Trial Title: Feasibility of sentinel lymph node biopsy in rectal cancer

Internal Reference Number / Short title: SentiRect

Ethics Ref: 15/SC/0317

NCT number: NCT02445456

Date and Version No: 15 March 2019, Version 1.3

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Sponsor: Oxford University Hospitals NHS Foundation Trust

Funder: Impact Acceleration Award Funding, NIHR Colorectal Therapies Healthcare Technology Cooperative, Leeds.

Funding for research consumables from Endomagnetics, Cambridge.

Chief Investigator Signature:

The investigators declare no potential conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY TRIAL CONTACTS

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2. SYNOPSIS

Trial Title	Feasibility study of sentinel lymph node mapping in rectal cancer	
Internal ref. no. (or short title)	SentiRect Study	
Trial Design	Feasibility: proof of principle	
Trial Participants	Rectal cancer patients	
Planned Sample Size	40 patients	
Treatment duration	Single pre-operative administration	
Follow up duration	2-3 weeks	
Planned Trial Period	12 months	
	Objectives	Outcome Measures/Endpoints
Primary	Establish feasibility of identifying sentinel lymph node in rectal cancer: [A] Adverse or hypersensitivity reactions after Sienna+ tracer injection in rectal cancer patients. [B] Establish whether tracer is still present and detectable at the tumour injection site and in the lymph nodes at the time of surgery. [C] Determine the impact of Sienna+ injection on subsequent	[A] Frequency of adverse or hypersensitivity reaction. [B] Frequency of tracer being detectable at the tumour injection site and in the lymph nodes at the time of surgery. [C] Surgeon's assessment of any difficulties encountered

	surgery (e.g. any difficulties due to discolouration or inflammation of tissue planes)	attributable to prior Sienna+ injection.
Secondary	[A] Correlate histopathology findings with tracer uptake in the lymph nodes. [B] Radiologically evaluate the drainage pattern of Sienna+ tracer using a high resolution MRI, to establish a baseline for individual variation. <u>EX-VIVO part of study:</u> [C] Measure the distribution and spread of the Sienna+ tracer using the SentiMag hand-held probe in mesorectal specimen after rectal cancer surgery (in the pathology department). This is to establish a baseline in individual variation between specimens. [D] Assess the accuracy of the SentiMag hand-held probe/Sienna+ tracer in identifying "sentinel" lymph nodes. This will be determined in conjunction with a pathologist who will systematically identify lymph nodes in the standard manner. The level of tracer activity in each lymph node will be measured and correlated with position and tumour cell involvement. <u>IN-VIVO part of study:</u> [E] Establish feasibility of sentinel lymph node biopsy during localised surgery for early rectal cancer using SentiMag hand-held probe to identify "peaks" of tracer activity intro-operatively.	[A] Correlation between histopathology findings and tracer uptake (determined by Sentimag probe) in the lymph nodes. [B] High-resolution MRI mapping of the drainage pattern of Sienna+ tracer. <u>EX-VIVO part of the study:</u> [C] Mapping of the distribution of Sienna+ tracer in the mesorectal specimen using the SentiMag hand-held probe after rectal cancer surgery. [D] Accuracy of the SentiMag hand-held probe/Sienna+ tracer in identifying "sentinel" lymph nodes, determined by correlation with histopathology findings. <u>IN-VIVO part of study:</u> [E] Number of TEM operations where the "sentinel" lymph node could be identified during surgery using the Sentimag probe, and then successfully removed.
Device name	Magtrace® tracer and SentiMag® magnetometer	
Device Manufacturer	Endomagnetics, Cambridge	
Device Classification	Class IIa device	

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction

CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CTRG	Clinical Trials and Research Governance
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
ERC	Early rectal cancer
GCP	Good Clinical Practice
GP	General Practitioner
IB	Investigators Brochure
ICF	Informed Consent Form
ICG	Indocyanine green
ICH	International Conference of Harmonisation
IMP	Investigational Medicinal Product
IRB	Independent Review Board
MDT	Multi-disciplinary team
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
NIR	Near infrared
NRES	National Research Ethics Service
OXTREC	Oxford Tropical Research Ethics Committee
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SNLB	Sentinel lymph node biopsy
SOP	Standard Operating Procedure
SPIO	Superparamagnetic iron oxide particles
SUSAR	Suspected Unexpected Serious Adverse Reactions
TEM	Transanal endoscopic microsurgery

TME	Total mesorectal excision
TMF	Trial Master File
TSG	Oxford University Hospitals Trust / University of Oxford Trials Safety Group

4. BACKGROUND AND RATIONALE

The scientific rationale mainly comes from studies involving sentinel lymph node biopsy (SLNB) in breast cancer (Krag et al, 2010). No difference was seen in overall survival or disease free survival when women had SLNB compared to women who had SLNB and axillary node dissection (AND) in women with disease negative sentinel nodes. A further study in breast cancer patients with positive SLNB but no clinical evidence of lymph node involvement demonstrated that there was no difference in outcome when comparing those who had no further axillary surgery versus those who had subsequent completion axillary lymph node dissection (Giuliano et al, 2011). In both studies, a high proportion of patients were treated with adjuvant systemic chemotherapy and external beam radiation. These data strongly support the need for further research into the role of organ-preservation surgery in other cancer types.

