trial Steering Committee.

Substantial amendments will be submitted to the REC and will not be implemented until REC approval is in place. It is the responsibility of the NCTU to submit substantial amendments. Non-substantial amendments may be made at any time with a record of the amendment held in the Trial Master File. Any non-substantial amendment that requires an update to the trial documentation will be submitted to the NHS REC for acknowledgement of the revised version of the document. It is the responsibility of the NCTU to submit non-substantial amendments.

Substantial amendments and those non-substantial amendments which may impact sites will be submitted to the relevant NHS R&D Departments for notification to determine if the amendment affects the NHS R&D confirmation of capacity and capability for that site. Amendment documentation will be provided to sites by the NCTU.

13.9. Post-Trial Care

Participants in both arms of the trial will receive the current post-operative standard of care for sarcoma patients.

13.10. Access to the Final Trial Dataset

The data set will be the property of the CI. Any requests to access the final trial dataset may be considered under the NCTU data sharing policy.

The final data set will be stored electronically in secure files on the Newcastle University server. Initially the final trial data set will be accessible only to the trial statisticians. Upon completion of the final analysis the final trial data set will be made available to the CI.

Following completion of the analysis, relevant copies of the data will be sent to the PI at each site. It will remain the responsibility of the PI to ensure that the site-specific data set is securely stored and retained for the specified arching period of five years.

13.11. NIHR Portfolio Adoption

The trial will have NIHR Clinical Research Network (CRN) support with the lead CRN support from North-East and North Cumbria (NENC).

14. DISSEMINATION POLICY

The data will be the property of the CI, Co-Is and PIs. Publication will be the responsibility of the CI and will follow published guidelines. Authorship of all publications will be on a named individual authorship basis. For each publication all individuals who fulfil the authorship definition for the publishing journal or site will be included as individually named authors. Authorship order will be decided by the CI. Any disputes regarding authorship will be adjudicated by the TSC. Non-author contributors will be acknowledged as part of the 'SarcoSIGHT trial group'.

To safeguard the integrity of the main trial, reports of explanatory or sub-studies will not be submitted for publication without prior agreement from the Trial Management Group and Trial Steering Committee.

It is planned to publish this trial in peer-reviewed articles and to present data at national and international meetings. Results of the trial will also be reported to the Sponsor, Funder and REC within one year of the end of trial. All manuscripts, abstracts or other modes of presentation will be reviewed by the Funder prior to submission. Trial participants will not be identified from any trial report.

Trial participants will be informed about the trial results at the end of the trial via a lay summary sent to them in the post or by email.

