OTIS

Optimising Treatment In Seminoma and dysgerminoma - Surveillance

OTIS-S: A phase II/III trial evaluating use of circulating serum miRNA as part of active surveillance for patients with stage I seminoma and dysgerminoma

PROTOCOL

Version: 1.2
Dated: 25 November 2025

Chief Investigator:	Dr Alison Reid
Sponsor:	The Institute of Cancer Research
Funders:	Cancer Research UK
Coordinating Trials Unit:	ICR Clinical Trials and Statistics Unit (ICR-CTSU) The Institute of Cancer Research

REC Reference Number:	25/LO/0836
IRAS Reference Number:	342479
ICR-CTSU Protocol Number:	ICR-CTSU/2025/10092
Sponsor Number:	CCR6237
ISRCTN:	
CRUK Reference Number:	CRUK/24/008

The OTIS-S study has been approved by Cancer Research UK's Clinical Research Committee (CRC) and is part of the National Institute for Health Research Clinical Research Network Trial Portfolio

ICR The Institute of Cancer Research

CANCER RESEARCH UK

ADMINISTRATION

Scientific Coordination

Dr Alison Reid (Chief Investigator)
Royal Marsden Hospital
Sutton, SM2 5PT

Professor Matthew Murrey (Scientific Lead)
University of Cambridge
Cambridge, CB2 1QP

Professor Robert Huddart (clinical coordinator)
Royal Marsden Hospital
Sutton, SM2 5PT

Dr Teresa Mele (Early Career Researcher)
Barts Health NHS Trust
London, E1 BB

Trial Coordination

ICR Clinical Trials and Statistics Unit (ICR-CTSU, a CRUK funded, UKCRC Registered Clinical Trials Unit (CTU)) is responsible for the central coordination of the trial on behalf of the Sponsor.

ICR Clinical Trials & Statistics Unit (ICR-CTSU),
Division of Clinical Studies,
The Institute of Cancer Research,
Sir Richard Doll Building,
Cotswold Road,
Sutton, Surrey SM2 5NG

ICR-CTSU Methodology Lead:
Dr Fay Cafferty

ICR-CTSU Clinical Trials Programme Manager:
Aude Espinasse

Trial Statistician:
Matthew Tyler

OTIS-S Senior Trial Manager:
Deborah Gardiner

Senior Data Manager:
Zaynah Gurreebun

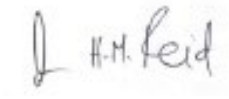
Any questions relating to this protocol should be addressed in the first instance to the OTIS-S Clinical Trial Manager within ICR-CTSU:

Email: OTIS-S-icrctsu@icr.ac.uk
General enquiries: 020 8722 4057
Study website: *https://go.icr.ac.uk/[shortURLextension]*

PROTOCOL DEVELOPMENT GROUP

Alison Reid	Chief Investigator	Consultant Medical Oncologist, The Royal Marsden NHS Foundation Trust
Fay Cafferty	Methodology lead	Lead Statistician, ICR-CTSU, The Institute of Cancer Research, London
Robert Huddart	Clinical Coordinator	Professor of Urological Cancer & Honorary Consultant Clinical Oncologist, The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research
Kyle Blain	PPI lead	Patient Advocate
Emma Hall	ICR-CTSU Director	ICR-CTSU Director, ICR-CTSU, The Institute of Cancer Research, London
Matthew Murray	Scientific lead	Honorary Consultant Paediatric Oncologist, University of Cambridge
Michelle Lockley	Dysgerminoma lead	Clinical Professor of Medical Oncology, Honorary Consultant, Queen Mary University of London and University College London Hospitals NHS Foundation Trust
Sara Stoneham	Childrens and TYA lead	Paediatric Oncologist, University College London Hospitals NHS Foundation Trust
Aude Espinasse	Senior Project Management lead	Clinical Trials Programme Manager, ICR-CTSU, The Institute of Cancer Research, London
Deborah Gardiner	Trial Management lead	Senior Trial Manager, ICR-CTSU, The Institute of Cancer Research, London
Daniel Stark	Patient-reported outcomes lead	Professor of Teenage and Young Adult Cancer Research, University of Leeds
Vijay Gc	Health Economic lead	Senior Research Fellow, University of York
Mike Hubank	Genomics expertise	Joint Head of Clinical Genomics (Research) & Honorary Faculty (TRANSGEN), The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research
Teresa Mele	Early career researcher	Consultant Medical Oncologist, Barts Health NHS Trust
Cinzia Scarpini	Lab coordinator	Senior Research Associate, University of Cambridge
Sam Withey	QA radiology reviewer	Consultant Radiologist, The Royal Marsden NHS Foundation Trust
Oliver Hulson	QA radiology reviewer	Consultant Radiologist, Leeds Teaching Hospitals NHS Trust
Clare Verrill	Pathology expertise	Professor of Cellular Pathology and Artificial Intelligence & Honorary Consultant Cellular Pathologist, University of Oxford and Oxford University Hospitals NHS Foundation Trust
Rupali Arora	Gynaepathology expertise	Lead Gynaepathologist, University College London Hospitals NHS Foundation Trust

Protocol Authorised by:

Name & Role	Signature	Date
Dr Alison Reid (Chief Investigator)		25/11/2025
Dr Fay Cafferty (ICR-CTSU Methodology Lead)		25/11/2025

This protocol describes the OTIS-S trial and provides information about procedures for entering participants into this trial. The protocol should not be used as a guide for the treatment of patients outside of this trial.

Every care was taken in the preparation of this protocol, but corrections or amendments may be necessary. Protocol amendments will be circulated to participating sites as they occur, but sites entering patients for the first time are advised to contact ICR-CTSU to confirm they have the most recent version.