Sentinel node interrogation has yet to be fully evaluated in rectal cancer surgery due to technical limitations of obtaining SLNB from the mesorectum. As for breast cancer, rectal cancers can be excised by either radical or organ-preservation surgical techniques. Decision for either method is currently based on the risk of local recurrence or presence of lymph node metastasis. Radical surgery involves removal of the whole of the mesorectum and is associated with significantly worse functional outcome, morbidity and mortality. Current guidelines recommend that full thickness removal of the rectal tumour by transanal endoscopic microsurgery, TEM, is appropriate for small T1N0 cancers (less than 3 cm) where the risk of local recurrence (LR) is relatively low (NCCN guidelines, 2014). This surgery avoids removal of the whole mesorectum. The risk of LR increases with the depth of penetration of the rectal wall. Results from the UK TEM database show that the risk of LR at 36 months trebles when comparing T1N0 to T2N0 cancers of the same diameter (4.4% versus 14.3% respectively) (Bach et al, 2009). However, in 85% of rectal cancers that are T2N0 (based on tumours that are 2.1-3 cm diameter, with no lymphatic invasion) there is no LR at 36 months. There is a risk of subjecting patients to over-treatment in terms of surgery resulting in a significant risk of morbidity, mortality and impaired quality of life. On the other hand under-treatment of some “high risk” cancers would lead to recurrence. SLNB at TEM would potentially allow clinicians to excise T2 cancers in order to further stratify cancers based on future risk. This would avoid surgical over-treatment and under-treatment.

Our research objective will be to assess the feasibility of using a magnetic non-radioactive iron-based tracer (Sienna+ or Magtrace) and a handheld magnetometer (SentiMag) to accurately define the position of sentinel lymph nodes in patients with operable rectal cancer. This technology has already been successfully used in breast cancer patients in a UK multi-centre trial (Thill et al, 2014). The tracer is non-radioactive and safe (there is an extremely low risk of a hypersensitivity reaction to iron-based compounds, magnetic tracer and superparamagnetic iron oxide (SPIOs)). Our research will be done in two stages. The first “ex-vivo” stage will involve mapping tracer activity in total mesorectal specimens from rectal cancer patients. These will be focused on patients with rectal cancers where standard radical surgery is recommended. In this group we will also inject indocyanine green dye (ICG) into the tumour during surgery to act as an adjunct to identifying the lymph nodes while we develop confidence with the Sentimag system. ICG is a widely-used dye with many applications in medicine, including SLN

identification, and has been used with some positive early results in rectal cancer (Cahill et al, 2012). The second stage would then involve an “in-vivo” study in patients with early rectal cancers (ERCs) that are suitable for TEM. We would use SentiMag technology to assess the feasibility and safety of SNLB in patients with ERC by taking lymph nodes with "peaks" of tracer activity from the mesorectum. After completion of this study, our intention will be to perform a clinical trial based on SLNB in rectal cancer patients.

There are no major ethical or legal issues associated with this study as SLNB is established in breast cancer practice. Our research will help to further define suitability for either localised or radical surgery in early rectal cancer patients.

5. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Objectives	**Outcome Measures/Endpoints**
Primary Objectives Establish feasibility of identifying sentinel lymph node in rectal cancer: [A] Adverse or hypersensitivity reactions after tracer injection in rectal cancer patients. [B] Establish whether tracer is still present and detectable at the tumour injection site and in the lymph nodes at the time of surgery. [C] Determine the impact of tracer injection on subsequent surgery (e.g. any difficulties due to discolouration or inflammation of tissue planes	[A] Frequency of adverse or hypersensitivity reaction. [B] Frequency of tracer being detectable at the tumour injection site and in the lymph nodes at the time of surgery. [C] Surgeon’s assessment of any difficulties encountered attributable to prior tracer injection
Secondary Objectives [A] Correlate histopathology findings with tracer uptake in the lymph nodes. [B] Radiologically evaluate the drainage pattern of tracer using a high resolution MRI, to establish a baseline for individual variation. <u>EX-VIVO part of study:</u> [C] Measure the distribution and spread of the tracer using the SentiMag hand-held probe in mesorectal specimen after rectal cancer surgery (in the pathology department). This is to establish a baseline in individual variation between specimens. [D] Assess the accuracy of the SentiMag hand-held probe/ tracer in identifying "sentinel" lymph nodes. This will be determined in conjunction	[A] Correlation between histopathology findings and tracer uptake (determined by Sentimag probe) in the lymph nodes. [B] High-resolution MRI mapping of the drainage pattern of tracer. <u>EX-VIVO part of the study:</u> [C] Mapping of the distribution of tracer in the mesorectal specimen using the SentiMag hand-held probe after rectal cancer surgery. [D] Accuracy of the SentiMag hand-held probe/ tracer in identifying "sentinel" lymph nodes, determined by correlation with histopathology