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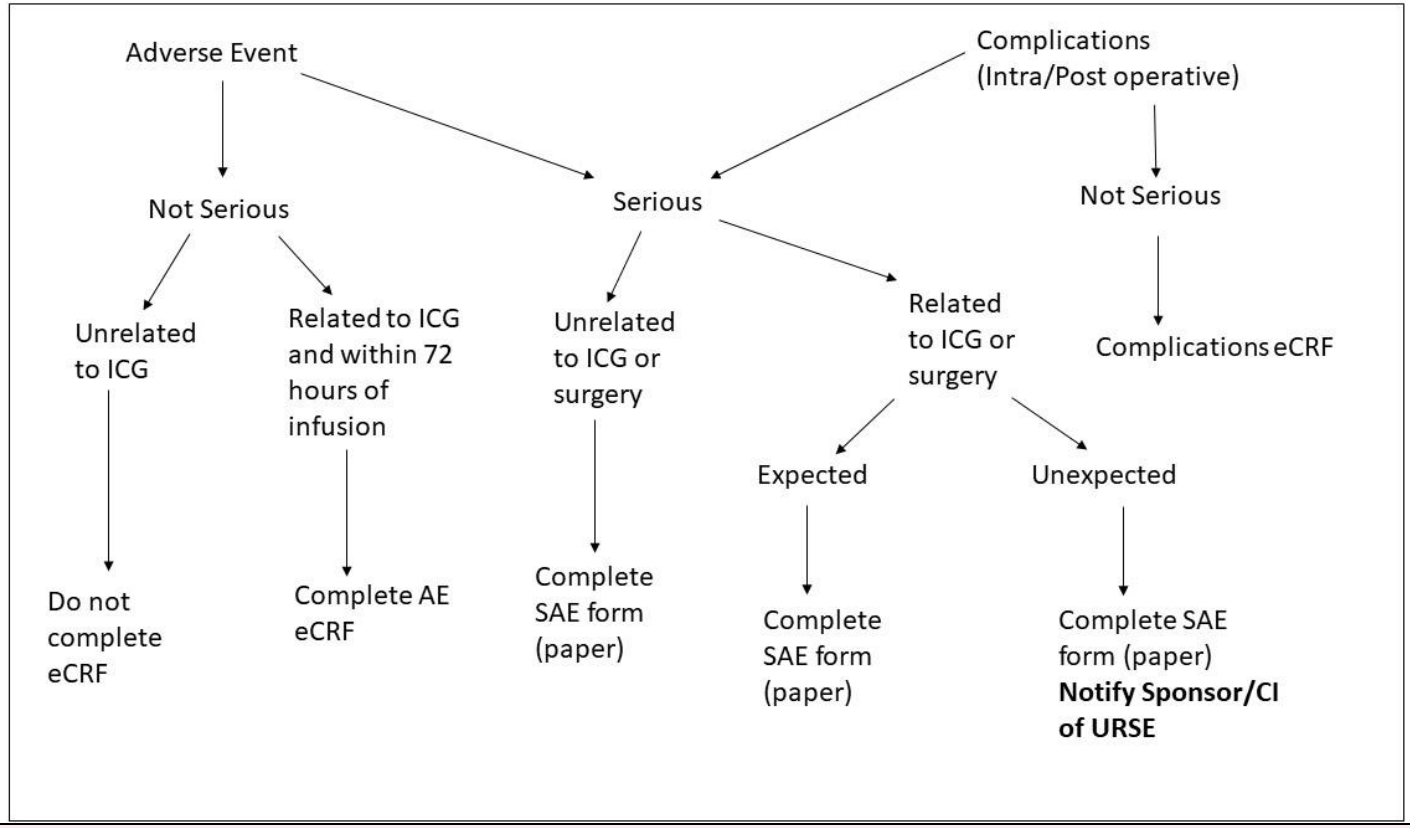
16 APPENDICES

16.1 Appendix 1 - Safety Reporting Diagram



Contact details for reporting SAEs and URSEs **Please send SAE form(s) via email to nctu.sarcosight.sae@nhs.net**

16.2 Appendix 2 – Amendment History

Amendment Number	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
SA01	3.0	16 JAN 2024	Stephanie Clutterbuck	<u>Section 8.4- Surgeon Training</u> • Section added to outline the updated training requirements for surgeons to complete before being signed off on delegation log. <u>Section 7.2 - Consent</u> • Update to who can take consent from patients. Consent can now be taken by the PI, or where delegated by the PI, other appropriately trained clinical and non-clinical site staff. • Update to clarify that participants will need to be re-consented to the trial in two instances: 1) if more than 28 days lapses between consent and surgery and 2) if a child participant turns 16 during their time on the trial (they will need to be re-consented as an adult). <u>Section 6.1 Inclusion Criteria</u> • Update to wording of inclusion criterion 4 to make it clearer which patients can be included in the trial. <u>Section 6.2 Exclusion Criteria</u> • Update to wording of exclusion criterion 1 to make it clearer which patients should be excluded from the trial. <u>Schedule of Events</u>

				• A minor error was amended to specify that it was participants who are 4-7 years old (not 3-7 years old) who would complete the EQ-5D-Y proxy version 1. <u>Site number</u> • Updated wording from 'up to 21 sites' to 'up to 22 sites' to account for an additional site included in the trial. <u>Typographical errors</u> • Typographical errors amended throughout the document.
NSA 01	4.0	13 MAR 2024	Stephanie Clutterbuck	<u>Section 8.1.2. Intervention Arm</u> • Wording added to clarify that due to the SPY-PHI camera software an unencrypted USB stick will need to be used to obtain images from the SPY-PHI camera. • Wording added to specify that it is a delegated member of the research team who will be responsible for collecting the images. • Wording added to remind sites this will be monitored via the USB and image data tracking log. <u>Section 11.2 Data Handling and Record Keeping</u> • Wording added to clarify that if the USB stick needs to be posted to the Newcastle Clinical Trials Unit via recorded delivery with the images, it will need to first be encrypted. The USB stick, with images should not leave the NHS site unencrypted. <u>Section 16.3 Appendix 3 (yellow text box)</u> • The word encrypted changed to unencrypted in the yellow text box.