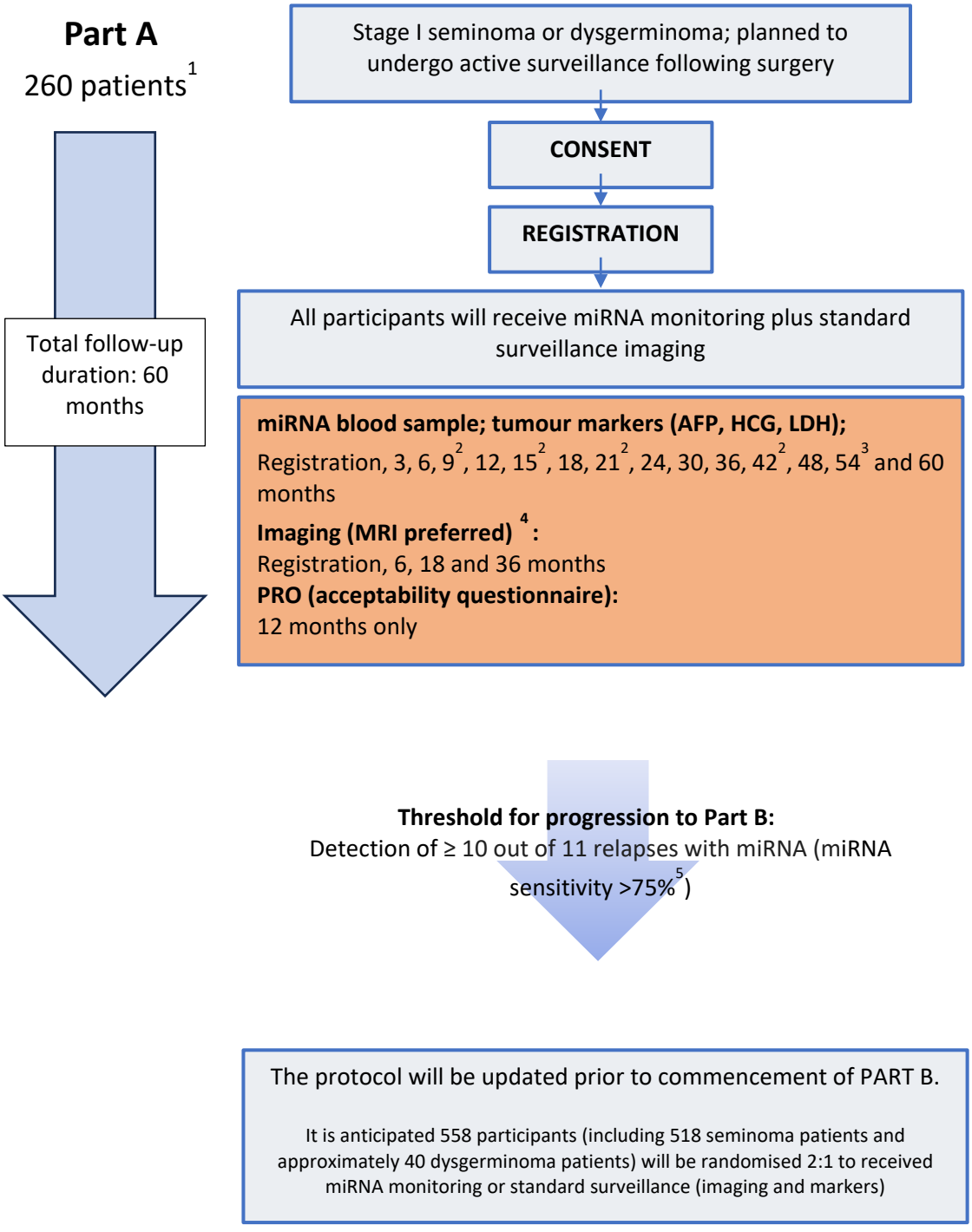
Contents

OTIS-S TRIAL SUMMARY

PROTOCOL TITLE	**OTIS-S**: A phase II/III trial evaluating use of circulating serum miRNA as part of active surveillance for patients with stage I seminoma and dysgerminoma.
TARGET DISEASE	Seminoma and dysgerminoma cancer.
TRIAL OBJECTIVES	The primary objective of **part A** is to demonstrate high sensitivity of miRNA for relapse detection in this setting. The primary objective for **part B** is to demonstrate non-inferiority of miRNA monitoring, when compared with standard imaging, for detection of advanced relapse in this setting.
TRIAL DESIGN	Multicentre, seamless phase II/III design. **Part A** (phase II) uses a single-arm approach and **part B** (phase III) will be randomised. Data from a previous trial in this setting will be used to augment the control arm in **part B**.
TRIAL POPULATION	People with stage I seminoma or dysgerminoma who have undergone orchiectomy or oophorectomy and have no adjuvant therapy planned.
RECRUITMENT TARGET	**Part A**: 260 patients, including approximately 250 seminoma and 10 dysgerminoma patients **Part B**: 558 patients, including approximately 518 seminoma and 40 dysgerminoma patients **Total**: 818 patients
TRIAL INTERVENTION	Serum miRNA monitoring, performed at baseline (post-surgery) and throughout follow-up.
PRIMARY ENDPOINT	**Part A:** Sensitivity of miRNA for relapse detection **Part B:** Advanced relapse (mass size ≥3cm or clinical stage (CS) III)
SECONDARY ENDPOINTS	**Part A** • Lead time (time of relapse detection by miRNA relative to imaging) • Specificity of miRNA for relapse detection • Time from blood draw to miRNA results being available to the clinical team and patient (feasibility) • Adherence to miRNA monitoring and imaging surveillance • Acceptability of miRNA monitoring to patients **Part B** • Cost-effectiveness of miRNA monitoring. • Radiation exposure • Adherence to miRNA monitoring • Acceptability of miRNA monitoring to patients • Anxiety and fear of recurrence • Health-related quality of life (HRQoL) • Disease-free survival • Relapse treatment

	• Overall survival • Second malignancies
FOLLOW UP	Patients will be followed for up to five years within the trial. Follow-up via routinely-collected datasets will be used to augment the trial data set.

TRIAL SCHEMA



Notes: *1: Approximately 245-250 seminoma patients, 10-15 dysgerminoma patients.*
2: miRNA samples at 9, 15, 21 and 42 months will be retrospectively analysed in the case of a recurrence occurring. All other miRNA samples will be analysed in real-time.
3: Tumour markers only.
4: Additional scans acceptable as per local standard of care
5: Designed to have 80% power to show sensitivity>75% with a one-sided 20% alpha.

1. INTRODUCTION

1.1. Background

1.1.1. Background to the OTIS programme

Malignant germ cell tumours (GCTs) are complex neoplasms affecting all age groups (paediatrics, teenagers and young adults (TYAs) and adults), occurring at different anatomical sites and comprising different histological subtypes. Testicular seminoma is the most common GCT and one of the most prevalent cancers in younger men (typically in their 20s and 30s; UK annual incidence ~1300 or ~4 per 100,000 men)[1,2]. Dysgerminoma is rarer (age-adjusted incidence 0.1 per 100,000 women in the US), but is the most common malignant ovarian GCT, typically affecting TYAs[3]. These tumours are comparable in biology and responsiveness to therapy[4,5] and survival outcomes are excellent, even for advanced disease[6,7]. However, patients are exposed to radiation (from serial CT scans or radiotherapy), and potentially cisplatin-based chemotherapy, which have associated long-term toxicities and health risks for this young population[8,9]. These include cardiological, neurological, audiological and psychological effects, as well as risks of second malignancy, with implications for health-related quality of life (HRQoL). Furthermore, both initial treatment and management of associated later events are costly for the NHS. Existing serum tumour markers (alpha fetoprotein (AFP), human chorionic gonadotrophin (HCG) and lactate dehydrogenase (LDH)) have limited sensitivity and specificity for seminoma[10], and there is a need for better risk stratification to facilitate more tailored approaches and reduce toxicity risks, particularly when recurrence risk is low.

The **OTIS (Optimising Therapy In Seminoma and Dysgerminoma) programme** seeks to identify alternative, effective approaches that reduce or avoid use of more intensive treatments for people with seminoma or dysgerminoma cancer whilst maintaining excellent outcomes. **OTIS-Surveillance** (**OTIS-S**) is one component of the programme which will focus on active surveillance in stage I disease.

1.1.2. Surveillance in early-stage disease

In current practice, most stage I seminoma patients are managed with active surveillance following surgery. This involves periodic cross-sectional imaging (typically 3-7 scans over 5 years), alongside tumour markers and clinical assessment. Around 10-20% of patients relapse, treatment is very successful, and survival approximately 100%[11]. In dysgerminoma, current international guidelines for early stage, completely resected stage I disease unanimously recommend surveillance, but approaches differ – even within the UK - and have not been prospectively evaluated[12,13]. Current biomarkers, HCG and AFP, have a low rate of sensitivity for seminoma relapse (at most 30%) and LDH lacks specificity, so is also of limited value[10]. As the main relapse site is in the para-aortic lymph nodes, in the absence of a reliable biomarker, current surveillance relies on imaging with CT or MRI. For the NHS, this is expensive and resource intensive; for patients, having scans is time-consuming and can induce anxiety[14]. Furthermore, CT scans expose patients to significant, cumulative diagnostic radiation[15]. Although there has been a move towards use of MRI in the UK following the TRISST trial results[16], this is limited by capacity.

Thus, there is a clear unmet need to strike the right balance between early detection of recurrent disease (in a minority of patients) and the intensity of radiologic surveillance. Reflecting this, the 2019 James Lind priority setting exercise for TYA cancers included identifying follow-up schedules which cause the least psychological and physical harm, whilst ensuring relapses are detected early[17]. A blood-based biomarker with good sensitivity for relapse detection, which might replace some or all imaging, is highly desirable. Consultation with patients has identified the potential benefits including increased sensitivity and safety, reduced time for testing and results, practical benefits and reduced anxiety[14]. For the NHS, there would be financial benefits[18] and increased imaging capacity for other patients. Furthermore, if the biomarker can detect recurrence earlier, this could reduce the need for subsequent, intensive chemotherapy (such as BEP; bleomycin,

etoposide and cisplatin). More relapses might be treated with local therapies or single-agent carboplatin, bringing further patient benefits and cost-savings for the NHS.

1.1.3. Evidence for use of circulating serum microRNA as a biomarker in GCTs

Unique and universal miRNA signature in malignant GCTs

MicroRNAs (miRNAs) are short, non-protein-coding RNAs that regulate gene expression and are dysregulated in cancer[19]. Previous work by **OTIS-S** co-applicants (MM, CS, Cambridge) showed that all eight miRNA members of the miR-371~373 and miR-302/367 clusters were highly expressed in all malignant GCTs, regardless of patient age, tumour site, or histological subtype[20]. Critically, they were not co-ordinately dysregulated in any other cancer or disease type[20]. With the development of highly sensitive, pre-amplified quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) methodology, the team demonstrated that these miRNAs could provide blood-based markers of malignant GCTs[21] and that circulating miRNA levels reflect disease-activity with the potential to detect malignant GCT recurrence[22]. Further studies have highlighted: (i) the potential clinical value of these markers, for GCTs developing at many body sites[22-27]; (ii) the expected cost savings (from health economic modelling) to healthcare systems for adopting circulating miRNA over imaging for surveillance of testicular GCT[18]; and (iii) with patient and public involvement (PPI), the acceptability of circulating miRNA testing to patients[14].