with a pathologist who will systematically identify lymph nodes in the standard manner. The level of tracer activity in each lymph node will be measured and correlated with position and tumour cell involvement. <u>IN-VIVO part of study:</u> [E] Establish feasibility of sentinel lymph node biopsy during localised surgery for early rectal cancer using SentiMag hand-held probe to identify "peaks" of tracer activity intro-operatively.	findings. <u>IN-VIVO part of study:</u> [E] Number of TEM operations where the "sentinel" lymph node could be identified during surgery using the Sentimag probe, and then successfully removed.
Tertiary Objectives Not applicable	Not applicable

6. TRIAL DESIGN

This is a clinical study assessing the feasibility of detecting and sampling sentinel lymph nodes in rectal cancer patients using a magnetic nanoparticle tracer (Sienna+ or Magtrace, class IIa device, CE-approved in Europe). See Appendix A for a flow diagram of the study schema.

The study will be conducted in two phases. The first (ex-vivo) phase will assess the feasibility of detecting sentinel lymph nodes using tracer/SentiMag probe in patients undergoing radical surgery for rectal cancer. The second (in-vivo) phase will assess the feasibility of removing sentinel lymph nodes identified using tracer/SentiMag probe in patients undergoing less radical surgery for early rectal cancer.

Expected duration of patient participation is 2-3 weeks, from pre-operative tracer injection till discharge from hospital following rectal cancer surgery. Standard practice includes a pre-operative clinic visit for discussion, visit to the endoscopy unit for endoscopic assessment of the tumour pre-operatively and hospital admission for surgery. There will be one additional visit for an MRI scan. This will be performed on the same day as the endoscopy visit or on the day of surgery.

Screening of patients and provision of information about the study will occur during the pre-operative clinic visit. Consent will be taken on visit to the endoscopy unit and Sienna+ tracer will be injected during endoscopy. The Sentimag probe will be used during surgery and for second phase participants the sentinel lymph node will be removed. Pathological assessment of the removed specimen will be carried out after surgery. Patients will be monitored on the colorectal ward post-operatively until discharge.

During each stage data will initially be recorded in hard copy on CRF then transferred to a secure electronic record.

7. PARTICIPANT IDENTIFICATION

7.1. Trial Participants

Participants diagnosed with rectal cancers that are operable and have no symptoms of intestinal obstruction.

7.2. Inclusion Criteria

- Participant is willing and able to give informed consent (in English) for participation in the trial.
- Male or Female, aged 18 years and below 90 years of age.
- Diagnosed with operable rectal cancer.
- Discussion of case at the Oxford Colorectal Cancer MDT.
- For in-vivo phase: Early rectal cancer and absence of lymph node involvement on staging MRI scan.
- In the Investigator's opinion, is able and willing to comply with all trial requirements.

7.3. Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

- Female participant who is pregnant or lactating.
- Known intolerance or hypersensitivity to iron or dextran compounds, magnetic tracers, or SPIOs.
- For ex-vivo phase for ICG injection: known intolerance of iodine
- For in-vivo phase: advanced rectal cancer or radiological nodal involvement on staging MRI scan
- Cancer involvement of anal sphincter complex on clinical, radiological or endoscopic assessment.
- Age less than 18 and greater than 90.
- Adults who are not able to give consent or who are deemed vulnerable.

8. TRIAL PROCEDURES

Methodology training for investigators: At the start of the study, the principal investigators will undergo methodology training and practice the use of the hand held SentiMag probe device. The chief investigator will standardize the sentinel lymph node biopsy technique during this feasibility study.

Screening and preliminary discussion of research project with potential participant: During the standard pre-operative clinic visit patients who have been identified as potential participants will be screened and informed about the study by a clinician or colorectal specialist nurse. They will have an opportunity to ask questions and will be provided with an information sheet.

Informed consent: In the endoscopy unit a clinician will discuss the study further and take consent if the patient is willing to proceed. A letter will be sent to the participant's GP informing them of the study.