SA02	5.0	25 JUN 2024	Stephanie Clutterbuck	Section 3.3.2 Secondary Outcomes • Additional check for complications at 1 month Section 4 Trial Design (Flow Chart) • 'Eligibility confirmed' changed to 'eligibility assessed' • 'Confirmation of Eligibility' moved to after 'Main Trial Consent' • Additional check for complications and AEs/SAEs added to 1 month follow up visit Section 5 Trial Setting • Change of reference from 22 sites to 20 sites taking part in the trial • Removal of two sites no longer taking part in the trial Section 6 Eligibility Criteria • Clarification that eligibility assessment is not the same confirmation of eligibility. The latter can only take place after consent to take part in the trial has been given by the patient/ parent or carer. Section 6.2 Exclusion Criteria • Clarification that it is women of child bearing potential (not all women) who will be pregnancy tested (as well as a definition of the criteria for child bearing potential) • Addition of patients with hyper-thyroidism or autonomic thyroid adenomas • Addition of premature infants/neonates with exchange transfusion indication due to hyperbilirubinemia

				Section 6.3 Other Clinical Considerations • This section was added to align with the SmPC for indocyanine green more closely. It highlights to clinicians specific factors to consider when deciding to invite a patient into the trial, including: - Patients with renal insufficiency - Timing of radio-active iodine uptake studies - The importance of a clinician being available to respond in the very rare instance of anaphylactoid and anaphylactic reactions. Section 7.1.3 Initial Approach • Additional option to send the PIS in the post to the patient if it is not possible to approach them during the clinic visit. Section 7.2 Consent • Removal of sentence outlining that eligibility assessment needs to be taken by a clinician delegated to the trial. This was moved to Section 7.3 Randomisation. Section 7.3 Randomisation • Addition of sentence outlining that eligibility assessment needs to be taken by a clinician delegated to the trial. • Clarification that only once a patient is confirmed eligible can they be randomised • Clarification that patients deemed ineligible at this stage will be entered as 'withdrawn' on the database • Additional advice to clinicians to document in the patient's medical notes the dosage requirements of ICG as 1mg/kg.

				Section 7.6 Trial Assessments • Addition of complication, adverse events and serious adverse events at 1 month follow up visit Section 7.7 Schedule of Events • Update to Schedule of Events in line with changes throughout protocol. Section 8.1.2 Intervention arm • Clarification that reconstitution of indocyanine green will be done in line with the manufacturer's patient information leaflet provided in the pack. • Reference to a dosing reference table • Wording regarding the transfer of images to the NCTU from sites has been updated to allow for a variety of methods of transfer Section 9.2 Recording AEs • 'ICG infusion' changed to 'ICG injection' • Additional check for AEs at 1 month follow up visit Section 9.5.1 Principal Investigator (or delegated person at site) • Additional check for complications, adverse events, serious adverse events at 1 month follow up visit. 11.2 Data Handling and Record Keeping • Clarification that pseudonymised image data will be stores at site on a shared folder on the NHS server where this is possible