Replication of methodology by other research groups showing that circulating miRNA (and specifically miR-371a-3p) is a highly promising biomarker for GCT

Numerous subsequent studies have utilised the Cambridge team's pre-amplified qRT-PCR methodology to show that miR-371a-3p are highly sensitive and specific for malignant seminoma diagnosis, disease-monitoring, and detection of recurrence. One investigation of 874 patients showed serum miR-371a-3p levels at primary diagnosis provided an area under the curve (AUC) of 0.97, with a specificity of 94% at a sensitivity of 90%, and a positive predictive value of 0.97[28]. In patients with clinical stage I disease, miR-371a-3p levels fell rapidly to normal within 24 hours following orchiectomy, indicating a much shorter half-life than AFP and HCG[28]. In patients with metastases, miR-371a-3p levels fell significantly between the first and subsequent courses of chemotherapy[28]. Further, a retrospective pooled analysis of four independent studies in which all four candidate circulating miRNAs (miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p) were assessed, demonstrated that no combination of markers provided additional diagnostic benefit to miR-371a-3p alone[29]. It was concluded that quantifying circulating miR-371a-3p alone is sufficient for malignant testicular GCT diagnosis, and, importantly, more cost-effective and easier to interpret than a larger marker panel, facilitating incorporation into routine clinical practice[29].

A small pilot study has shown that, in the follow-up of patients with stage I testicular GCTs[30], all 10 patients who had a recurrence exhibited increased miR-371a-3p levels. Levels remained non-elevated in all but one of 23 patients without recurrence[30]. The authors concluded that miR-371a-3p detects recurrence reliably and usually several months earlier than standard investigations (markers and imaging)[30]. Together, these data show that circulating miR-371a-3p levels accurately reflect malignant GCT burden and response to treatment. Consequently, the miR-371a-3p assay has the potential to replace scans in follow-up with estimated cost-savings of up to $69M per year in the US[18]. Similar gains are expected in the UK.

Refining the circulating miR-371a-3p assay to allow clinical adoption.

Given the evidence for patient benefit, the Cambridge team and collaborators from the Malignant Germ Cell International Consortium (MaGIC) have refined the circulating miR-371a-3p assay to allow clinical adoption, particularly for the stage I surveillance setting[31], which is the planned use in **OTIS-S**. The MaGIC team showed that assay performance was comparable when thresholding based on raw quantification value (Cq) miR-371a-3p compared with historical normalized (ΔCq) values. Interlaboratory concordance of miR-371a-3p was high, but reference genes miR-30b-5p (endogenous) and cel-miR-39-3p (exogenous spike-in) were discordant[31]. Based on raw Cq, levels >35 were negative, and levels <28 positive. For any initial run with a Cq 28-35, a repeat run was performed. Samples falling in the Cq 28-35 range again were labelled indeterminate. Introduction of this indeterminate category improved assay accuracy from 0.84 to 0.92 in a group of patients

suspected of occult GCT[31]. This work has been extended further to confirm the utility of the indeterminate range in 60 control patients without malignant GCT. Of these, using the thresholds defined above, 58 cases were negative (96.7%); 2 were indeterminate (3.3%); and none were positive (0.0%) (Scarpini, personal communication). Importantly, there were no significant differences in circulating miR-371a-3p levels by sex or age group, demonstrating the validity of the assay for the whole patient population. Together, these data confirm that the refinements to the methodology have led to a clinical grade assay which minimises risks of false positive results and is now ready for clinical use.

Whilst the evidence-base for a role of miRNA in the management of GCT has primarily focused on testicular cancers, biological similarities lead to the hypothesis that miRNA testing may also have utility in managing other GCTs, and inclusion of dysgerminoma patients within the OTIS-S trial will allow exploration of this hypothesis.

1.1.4. Parallel programme of work for clinical accreditation

The **OTIS-S** trial will run in parallel with a funded programme of work to transfer the assay into a clinically accredited test following a successful bid to NHS England/Genomics England. The `Circulating miR-371a-3p Assay Work Package' forms part of the NHS Cancer Genomic Network of Excellence 'Circulating tumour biomarker testing for rapid, effective cancer diagnostics and treatment monitoring'. Running between 2024-2026, this work will ensure that miRNA biomarker testing will be ready for routine clinical implementation for all patients with GCTs. **OTIS-S** will provide a key test case for the work, demonstrating feasibility of real-time circulating miRNA follow-up of stage I testicular seminoma and dysgerminoma and, in turn, the accreditation work will facilitate rapid implementation into clinical practice if the trial is able to demonstrate clinical utility of the biomarker in this setting.

1.2. Description of Population

Patients with histologically confirmed stage I seminoma or dysgerminoma of any extra-cranial site, who have undergone orchiectomy or oophorectomy, and have no adjuvant therapy planned.

1.3. Study Rationale

Circulating miRNAs offer exciting possibilities to transform GCT management for patient benefit. The clinical validity of the assay has been demonstrated in previous studies. **OTIS-S** aims to provide evidence for clinical utility of the biomarker, in place of regular surveillance imaging (MRI/CT), in stage I seminoma and dysgerminoma. Potential benefits of the approach for patients, include reduced practical burden, anxiety and radiation exposure associated with scans, as well as increased confidence and reassurance from regular and prompt test results. There is also the possibility of earlier relapse detection which could avoid the need for intensive cisplatin-containing chemotherapy in some cases. For the NHS, there are potential cost-savings and increased imaging capacity for other patient groups. Running alongside the ongoing accreditation work, if the trial is able to demonstrate feasibility of the approach and associated benefits for patients and the NHS, it is expected to facilitate rapid adoption of miRNA testing into routine practice.

2. TRIAL OBJECTIVES

2.1. Primary Objective

Part A:

- To demonstrate high sensitivity of miRNA for relapse detection in patients who have undergone surgery for stage I seminoma or dysgerminoma

Part B:

- To demonstrate non-inferiority of miRNA monitoring, when compared with standard imaging, for detection of advanced relapse in patients who have undergone surgery for stage I seminoma or dysgerminoma

2.2. Secondary Objectives

Part A:

- To demonstrate high specificity of miRNA for relapse detection in this setting
- To demonstrate feasibility and acceptability to patients of miRNA monitoring in this setting
- To quantify the lead time (extent to which relapse diagnosis can be bought forward) associated with miRNA monitoring when compared with standard surveillance

Part B:

- To demonstrate cost-effectiveness of miRNA monitoring compared with standard surveillance
- To demonstrate reduced use of imaging and subsequent impact on radiation exposure with miRNA monitoring compared with standard imaging-based surveillance
- To further evaluate acceptability to patients of miRNA monitoring in this setting
- To quantify anxiety, fear of recurrence and health-related quality of life for patients managed with the two different approaches to surveillance
- To understand any impact on disease outcomes and subsequent treatment associated with use of miRNA monitoring in place of standard imaging
- To understand the risk-benefit balance of a miRNA monitoring approach (when compared to standard surveillance) from the perspective of different stakeholders, including patients

2.3. Exploratory Objectives

Whilst primary and secondary objectives relate to the cohort as a whole, they will also be considered separately for seminoma and dysgerminoma patients to identify and explore any differences. Due to the small numbers of dysgerminoma patients expected, analyses pertaining to this cohort are considered exploratory and evidence will be gained from considering consistency with results for the seminoma cohort[32].

3. TRIAL DESIGN

This is a multicentre, seamless phase II/III trial which will recruit a total of 818 participants with stage I seminoma or dysgerminoma. The intervention is the collection of blood samples for the purpose of miRNA monitoring. The staged approach to evaluation will allow experience and confidence with miRNA monitoring to build over time in both patient and clinical communities and facilitate implementation into routine practice, if warranted by the results.

Part A (phase II) will use a single-arm approach in which approximately 260 participants will undergo miRNA monitoring alongside standard surveillance with the aim of demonstrating that miRNA exceeds a pre-specified level of sensitivity for relapse detection.

If warranted by results from part A, 558 participants will be enrolled in part B (phase III) and will be randomly allocated 2:1 to miRNA monitoring with triggered imaging only (no routine imaging) OR standard surveillance imaging. Control arm data will be augmented by data from a previous trial in this setting, utilising Bayesian methods, as an efficient approach which allows for a smaller within-trial control group[32-34].

Inclusion of two biologically-similar tumour types (seminoma and dysgerminoma) within a single protocol will allow evidence to be gained for a rare tumour type (dysgerminoma, where a separate, fully-powered trial would not be feasible), through assessing consistency with the seminoma cohort in terms of primary and secondary endpoints[32].