Endoscopic injection of tracer and post-injection monitoring: The magnetic tracer will be injected approximately 5 days before surgery during endoscopic assessment of the tumour. The rectal tumour

will be visualized by endoscopy, as per standard clinical practice. Up to 2ml tracer solution will be infiltrated systematically (in 4 quadrants) into the submucosa around the rectal tumour. After the procedure, patients will be monitored for 1 hour to ensure no hypersensitivity or adverse reactions occur and provided with a contact telephone number in case of any delayed reaction or side-effects on discharge.

MRI scan of pelvis: On the same day as the endoscopy unit visit or the day of surgery, patients will undergo an MRI scan of the pelvis. This will be done 2-3 hours after the endoscopic injection of tracer or on the day of surgery.

Rectal cancer surgery: Patients will undergo rectal cancer surgery according to their clinical indication. During surgery for the "ex-vivo" group, indocyanine green dye (ICG) will be injected near the tumour. At the conclusion of surgery the surgeon will make an assessment as to whether the tracer injection affected surgery, for example by causing inflammation in the tissue planes.

Intra-operative detection of the sentinel lymph node using the Sentimag probe and excision of the identified node (second part participants only): During rectal cancer surgery, following excision of the tumour, the surgeon will use the hand held SentiMag probe to identify the sentinel lymph node. This will then be dissected and removed for subsequent histological analysis.

Post-operative monitoring: Following surgery patients will be admitted to the colorectal ward for routine post-operative care. During this time they will be monitored and any adverse reactions or post-operative complications will be recorded. The patients' participation in the study will cease when they are discharged from hospital.

Ex-vivo detection of the sentinel lymph node in the surgical specimen using the Sentimag probe (first part only): The Sentimag probe will be used to measure the distribution of the tracer and to identify the sentinel lymph node in the specimen removed during the standard surgical procedure. A Near InfraRed (NIR) imaging system will also be used to assess the specimen to detect the distribution of ICG as an adjunct to identifying lymph nodes.

Ex-vivo histological assessment of the surgical specimen: A colorectal pathologist will examine the tissue removed during surgery to identify all the lymph nodes present and whether or not they contain metastatic cancer deposits, according to standard practice. At the conclusion of the study the samples will be held in the cellular pathology department, in line with standard practice for diagnostic surgical specimens.

8.1. Recruitment

The identification of patients suitable for this study will take place at colorectal cancer multi-disciplinary meetings. This will involve reviewing the clinical, histological and radiological investigations with other surgeons, oncologists, radiologists and pathologists as part of the normal clinical review process. The principal investigators, who are part of the standard clinical care team for rectal cancer patients, are routinely present at this meeting.

Identified potential participants will then be approached by the principal investigator during their routine pre-operative clinic visit, and screening will be performed at this time using a checklist. They will be

provided with an information sheet and given time to ask questions. Patients agreeing to participate will be recruited on their subsequent pre-operative visit to the endoscopy unit.

8.2. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant and a copy will go into the patient's medical notes. The original signed form will be retained at the trial site.

8.3. Screening and Eligibility Assessment

Not applicable. Participation will be sought from patients that are diagnosed with rectal cancer and are discussed at the Colorectal MDT meeting. A simple checklist of inclusion and exclusion criteria will be used to screen the patients during their pre-operative clinic visit.

8.4. Randomisation, blinding and code-breaking

Not applicable

8.5. Baseline Assessments

Confirmation of operable rectal cancer at the Oxford Colorectal MDT discussion.

8.6. Subsequent Visits

[Visit 1: clinic] Potential participants will be informed about the study by the study investigators or the colorectal nurse specialists at their initial hospital clinic visit with the operating surgeon. This is part of the clinical work-up of a patient and therefore would not involve an additional visit for the participant. This visit will take place approximately 2-3 weeks prior to surgery. Information will only be given to patients who are already aware of their diagnosis. Patient information sheets will be given to the patient at this stage. Patients will be screened using the inclusion and exclusion checklist.

[Visit 2: endoscopy suite] Surgeons assess rectal cancers by endoscopic means in order to plan surgery. This is part of normal practice and will not involve an additional visit for the potential participant. This usually occurs a week before surgery. Informed consent will be obtained from participants at this stage. During the endoscopic procedure the tracer will be injected around the rectal tumour. Patients will be monitored for 1 hour following this for any adverse effect of the injection.

[Visit 3: Radiology suite] Patients will have an MRI scan to assess tracer uptake into the mesorectum. This is not part of the standard routine care and will involve an additional hospital visit for the patient. This will be undertaken 2-3 hours after the endoscopic injection, on the same day as the endoscopy visit or prior to surgery on the day of surgery.