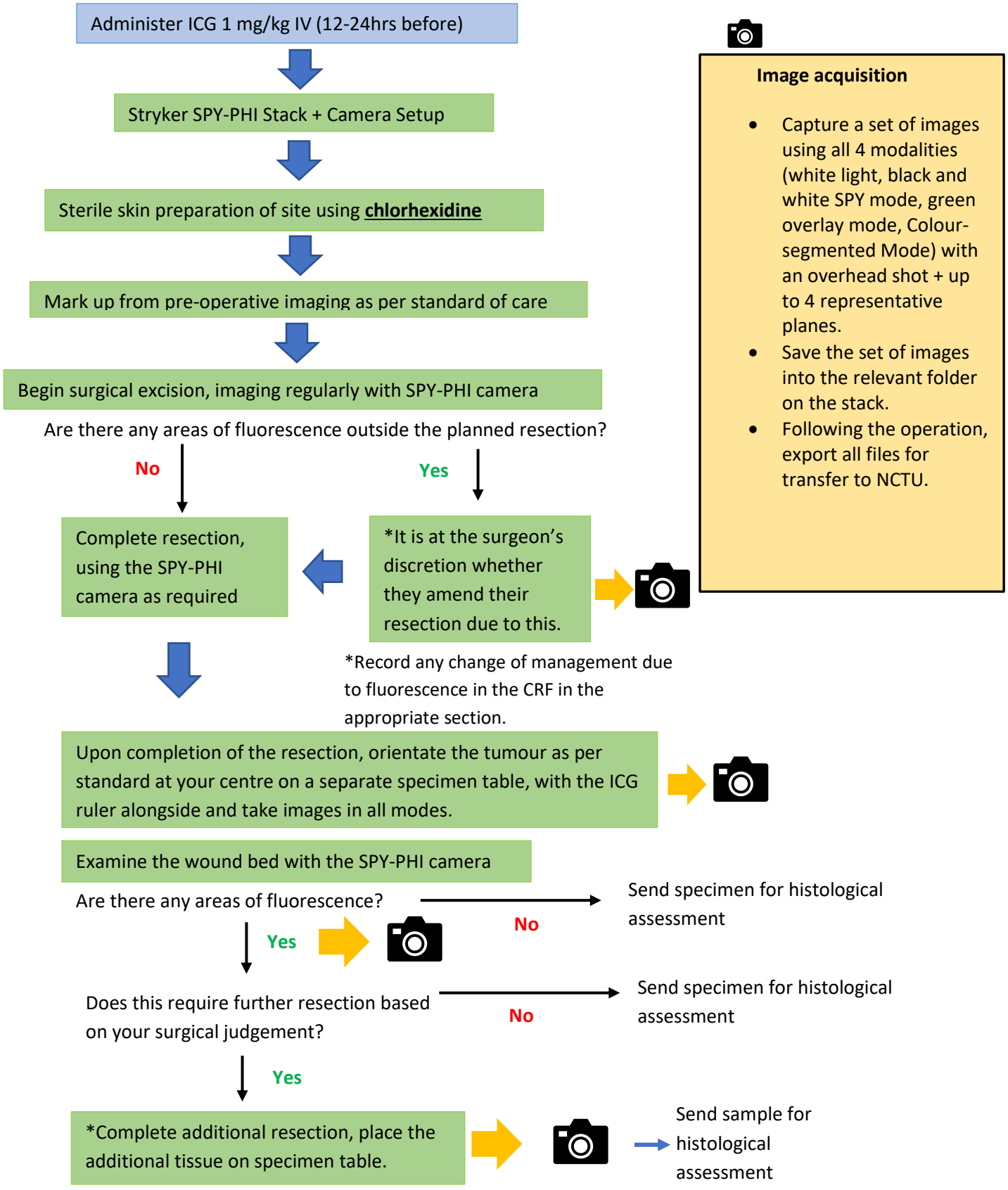
				13.1 Research Ethics Committee Review and Reports • Removal of reference to submission of annual progress report to REC as this is no longer a requirement. 16.3 Appendix 3- (yellow text box) • Removal of reference to 'unencrypted USB from yellow text box. Throughout Sections • Change from 'anonymised' to 'pseudonymised' • Correction of grammar and typographical errors

All amendments to the protocol, substantial or non-substantial, are listed in this table. Substantial amendments have been approved by the NHS REC. Non-substantial amendments have been sent to the NHS REC for acknowledgement only.

Day before surgery

Day of Surgery

16.3 Appendix 3- Surgery Procedure for participants randomised to FGS using ICG



16.4 Appendix 4. Calvien-Dindo Classification of Surgical Complications

Grade	Description
Grade I	Any deviation from the normal post-operative course not requiring surgical, endoscopic, or radiological intervention. This includes the need for certain drugs (e.g., antiemetics, antipyretics, analgesics, diuretics, and electrolytes), treatment with physiotherapy and wound infections that are opened at the bedside
Grade II	Complications requiring drug treatments other than those allowed for Grade I complications; this includes blood transfusion and total parenteral nutrition (TPN)
Grade III	Complications requiring surgical, endoscopic, or radiological intervention
Grade IIIa	Intervention not under general anaesthetic
Grade IIIb	Intervention under general anaesthetic
Grade IV	Life-threatening complications: this includes central nervous system complications (e.g., brain haemorrhage, ischaemic stroke, subarachnoid haemorrhage) which require intensive care, but excludes transient ischaemic attacks (TIAs)
Grade IVa	Single-organ dysfunction (including dialysis)
Grade IVb	Multi-organ dysfunction
Grade V	Death of the patient

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