3.1. Primary Endpoint

Part A: Sensitivity of miRNA for relapse detection defined as the number of relapses detected on miRNA before or at the same time as detection on imaging (or via other components of standard surveillance) as a proportion of the total number of relapses identified via standard investigations.

Part B: The number of relapses of mass size ≥3cm or clinical stage (CS) III.

3.2. Secondary Endpoints

Part A:

- Lead time afforded by use of miRNA for relapse detection (time of relapse detection by miRNA relative to time of relapse detection on imaging)
- Specificity of miRNA for relapse detection
- Time from blood draw to miRNA results being available to the clinical team and patient, and the number of results available within a 2-week target period
- Adherence to both miRNA monitoring and surveillance imaging schedules
- Acceptability of miRNA monitoring to patients (patient-reported, using a trial-specific questionnaire based on a validated, generic instrument for evaluating acceptability of healthcare interventions[35,36])

Part B:

- Cost-effectiveness based on patient-completed questionnaires (EQ-5D-5L/EQ-5D-Y-5L, resource-use) supplemented by data from national routine data sources to identify secondary care NHS services, as well as capturing significant medical events and deaths in the longer term
- Radiation exposure (estimated based on number and type of imaging investigations)
- Adherence to both miRNA monitoring and surveillance imaging schedules
- Acceptability of miRNA monitoring to patients (patient-reported)*
- Patient-reported health-related quality of life, anxiety and fear of recurrence (using EORTC survivorship module, SURV100, Fear of Recurrence short form, FCR4)[37 38]
- Disease-free survival
- Treatment for relapse
- Overall survival
- Incidence of second malignancies

*The extent and methods for evaluation of acceptability during part B will be dependent on findings relating to acceptability during part A.

4. PARTICIPANT SELECTION & ELIGIBILITY

4.1. Number of Participants

The aim is to recruit 818 participants into OTIS-S; approximately 260 participants into part A and 558 into part B.

4.2. Source of Participants

Participants will be recruited from approximately 20 participating sites in the UK. Potential participants will be identified in oncology clinics and discussed at Multi-Disciplinary Team (MDT) meetings. Eligibility criteria are the same for both Part A and Part B. Depending on part A recruitment rates, further sites may be opened for part B in the UK as well as internationally (subject to local approvals and funding).

ICR-CTSU encourages investigators to consider equality, diversity and inclusion when recruiting participants into its trials, to ensure that everyone eligible is offered the opportunity to consider participation. Note that there are no age restrictions since the trial may be relevant to children, teenagers and young adults, and older adults.

Protocol waivers will not be permitted.

4.3. Inclusion Criteria

1. Histologically confirmed testicular pure seminoma or extra-cranial pure dysgerminoma*
2. Primary cancer managed by orchiectomy or complete surgical removal of dysgerminoma (trial entry is permitted within 8 weeks following surgery, up to a maximum of 10 weeks under exceptional circumstances).
3. Stage I based on CT or MRI abdomen and pelvis and CT chest. For dysgerminoma participants under the age of 19, pelvic ultrasound (US) may be used at physician's discretion where MRI is not possible or where US is used routinely at site.
4. Normal post-operative tumour markers as follows (may have been raised pre-operatively and immediately post-operatively, but have returned to normal range):
    a. AFP≤10 ng/ml [AFP >10 and ≤25 ng/ml and confirmed as not rising on three successive tests may be accepted as eligible after discussion with Chief Investigator or delegate]
    b. β-HCG≤4 IU/L
    c. LDH<1.2 x upper limit of normal
5. No adjuvant therapy planned.
6. Participant (or, where the participant is under 16 years old, their parent or guardian) has given written informed consent prior to any study-specific procedures.

*Patients with any non-seminomatous elements, including teratoma, are not eligible

4.4. Exclusion Criteria

1. Previous contralateral testis tumour within 3 years of trial entry.
2. Previous or concurrent illness or condition which, in the investigator's opinion, would interfere with participation in the trial.
3. Pregnancy (pregnancy can be excluded on the basis of β-HCG result).
4. Unable/unwilling to comply with trial visit schedule/trial assessments.

5. SCREENING

5.1. Screening Log

All participating sites will be required to keep a log of all participants with stage I seminoma or dysgerminoma that are potentially eligible for this study. The information collected on the log will include:

- Date potential participant identified
- Screening outcome (approached/accepted participation/declined participation)
- Reasons for not approaching / declining participation (if available)
- Trial ID (if applicable)

This information will be used by the Trial Management Group (TMG) to monitor recruitment activity. No participant identifiable data will be sent to ICR-CTSU at this stage.

5.2. Procedure for Receiving Informed Consent

The Principal Investigator (or designated individual) must ensure that each trial participant (and/or the participant's parent/guardian if patient is under 16), is fully informed about the nature and objectives of the trial and possible risks associated with participation. The appropriate current ethics approved OTIS-S participant information sheet (PIS) should be provided for their consideration. Potential participants (or their parent/guardian for patients under 16) should only be asked to provide informed written consent to the study after they have had sufficient time to consider the trial and the opportunity to ask any further questions. Where appropriate, potential participants under 16 should also be given the opportunity to assent to the trial.

Remote consent is permitted providing the following steps are taken:

- A member of the research team should contact the potential participant (and/or the patient's parent/guardian if patient is under 16) to introduce the trial and to organise a telephone appointment with an Investigator in order for the Investigator to discuss the trial with the potential participant in detail.
- A copy of the appropriate current ethics approved PIS and Informed Consent Form (ICF) should be sent to the potential participant (and/or the patient's parent/guardian if patient is under 16) by email or post ahead of the scheduled appointment so that they have sufficient time to review the trial information. (If sending by post, two copies should be sent – one for the patient to retain and one to return to the site).
- During the telephone appointment the Investigator should complete the informed consent process remotely, discussing the study and ensuring that the potential participant (and/or the patient's parent/guardian if patient is under 16) is fully informed.
- If the potential participant (or the patient's parent/guardian if patient is under 16) agrees to consent the Investigator should ask them to initial the sections of the ICF and sign and date two copies. They should ask for one copy to be emailed/posted back to the site as soon as possible.
- The Investigator should record the date of verbal consent in the participant's clinical notes and confirm to the participant (and/or the patient's parent/guardian if patient is under 16) that this consent has been noted.

- The ICF should be received from the participant (or their parent/guardian) within a week of consent. Sites should follow up on any consent forms not received until they have been received, signed and filed.
- On receipt of the signed copy of the ICF from the participant (or the patient's parent/guardian if patient is under 16) the consenting Investigator should add their signature and date of signing and place in the patient file.
- A completed copy should be emailed/posted to the participant (or the patient's parent/guardian if patient is under 16) for their records.

No protocol-required assessments should be conducted until the OTIS-S consent form has been signed and dated by both the participant (or parent/guardian for participants under 16) and the Investigator, unless they are performed routinely as part of standard patient care.

Confirmation of the participant's consent (or parent/guardian's consent for patients under 16) and the informed consent process must be documented in the patient's medical notes. A copy of the signed consent form should be provided to the participant (or parent/guardian for patients under 16) and the original retained in the investigator site file, which must be available for verification by ICR-CTSU study staff or for regulatory inspection at any time.

Participants entering the trial, aged under 16 with parent/guardian's consent, who turn 16 years old during the trial will need to be reconsented using the appropriate participant information sheet/consent form. This new consent must be documented in the patient's medical notes. A copy of the new signed consent form should be provided to the participant and the original retained in the investigator site file, which must be available for verification by ICR-CTSU study staff or for regulatory inspection at any time.

5.3. Participation in other Clinical Trials

Patients who fulfil the eligibility criteria will be given the opportunity to participate in OTIS-S if they have previously participated in other clinical trials prior to recruitment.

Participation in other research should be discussed with the OTIS-S Trial Manager prior to co-enrolment.

6. TRIAL ENTRY

6.1. PART A

This section describes trial entry procedures during Part A. Procedures for trial entry and randomisation during part B will be confirmed in a protocol amendment prior to the commencement of Part B.

No protocol required assessments should be conducted until the OTIS-S consent form has been signed and dated by both the participant and the Investigator unless the assessments are performed routinely as part of standard patient care.

Trial enrolment will be performed by site staff who will log onto the trial database to register the participant. An eligibility checklist must be completed prior to registration.