[Visit 4: Admission to hospital for surgery]: This is part of normal practice and will not involve an additional visit for the potential participant. Sentinel node sampling will take place after surgery from the resected specimen ("ex-vivo" phase) or during surgery ("in-vivo" phase). Participants will be monitored during their post-operative stay in hospital as part of normal practice. The incidence of post-operative complications will be recorded on discharge.

8.7. Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the trial at any time. In addition, the Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the device or results in inability to continue to comply with trial procedures
- Withdrawal of Consent
- Loss to follow up

No additional procedures or observations will continue to be required after the end of the study.

Withdrawal from the study will result in the exclusion of the data for that participant from analysis.

Withdrawn participants will be replaced by further recruitment to the study.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

8.8. Definition of End of Trial

The end of trial is the date of the last participant discharged from hospital.

9. IDENTIFICATION & DESCRIPTION OF THE INVESTIGATIONAL DEVICE

9.1. Device description

This study will use Sienna+® or Magtrace® tracer which contains magnetised nanoparticles and a hand-held SentiMag® probe within its CE market intended purpose for sentinel lymph node mapping. Lymph node mapping is used in cancer patients to identify the first lymph nodes that would be affected by spread of the cancer. Specifically, in this study, the device will be used to identify sentinel lymph nodes in the mesorectum of patients with rectal cancer.

The tracer is a dark brown aqueous suspension of organically coated superparamagnetic iron oxide particles, supplied in a vial. It will be diluted and injected during endoscopy. The vials will be supplied directly and will not involve pharmacy.

The manufacturer in Endomagnetics Limited, 325 Cambridge Science Park, Cambridge CB4 0WG. The Sienna+ product has been in use since 29 February 2012. On 31 October 2018 Endomagnetics discontinued Sienna+ and replaced it directly with Magtrace, which contains the same nanoparticle, in the same amount and concentration, but formulated in 0.3% saline rather than water.

At the start of the study, the principal investigators will undergo methodology training and practice the use of the hand held SentiMag probe.

9.2. Device Safety

The tracer is supplied in vials with a long shelf life. The storage and handling procedures are straightforward; there are no temperature issues. The vials will be held in a secure storage area in the endoscopy department. The vial details and expiry date will be checked prior to administration in accordance with standard procedures. Details of the batch and injection will be recorded on the standard endoscopy documentation. Details of the injection will also be recorded on the CRF.

The Sentimag includes a probe and a portable base unit that it connects to. There are no safety issues with either the tracer or the probe.

9.3. Device Accountability

The manufacturer (Endomagnetics) is responsible for the quality control of the tracer and probe.

10. NON-INVESTIGATIONAL MEDICINAL PRODUCT (NIMP)

10.1. NIMP Description

Indocyanine green (ICG) dye is a tricarbocyanine dye with a peak spectral absorption in the near infrared (NIR) range. Following injection it can be visualised in the tissues using an NIR imaging system. ICG has a low molecular size so when injected subserosally or submucosally into the bowel it is rapidly taken up by the lymphatics and deposited in the local lymph nodes. It has been widely used for sentinel lymph node mapping in colorectal and other cancers, and is also commonly used as an indicator dye in many other

areas of medicine. ICG contains sodium iodide. It is safe for general use, but can cause allergic reactions in people with iodine sensitivity.

ICG is supplied as a sterile water-soluble powder in 25mg vials. The vials are stored at room temperature. Prior to use the powder is dissolved in 10ml of sterile water. Once dissolved the ICG must be used within 6 hours.

11. SAFETY REPORTING

11.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory findings) in participants, users or other persons whether or not related to the investigational medical device. This includes events related to the investigational device or comparator, events related to the procedures involved (any procedure in the protocol). For users or other persons this is restricted to events related to the investigational medical device.
Adverse Device effect (ADE)	An adverse event related to the use of an investigational medical device. This definition includes any events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device. This definition also includes any event resulting from user error or form intentional misuse of the investigational device.
Serious Adverse Event (SAE)	An adverse event that: • Led to death • Resulted in serious deterioration in the health of the subject that: o resulted in a life-threatening illness or injury o resulted in a permanent impairment of a body structure or a body function o required in-patient care or prolongation of hospitalisation o resulted in medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function. This includes device deficiencies that might have led to a serious adverse event if: a) suitable action had not been taken or

	b) intervention had not been made or c) circumstances had been less fortunate. These are handled under the SAE reporting system. Planned hospitalisation for a pre-existing condition, or a procedure required by the trial protocol, without serious deterioration in health, is not considered a serious adverse event.
Serious Adverse Device Effect (SADE)	Any untoward medical occurrence that can be attributed wholly or partly to the device, which resulted in any of the characteristics of a serious adverse event as described above. *Unanticipated Serious Adverse Device Effects (USADE)* Any serious adverse device effect which, by its nature, incidence, severity or outcome, has not been identified
Device deficiency	Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling. Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate
User error	Act or omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user. Use error includes slips, lapses and mistakes. An unexpected physiological response of the subject does not itself constitute a use error.