The following information will be required at registration:

- Name of hospital, consultant and person registering participant
- Confirmation that participant (or parent/guardian if appropriate) has given written informed consent for trial participation

- Confirmation that participant is eligible for the trial by completion of the eligibility checklist
- Participant's full name, hospital number, date of birth, postcode, NHS/CHI number
- Participant postal or email address (depending on participant' choice for the provision of PRO questionnaires)

Site staff should register the participant by logging onto the trial database, selecting OTIS-S and completing the questions. The Trial ID will be issued, and the system will confirm participant entry into the trial.

As this is an electronic system, sites will have access at all times, but the OTIS-S trial team will only be available between 09.00-17:00 (UK time) Monday to Friday, excluding bank Holidays.

In Part A, all registered OTIS-S participants will undergo serum miRNA monitoring alongside standard surveillance of MRI/CT *(see section 9* - STUDY INTERVENTION*).*

7. TRIAL ASSESSMENTS

The following sections describe trial assessment during Part A. Assessments during part B of the trial will be similar to part A, except that patients will either have the miRNA monitoring (with no routine cross-sectional imaging) or the cross-sectional imaging (no miRNA monitoring) according to random allocation. Both groups will have regular routine markers as in the part A schedule. There will be some additional patient-completed questionnaires (see section 8). Full details of Part B assessments will be confirmed in a protocol amendment prior to commencement of Part B.

7.1. Post Trial Entry Assessments

7.1.1. PART A

The following assessments should be conducted within 14 days **after** registration:

- Medical history
- Physical examination
- Routine tumour markers: AFP, β-HCG and LDH (investigations undertaken as part of standard care within 3 weeks prior to registration are acceptable)
- Radiological assessment as follows (investigations undertaken as part of standard care within 6 weeks prior to registration are acceptable). Ideally the same imaging modality should be used for surveillance throughout the trial.:
    - ***For seminoma participants:*** MRI/CT abdomen as per local practice.
    - ***For dysgerminoma participants***: MRI and/or abdominal ultrasound.
- Collection of blood sample for miRNA analysis (See section 9 - STUDY INTERVENTION)

7.2. Surveillance Assessments

7.2.1. PART A

The following assessments should be conducted at the time points indicated. For scheduling purposes, a 2-week window either side of the actual time point is acceptable. Imaging should be performed within 2 weeks either side of the blood sample being taken for miRNA analysis.

3 months (m), 6m, 9m, 12m, 15m, 18m, 21m, 24m, 30m, 36m, 42m, 48m, 54m and 60m after registration in the trial.

- Routine tumour markers (AFP, β-HCG and LDH)
- Review/Reporting of SAEs as appropriate (see section 10)
- Collection of blood sample for miRNA analysis (except at 54m)* - described further in section 10
- **Required radiological assessments** (Ideally the same imaging modality should be used for surveillance throughout the trial.):

    - ***For seminoma participants:*** Cross-sectional abdominal imaging at 6m, 18m and 36m, preferably with MRI but CT can be used according to local practice.
    - ***For dysgerminoma participants:*** Cross-sectional abdominal+/-pelvic imaging at 6m, 18m and 36m, preferably with MRI. For participants under the age of 19, pelvic ultrasound (US) may be used at physician's discretion where MRI is not possible or where US is used routinely at site.
- **Additional imaging and assessments according to local practice**: In addition to the required radiological assessments above, the following can also be performed according to local practice:
    - cross-sectional imaging (abdominal+/-pelvic MRI or CT) can be performed at up to 4 further time points over the 5-year follow-up period
    - Physical examination
    - For seminoma, chest x-ray can be performed as per local practice
    - For dysgerminoma participants, low dose chest CT and transvaginal US can be performed as per local practice.
- Acceptability questionnaire to be completed by participant at 12m** (for participants 16 years old and over only)

*Note: miRNA samples taken at 9m, 15m, 21m and 42m will be analysed retrospectively in case of relapse only, and results will not be fed back to centres in real time.

**Note: Acceptability questionnaire (16 and over only) emailed or posted to participant's home address directly from ICR-CTSU, based on participant's preference, but site teams are asked to remind the participant about completion in order to maximise return rates.

7.3. Procedure for follow-up of positive routine tumour marker or finding on physical exam

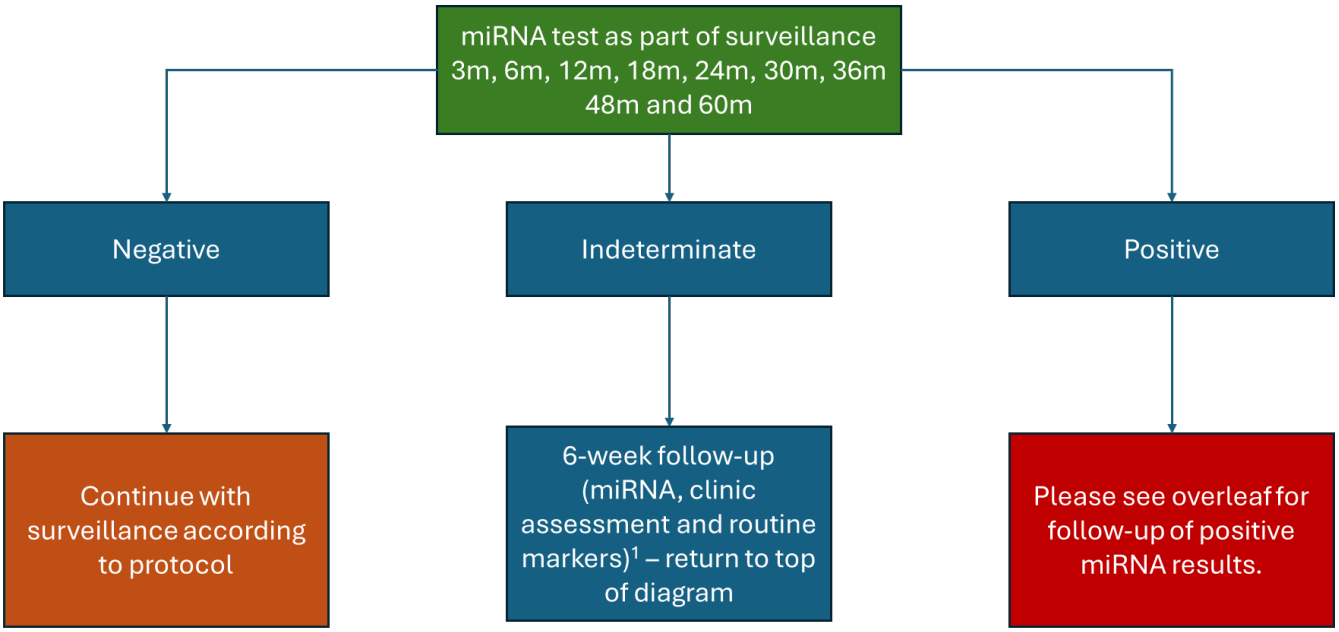
Participants found to have raised markers (according to one or more of the criteria below) should have all tumour markers repeated within 1-4 weeks. If persistently elevated, this should be further investigated as per section 7.4.2:

- β-HCG>4 IU/L
- AFP >10 (if ≤10 at baseline) OR increase of >25% above baseline (where >10 at baseline)
- LDH >1.2 x upper limit of normal

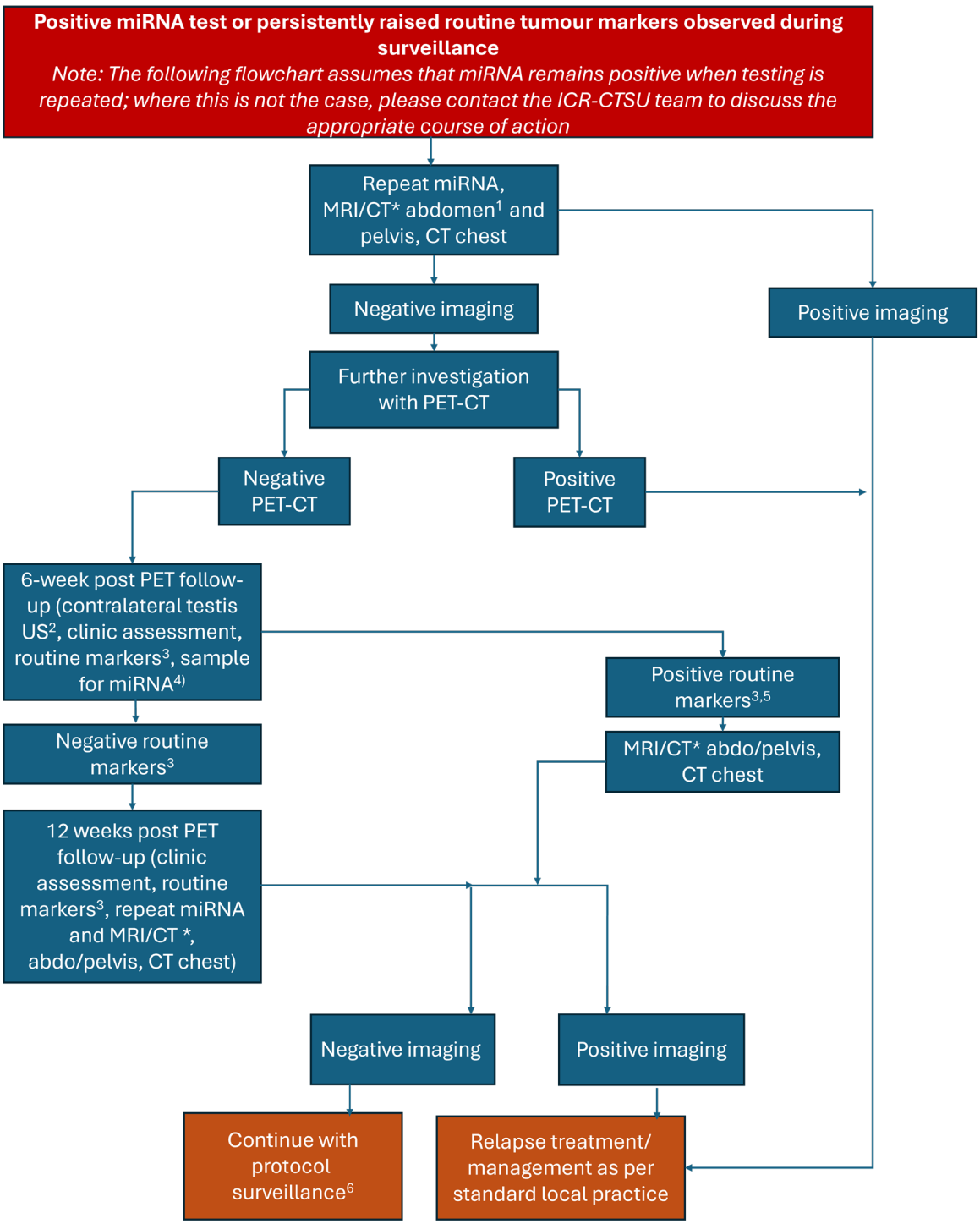
Any abnormal finding on physical examination should be followed up as per local practice.

7.4. Procedure for follow-up of different miRNA results

Please refer to the flowchart below describing follow-up procedures according to miRNA results. In the event of a negative result, surveillance should continue as described in section 7.2.



1. AFP, β-HCG and LDH



* For dysgerminoma patients under the age of 19 US may be used at physician's discretion.

1. This would be the routine surveillance MRI/CT if the positive miRNA result occurred at a time point when routine imaging was planned (6, 18 or 36m).
2. US results managed as per local practice.
3. 1. AFP, β-HCG and LDH
4. miRNA sample to be analysed retrospectively.
5. Confirmed on repeat within 4 weeks.
6. In cases where the miRNA remains positive and no other scans are planned within the routine surveillance schedule, MRI/CT should be repeated 6 months after the 3m follow-up scan. If the miRNA remain positive with negative imaging, please contact the trial team to discuss.

7.4.1. Procedure on receipt of an indeterminate miRNA result

If the miRNA test returns an indeterminate result, participants should be followed up after 6 weeks with the following assessments:

- Physical examination
- Routine tumour markers (AFP, β-HCG and LDH)
- Collection of blood sample for miRNA analysis

Results of the miRNA test (and other investigations) will then determine subsequent management as per the flowchart above.

7.4.2. Procedure on receipt of a positive miRNA result or routine tumour marker

If the miRNA test returns a positive result or routine markers are positive (and remain positive on repeat testing as per section 7.3), the potential relapse should be confirmed by MRI or CT (as per local practice, ideally the same imaging modality should be used throughout the trial) of the abdomen and pelvis and CT of the chest. For dysgerminoma participants under the age of 19 US may be used at physician's discretion. A repeat miRNA test should also be performed.

- If imaging is positive, procedures at disease recurrence, as described in section 7.6, should be followed.
- If the imaging is negative, further investigation should be conducted with 18 Fluoro-deoxy glucose (FDG) PET-CT. Ultrasound of contralateral scrotal testis should also be considered in seminoma participants.
- If the FDG PET-CT is negative, participants should be assessed 6 weeks after that scan with the following assessment:
    - Routine tumour markers (AFP, β-HCG and LDH)
    - Collection of blood sample for miRNA analysis (to be analysed retrospectively)
    - For seminoma patients, ultrasound of contralateral scrotal testis if not performed at time of PET-CT.
- If the routine tumour markers are elevated, this should be confirmed as described in section 7.3, and then imaging should be repeated (MRI or CT of the abdomen and pelvis and CT of the chest, Ideally the same imaging modality should be used for surveillance throughout the trial).
- If the routine tumour markers are not elevated, participants should be assessed again after a further 6 weeks (i.e. 12 weeks after PET-CT) with the following assessments:
    - Physical examination
    - Routine tumour markers (AFP, β-HCG and LDH)
    - Collection of blood sample for miRNA analysis
    - Imaging (chest CT and with either MRI or CT abdomen and pelvis as per local practice, consider including sanctuary sites such as brain). For dysgerminoma participants under the age of 19, US may be used at physician's discretion.

In the event that the miRNA result returns to normal during repeat testing as part of the above process, please contact the trial team to discuss ongoing management on a case-by-case basis.

In the event that miRNA remains positive, but relapse has not been confirmed on imaging as part of the above process, this should also be discussed with the trial team to determine appropriate management on a case-by-case basis. Where no more surveillance imaging is scheduled (i.e. >36 months post-randomisation),

it may be appropriate to schedule a further imaging investigation 6 months after previous scan (i.e. approximately 9m after the first positive miRNA result).

Please note that the investigations detailed above are the minimum requirements for trial purposes; other investigations can be carried out on a case-by-case basis as deemed appropriate by the local investigator.

7.5. Procedure for follow-up of positive or equivocal findings on surveillance imaging

In the case of relapse detection on surveillance imaging, procedures for disease recurrence should be followed as below (section 7.6) regardless of miRNA results. In the event that a recent miRNA test has not been performed (i.e. within +/- 2 weeks of imaging), a sample should be collected for this purpose as soon as possible.

In the case of an equivocal result on imaging, imaging should be repeated at 3 months or as per local practice.

7.6. Procedure at Disease recurrence

On confirmation of disease recurrence by imaging, participants should receive relapse treatment/management as per standard of care.

At the time of disease recurrence, participants will discontinue protocol surveillance but follow-up data collection documenting relapse details, relapse treatment, any subsequent relapse/progression, and survival should continue unless the participant has explicitly withdrawn consent for this. Follow-up should be approximately 6-monthly, but can be timed to align with routine care visits.

7.7. Change in Participation Status

Participants may choose to change, reduce or stop their participation after joining the trial. Changes may also be made at the discretion of the Principal Investigator. If a participant chooses to change their participation in OTIS-S, the local investigator should explore what level of engagement the participant is willing to continue (with a preference for collecting as complete data as possible).

Within OTIS-S the following changes in participation are possible:

- Stopping miRNA surveillance - but agrees to data being submitted from trial visits.
- Stopping trial-specific follow up – but agrees to data being submitted from routine visits/medical records.
- Stopping donated samples being used in OTIS-S research/analysis (this only applies to miRNA samples used for retrospective analysis).
- Stopping future sharing of data/samples.
- Withdrawal of consent for any further data to be submitted – data up to the point of withdrawal will be retained as described in the participant information sheet.

Changes in participation should be led by the participant and no assumptions should be made on their behalf. A change in participation status form should be submitted to ICR-CTSU to report the details of the reduction in participation. For further guidance on the types of change in participation status, and guidance on loss of contact, please refer to trial guidance notes.

Note that, if a patient becomes pregnant during participation in the trial, they are able to continue in the trial (unless they wish to withdraw as above). However, this should be discussed with the Chief Investigator or delegate to agree appropriate adaptations to the imaging protocol.

7.8. Schedule of Assessments

The following table describes the trial assessments during Part A. Full details of required assessments during Part B of the trial will be confirmed in a protocol amendment prior to commencement of Part B. Part B assessment schedules will be similar, except that patients will have miRNA monitoring (without routine radiological assessment) or standard imaging-based surveillance (no miRNA monitoring) according to random allocation. Full details will be confirmed in a protocol amendment prior to commencement of Part B.