Severity definitions

The following definitions will be used to determine the severity rating for all adverse events:

Mild: awareness of signs or symptoms, that does not interfere with the subject's usual activity or is transient that resolved without treatment and with no sequelae.

Moderate: a sign or symptom, which interferes with the subject's usual activity.

Severe: incapacity with inability to do work or perform usual activities.

11.2. Causality

The relationship of each adverse event to the trial device may be determined by the manufacturer and/or a medically qualified Investigator according to the following definitions:

Not related: The event is clearly related to other factors such as the patients/participants clinical condition, therapeutic intervention, concomitant medication.

Unlikely: The event is probably produced by other factors such as the patients/participants clinical condition, therapeutic intervention, concomitant medication and does not follow a known response pattern to the device

Possibly: The event follows a reasonable temporal relationship form the time of placement/administration and/or follows a known response pattern to the device but could have been caused by other factors such as the patients/participants clinical condition, therapeutic intervention, concomitant medication.

Most probable: The event follows a reasonable temporal relationship form the time of placement/administration and/or follows a known response pattern to the device and could not have been caused by other factors such as the patients/participants clinical condition, therapeutic intervention, concomitant medication. Further the event immediately follows the administration/placement of the device and improves on stopping or removing the device.

11.3. Procedures for Recording Adverse Events

All adverse events (including ADEs) and device deficiencies occurring during the course of the study will be recorded on the CRF whether or not attributed to the trial device. The information recorded will include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the device
- Whether the AE is serious or not
- Whether the AE arises from device deficiency
- Whether the AE arises from user error

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs/ADEs considered related to the device as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE/ADE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE/ADE. If either of these occurs, the participant must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

11.4. Reporting Procedures for Serious Adverse Events

Reporting of all Serious Adverse Events will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010).

SAEs/SADEs that pose an immediate risk to patient health or safety, will be reported to R&D immediately or no later than 24 hours after the Investigator is aware and to the device manufacturer, competent authority and the REC within 2 calendar days of the Chief Investigator becoming aware of the event.

All other reported SAEs/SADEs will be reported to R&D and competent authority within 7 calendar days of notification, if appropriate. This will not include SAEs that may be expected as part of the risks of routine care. Adverse device events (SADEs, USADEs) and device deficiencies will also be reported to the device manufacturer. All SAEs will be followed up to resolution.

SAEs/SADEs will be considered as any adverse event occurring from the injection of Sienna+ tracer during endoscopy to patient's discharge from hospital following their scheduled surgery.

11.5. Expectedness

Expectedness will be determined according to the Manufacturers risk analysis report.

Expected adverse reactions are uncommon but may include hypersensitivity reaction such as rash, itching, dizziness and lightheadedness. If inadvertent injection into the vascular system occurs anaphylactic reaction is possible. There may be brownish discolouration of the tissue around the injection site that may be noticeable at surgery.

11.6. Safety Monitoring Committee

The Oxford University Hospitals Trust Trials Safety Group (TSG) will conduct a review of all SAEs/SADEs for the trial reported during the quarter and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the trial continuing and take appropriate action where necessary

12. STATISTICS

12.1. Description of Statistical Methods

All data will be stored in an Excel worksheet and analysed using Excel and R.

Frequency of event (adverse, hypersensitivity or complication) will equate to: number of adverse event/total number of recruited patients.

Frequency of tracer detection at surgery (at tumour site and in lymph nodes) will equate to: number of operations where tracer seen/ total number of operations on recruited patients.

Impact of tracer injection on subsequent surgery will be assessed by the surgeon's binary assessment as to whether surgery was more difficult than expected, and also free text comments to be analysed qualitatively.

A sentinel lymph node will be defined as the first lymph node draining the tumour and positivity defined according to the manufacturer's instructions. A "positive" node will be defined as having over 10% of the tracer activity of the site of initial submucosal injection. A "negative" node will be defined as having less than 10% of the tracer activity of the site of initial submucosal injection.

The detection rate for sentinel lymph nodes on MRI scan and Sentimag probe assessment will equate to: number of patients/specimens in whom a sentinel lymph node is identified/total number of examined patients/specimens.

MRI and Sentimag mapping will be qualitatively compared for all relevant patients/specimens (as appropriate) to establish a baseline of individual variability.

The presence and location of lymph nodes in the mesorectal specimen will be compared with the pathology findings for each patient in the ex-vivo part using correlation.