7.8.1. PART A

		Timepoint post registration													
Visit/Assessment	Registration[3]	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months	30 months	36 months	42 months	48 months	54 months	60 months	Disease recurrence
Medical history	X															
Physical examination	X															
Tumour markers (AFP, β-HCG, LDH)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
miRNA blood sample[1]	X	X	X	X[4]	X	X[4]	X	X[4]	X	X	X	X[4]	X		X	X[4,5]
Radiological assessment[2]	X		X				X				X					
Serious Adverse Event assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PRO questionnaires (Acceptability questionnaire)					X[6]											
Radiological scan and report upload																X

1. BD Vacutainer™ SST™ II Advance Tubes, 5ml tube.
2. Cross-sectional abdominal imaging (MRI preferred). For dysgerminoma participants under 19, US may be used at physician's discretion. Some additional imaging, as detailed in section 7.2, in accordance with local practice is permitted. Ideally the same imaging modality should be used for surveillance throughout the trial.
3. Assessments to be completed within 14 days after participant registered into OTIS-S (see section 7.1.1 for exceptions).
4. Samples analysed retrospectively in event of relapse; results not sent to centres in real time.
5. miRNA sample to be taken if not performed within 2 weeks of the confirmatory imaging.
6. Acceptability questionnaire (16 and over only) emailed or posted to participant's home address directly from ICR-CTSU, based on participant's preference.

8. PATIENT-REPORTED OUTCOMES

8.1. Administration of (e)PRO booklets

Participants will be asked at trial entry whether they prefer electronic completion or paper completion of the questionnaire. The PRO questionnaire will only be administered to participants 16 and over only.

Paper PRO completion
Paper versions of questionnaires will be sent out to the participant's address by the ICR-CTSU team. A pre-paid envelope will be included for the participant to return the questionnaire to the OTIS-S trials office.

Electronic PRO completion (ePRO)
Participants who have indicated a preference for electronic completion will be provided with login details and will receive email notifications and reminders when the questionnaire needs completing.

8.2. Part A PRO Schedule

During Part A the only PRO assessment is an acceptability questionnaire to be completed by participants at 12 months post-registration.

8.3. Part B PRO schedule

Additional PRO assessments will be added during Part B of the trial, aimed at assessing the impact of different monitoring approaches on anxiety, fear of recurrence and health-related quality of life. Full details of the PRO schedule will be confirmed in a protocol amendment prior to commencement of Part B. Administration will be coordinated by the ICR-CTSU following the process described above.

9. STUDY INTERVENTION

9.1. miRNA blood sample collection

All blood samples taken for miRNA testing will comprise of 1 blood sample (5ml) collected in 1 tube at each time point. The majority of blood samples will be taken at the same time as routine blood collection.

9.1.1. PART A Sample scheduling

Blood samples for miRNA testing will be collected at all protocol-mandated visits, with the exception of the 54-month visit, timed from registration as follows: registration, 3 months (m), 6m, 9m, 12m, 15m, 18m, 21m, 24m, 30m, 36m, 42m, 48m, 60m.

Samples collected at registration, 3m, 6m, 12m, 18m, 24m, 30m, 36m, 48m and 60m will be analysed in real time and results fed back to the centre. Samples collected at the other time points will be analysed retrospectively at the end of Part A for participants who relapsed during the trial. Results from these samples will not be fed back but will be used to provide more detailed information of the potential lead time for relapse detection on miRNA when compared with imaging.

9.1.2. Storage, labelling and postage of miRNA blood samples

All OTIS-S trial samples should be collected, processed, labelled, stored and shipped as detailed in the OTIS-S Laboratory Manual. All samples must be labelled with the participant's unique trial identifier (Trial ID, as applicable), date of birth and date of sample collection to enable cross-referencing.

9.1.3. Reporting of miRNA blood sample results to centres

For all samples analysed in real-time, miRNA results will be available on the dedicated section of the trial clinical database normally within 2 weeks of the samples being received by the central lab. More details on the data entry by the lab and access procedures for the centres will be available in the trial guidance notes.

9.2. Imaging

For participants who relapse and participants with a positive miRNA result not confirmed by imaging, copies of the MRI/CTs performed throughout the course of the trial will be collected for central review. Centres will transfer the anonymised scans and associated radiology reports by IEP (Image Exchange Portal) to their designated reviewer as described in the OTIS-S trial guidance notes. Ideally the same imaging modality should be used for surveillance throughout the trial.

9.3. Supportive care

All medication considered necessary for the participants' welfare and which is not expected to interfere with the evaluation of the intervention may be given at the discretion of the investigator. All medications (including start/stop dates, dose frequency, route of administration and indication), must be recorded in the participant's clinic notes, as well as the appropriate pages of the eCRF.

10. Safety Reporting

As the study intervention in OTIS-S is limited to blood draws, imaging scans and questionnaire completion, reporting requirements for OTIS-S are limited to direct study-related procedure SAEs as defined in Section 10.1.

10.1. Definitions

Adverse Event (AE)
An AE is any untoward medical occurrence in a trial participant receiving a study intervention; the event does not necessarily have a causal relationship with the procedure. Study interventions for OTIS-S are limited to blood draws and scans, i.e. CT, MRI or ultrasound scans. Adverse events not meeting the criteria of serious will not be collected.

Serious Adverse Event (SAE)
An SAE is any untoward medical occurrence that occurs from baseline and within 30 days of the last study intervention (in OTIS-S this will be the blood draws and imaging assessments performed during follow-up) and:

- results in death

- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect

Important adverse events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, may also be considered serious.

Progression of the indicated disease and death due to progression of the indicated disease are not considered SAEs.

Definitions of causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial procedure.
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial procedure). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial procedure). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

Related Unexpected Serious Adverse Event (RU-SAE)
An adverse event that meets the definition of serious and is assessed by the CI or nominative representative as:

- "Related" – that is, it resulted from administration of any of the research procedures, and
- "Unexpected" - for the purposes of OTIS-S all serious and related adverse events will be considered unexpected

10.2. Reporting Related Serious Adverse Events to ICR-CTSU

Safety reporting requirements for OTIS-S are limited to only related (to study procedure) SAEs as defined in Section 10.1. Study procedures are limited to the imaging interventions and the blood draws for the miRNA testing. Any related SAE that occurs from the time of baseline imaging and up to 30 days following the last blood draw must be reported.

The severity of AEs should be graded according to the NCI-CTCAE criteria version 5.

Whenever one or more sign/symptom corresponds to a disease or a well-defined syndrome only the main disease/syndrome should be reported.

All related SAEs should be reported to ICR-CTSU within 24 hours of the Principal Investigator (or designated representative) becoming aware of the event, by completing the OTIS-S SAE form in the trial database. As much information as possible, including the Principal Investigator's assessment of causality, must be reported to ICR-CTSU in the first instance. Additional follow-up information should be reported as soon as it is available.

All SAE forms must be completed, signed and dated by the Principal Investigator or designated representative.

Data on serious adverse events that do not meet the above definition of a related SAE will not be collected.

10.3. Review of Related Serious Adverse Events

The Chief Investigator (or designated representative) will assess all reported related SAEs for causality (NB. The Chief Investigator cannot down-grade the Principal Investigator's assessment of causality) and, for OTIS-S, all serious and related AEs will be considered unexpected.

RU-SAEs will undergo expedited reporting to the relevant authorities and all other interested parties by ICR-CTSU.

Sites should respond as soon as possible to requests from the Chief Investigator or designated representative (via ICR-CTSU) for further information that may be required for final assessment of a related SAE.

10.4. Expedited Reporting of Related Unexpected SAEs

All RU-SAEs will be reported by ICR-CTSU to the main REC, the Sponsor and all other interested parties within 15 days of being notified of the event.

The Principal Investigators at all actively recruiting sites will be informed of RU-SAEs occurring within the trial at appropriate intervals.

10.5. Follow up of Serious Adverse Events

The PI should actively seek follow up information on reported SAEs. All related SAEs should be followed up until clinical recovery is complete or until disease has stabilised. Related SAE outcomes should be reported to ICR-CTSU using the relevant section of the SAE form as soon as the Principal Investigator or designee becomes aware of the outcome.

10.6. Reporting Pregnancies

If any trial participant becomes pregnant while taking part in the OTIS-S trial, this should be reported to ICR-CTSU using the pregnancy reporting form. Participants who become pregnant should be discussed with the Chief Investigator or delegate and may continue in the trial but with adaptations to the imaging protocol as appropriate. The participant should not be exposed to ionising radiation until the pregnancy is completed. Pregnancies should be followed up until conclusion and all follow-up information should be reported to ICR-CTSU. If the outcome of the pregnancy meets the definition of serious (i.e. congenital abnormality)

this should be reported to ICR-CTSU following the serious adverse event reporting procedures described above.