The success rate for removing the sentinel lymph node during TEM surgery will equate to: number of patients in whom the sentinel lymph node was removed/total number of patients undergoing TEM operation.

As this is a feasibility study, results will be continuously evaluated and adjustments made to the tracer dosage and timing of injection if indicated.

12.2. The Number of Participants

The first (ex-vivo) part of the study will recruit 20 patients due to have radical surgery for rectal cancer in order to establish the accuracy of sentinel node detection using Sienna+ tracer by ex-vivo specimen analysis. This number is based on previously published sentinel node studies in breast cancer and melanoma (O'Hea et al, 1992; Brouwer et al, 2012). The second (in-vivo) part will recruit a further 20 patients, but these will be people with early rectal cancer scheduled for a less invasive TEM operation to treat their rectal cancer.

12.3. The Level of Statistical Significance

Not applicable for this proof of concept study

12.4. Criteria for the Termination of the Trial

Any serious adverse event will prompt a review of the study and possible termination.

12.5. Procedure for Accounting for Missing, Unused, and Spurious Data.

All available data will be used. Missing data will not be imputed. Spurious data will be re-checked, and if valid will be included in the analysis.

12.6. Inclusion in Analysis

All patients who receive the tracer injection will be included in the analysis.

12.7. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Not applicable. The analysis involves only simple statistical measures, and all proposed measures are necessary to determine feasibility.

13. DATA MANAGEMENT

13.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

13.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections. Data will not be transferred outside of the UK.

13.3. Data Recording and Record Keeping

Data will be recorded using paper CRFs and entered into an Excel spreadsheet. Data from this study will be generated and analysed at the Oxford University Hospitals by the research investigators. Personal data will be stored and accessed by the investigators for less than 3 months. Research data will be stored for 10 years after the completion of the study.

The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will NOT be included in any trial data electronic file.

All electronic data will be password-protected and anonymised. All hard copy files and data will be kept in a locked cabinet within a locked office with restricted access. Access to the data will be restricted to study team members. The NHS Code of Confidentiality will be followed. This study will follow the sponsor organisation's policy regarding data storage and the NHS Code of Confidentiality. Information derived from the study will be stored on a single, secure (password encryption) NHS computer. This will be positioned within a secure location in the hospital.

All participants will be informed using patient information sheets of how data will be stored. The principal investigators will constantly review the security of research data files.

14. QUALITY ASSURANCE PROCEDURES

The trial will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

15. SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to 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".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki. These details are provided in the protocol.

16.2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

16.3. Medical Device regulations

The Investigator will ensure that this trial is conducted in full conformity with:

- European Commission Medical Device Guidelines relating to the application of the EU Directives on Medical Devices
- Guide to European Medical Device Trials and BS EN ISO 14155

16.4. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.5. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

16.6. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

16.7. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

16.8. Other Ethical Considerations

No other ethical declarations to declare in relation to this study.

17. FINANCE AND INSURANCE

17.1. Funding

This study has received a £5000 Impact Acceleration Award from the NIHR Colorectal Therapies Healthcare Technology Cooperative, Leeds.

Funding for the use of research devices and consumables will be provided by Endomagnetics, Cambridge

17.2. Insurance

NHS bodies are legally liable for the negligent acts and omissions of their employees. If you are harmed whilst taking part in a clinical trial as a result of negligence on the part of a member of the trial team this liability cover would apply.

Non-negligent harm is not covered by the NHS indemnity scheme. The Oxford University NHS Trust, therefore, cannot agree in advance to pay compensation in these circumstances.

In exceptional circumstances an ex-gratia payment may be offered.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge the source of funding for the study. Authorship will be determined in accordance with the ICMJE guidelines and all other contributors will be acknowledged.

19. REFERENCES

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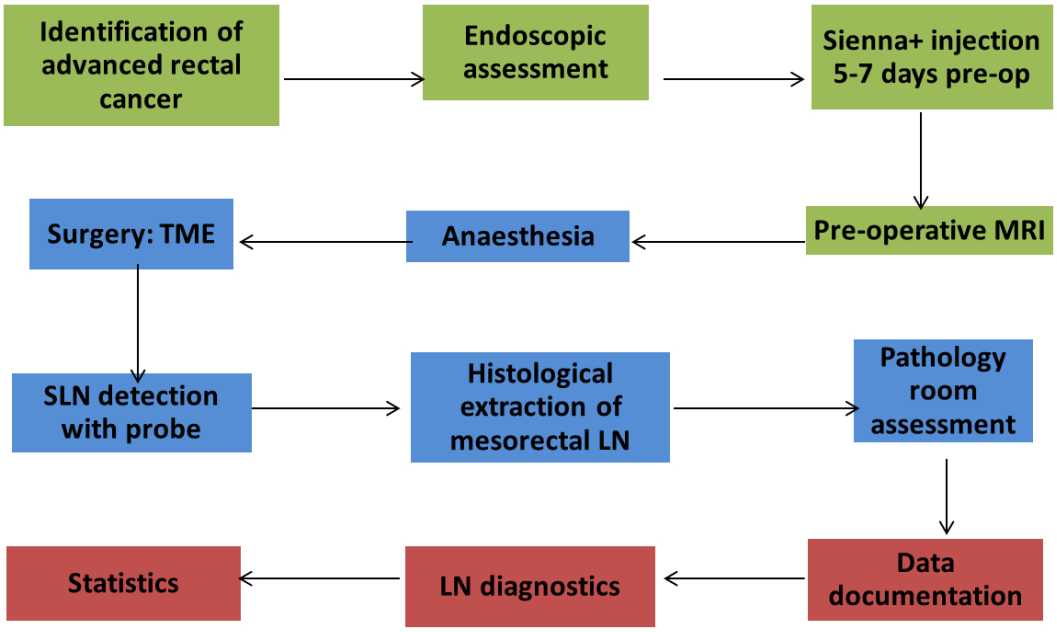
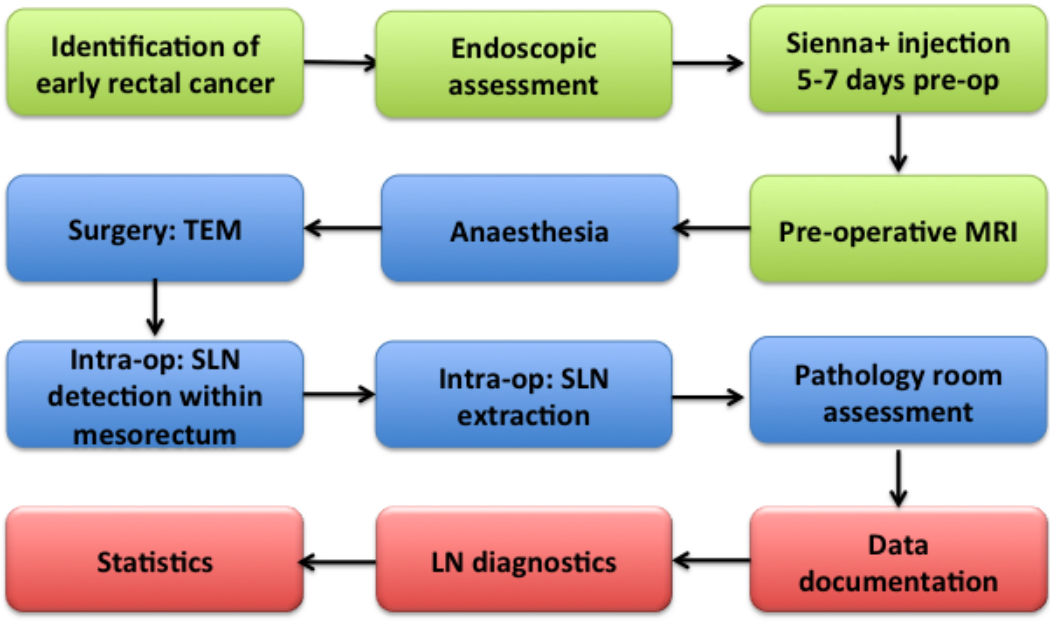
20. APPENDIX A: TRIAL FLOW CHART



Study schema 1: Sentinel lymph node biopsy in rectal cancer, "ex-vivo" assessment



Study schema 2: Sentinel lymph node biopsy in rectal cancer, "in-vivo" assessment

21. APPENDIX B: SCHEDULE OF PROCEDURES

Procedures	Visit 1	Visit 2	Visit 3	Visit 4
Informed consent		√		
Demographics				
Medical history				
Concomitant medications				
Laboratory tests				
Eligibility assessment	√			
Initial visit and discussion at Colorectal clinic	√			
Endoscopy suite (Sienna+ injection)		√		
Radiology, MRI pre-op assessment			√	
Admission for surgery				√
Adverse event assessments		√		√

22. APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.1	25 June 2015	H Jones	Addition of comment in 11.2 that data will not be transferred outside the UK
2	1.2	2 April 2017	H Jones	Addition of MRI scan and ICG injection during surgery for Group A patients, removal of interim analysis after 10 patients, change in tracer dosage specification
3	1.3	15 March 2019	H Jones	Sienna tracer has been withdrawn by the company and directly replaced by Magtrace. Time for patient to wait in endoscopy after injection reduced to 1 hour.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee or MHRA.