11. STATISTICAL CONSIDERATIONS

11.1. Statistical Design, Intervention Allocation and Sample Size Justification

11.1.1. Part A Statistical Design

Part A will use a phase II, A'Hern single arm design[37] with all participants receiving the intervention (miRNA monitoring) alongside standard surveillance imaging.

The aim is to demonstrate that miRNA sensitivity exceeds a pre-specified threshold and, hence, sample size is driven by the number of relapses. The sample size is based on the combined cohort (seminoma and dysgerminoma), since differences are not anticipated, and this will allow more timely progression to Part B.

Sensitivity of miRNA for relapse detection is expected to be at least 95% based on previous studies[30]. Sensitivity >75% is deemed appropriate to warrant further study (i.e. progression to Part B) and, ultimately, the aim is to demonstrate that sensitivity exceeds 85%. For this phase II evaluation, a one-sided alpha of 0.2 will be used. The higher "false positive" risk associated with using a higher alpha than might traditionally be used will facilitate a timely decision regarding initiation of the Part B which will provide a confirmatory evaluation. Subsequent analyses of the Part A cohort, when more relapses have been observed, will achieve greater precision in estimating sensitivity.

In order to have 80% power to show sensitivity >75%, with one-sided alpha of 0.2, a total of 11 relapses need to be observed. If at least 10 of these are correctly identified on miRNA at the same time or before being identified on imaging, a sensitivity ≤75% could be excluded. Two further analyses will be performed when 21 and 28 relapses have occurred with the aim of showing sensitivity >80% and >85% respectively, requiring at least 19 of 21 and at least 26 of 28 relapses to be detected on miRNA. Power for both of these analyses (using one-sided alpha of 0.2) will be >80%. Calculations were performed using Stata version 17.0.

In order to determine required sample size, projected numbers of relapses have been based on TRISST relapse patterns and recruitment rates; 12% of participants are expected to relapse, nearly all within 36 months[16]. Although relapse rates may be slightly higher for dysgerminoma, these patients are expected to only make up a very small proportion of the cohort[38]. In order to achieve a relatively seamless transition to Part B, recruitment to part A will continue up until 11 relapses are observed at which point the primary analysis, aimed at demonstrating sensitivity >75%, will be performed. The expected sample size to be recruited during this period is **approximately 260 participants**. Further follow-up of this cohort will allow the later, updated analyses to be performed, increasing the precision with which sensitivity can be estimated.

11.1.2. Part B Statistical Design

Part B will use a phase III, randomised design to test the non-inferiority hypothesis that there is no unacceptable increase in risk of advanced relapse (≥3cm or CSIII) when miRNA monitoring is used in place of standard surveillance imaging.

Seminoma participants will be allocated 2:1 to miRNA monitoring (intervention) or standard surveillance imaging (control) using computer-generated random permuted blocks.

Randomisation will be stratified by surveillance imaging type (MRI/CT), age group (≤29, 30-39, ≥40 years) and prognostic group (low, intermediate or high, based on European Association for Urology risk classification[39]). Dysgerminoma participants will be allocated 1:1 between miRNA monitoring or standard surveillance imaging (control) with randomisation stratified by age group only (≤18 years or ≥19 years).

As for Part A, the primary analysis will consider the combined seminoma and dysgerminoma cohort but, for this confirmatory component, a separate analysis of the seminoma cohort will also be adequately powered. For the dysgerminoma cohort, where there is more uncertainty around event rate and it would not be feasible to achieve a fully powered cohort, evidence pertaining to this group will be based on consistency of outcomes (primary and secondary) between the two cohorts.

Based on the TRISST trial, 4.5% of seminoma patients are expected to relapse with advanced disease on a 3-scan imaging schedule[16]. Part B will be designed to have 80% power to exclude a doubling of this rate (non-inferiority margin 4.5%) with a one-sided 5% alpha. Allowing for a 15% dropout rate (again, based on TRISST), this requires 345 seminoma participants per arm based on a 1:1 randomisation. This was calculated in Stata version 17.0 using the ART (Analysis of Resources for Trials) package, binary version 2.1.0.

A 1:1 randomisation would lead to a prohibitively large sample size; however, the TRISST trial provides robust data on outcomes in a large, contemporary cohort undergoing standard surveillance, in a population expected to be very similar to the seminoma participants recruited to OTIS-S. This data will be used to supplement control data from within OTIS-S, allowing for a smaller within-trial control arm[33,40]. As such, **518 seminoma participants** will be allocated 2:1 to miRNA (n=345) or standard surveillance (n=173). Since previous datasets on outcomes for dysgerminoma patients are less robust, allocation will be 1:1 for this group. **40 dysgerminoma participants** are expected to be recruited in the timeframe required for seminoma recruitment. Thus, the **total target sample size for part B is 558**.

11.2. Endpoint Definitions

11.2.1. Part A Primary Endpoint

The primary endpoint for Part A is sensitivity of miRNA for relapse detection, defined as the number of relapses detectable on miRNA testing before or at the same time as detection on imaging (or via other components of standard surveillance) as a proportion of the total number of relapses identified via standard investigations. In all cases, date of detection will be taken to be the date of the sample collection or imaging/investigation (rather than the date the results were available) and a 2-week window will be allowed from detection on imaging/standard investigations to allow for scheduling of miRNA testing sometimes occurring after imaging. Detection on miRNA requires a positive result according to lab-specific normal ranges, and the date of sample collection will be taken to be the date of relapse detection.

11.2.2. Part A Secondary Endpoints

The following secondary endpoints will be evaluated during part A:

- Lead time for relapse detection on miRNA, defined as time between relapse detection on miRNA (date sample taken) and relapse detection on imaging (date of scan).

- Specificity of miRNA for relapse detection, defined as number of non-relapsing participants (based on imaging) whose miRNA levels remained negative (or indeterminate) throughout the follow-up period, as a proportion of all non-relapsing participants.
- Time from blood draw for miRNA testing to miRNA results being available to the clinical team (and participant), and the number (proportion) of results available within 2 weeks.
- Adherence to miRNA testing, defined as the number of samples taken for miRNA testing as a proportion of all scheduled tests; numbers of tests performed more than a 4-week window either side of the schedule time point and number of samples that could not be analysed will also be reported.
- Adherence to imaging schedules defined as the number of attendances for imaging as a proportion of all scheduled imaging; the number of scans performed; numbers of scans performed more than a 4-week window either side of the schedule time point will also be reported.
- Patient-reported acceptability of miRNA monitoring without routine surveillance imaging (using a trial-specific questionnaire based on a validated, generic instrument for evaluating acceptability of healthcare interventions[35,36]).

11.2.3. Part B Primary Endpoint

The primary endpoint for Part B is the number of advanced relapses, defined as those with mass size (maximum short axis diameter) ≥3cm and/or clinical stage III. The time point of interest is 3 years and Kaplan-Meier estimation will be used, with censoring at the last disease assessment for those participants lost to follow-up before 3 years.

11.2.4. Part B Secondary Endpoints

The following secondary endpoints will be evaluated during part B:

- Number and type of imaging investigations performed
- Patient-reported acceptability of the different surveillance approaches
- Patient-reported anxiety and fear of recurrence (from Fear of Recurrence short form, FCR4[41])
- Patient-reported health-related quality of life (from EORTC survivorship module, SURV100[42])
- Disease-free survival (DFS), defined as time from randomisation to disease recurrence or death from any cause with observations censored at most recent follow-up assessment for participants who remain alive and disease-free
- Overall survival (OS), defined as time from randomisation to death from any cause with observations censored at date last known to be alive for surviving participants
- Treatment for relapse
- Incidence of second malignancies
- Cost-effectiveness based on patient-reported health status and health resource usage

11.3. Statistical Analysis Plan

All statistical analyses will be performed at the ICR-CTSU. Planned analyses for Part A are outlined here in brief. Details of the planned analyses in Part B, including timing and analysis sets, will be added via an amendment prior to commencement of Part B. Full details of analysis methods will be specified in Statistical Analysis Plans (SAPs) for Part A and Part B in accordance with ICR-CTSU Standard Operating Procedures.

The Part B health economics analysis will be carried out by the University of York. A summary of the planned analyses will be added to the protocol prior to commencement of Part B and will be detailed in full within a separate Health Economics Analysis Plan (HEAP).

11.3.1. Part A Analysis Timing and Decision Rules

The primary analysis for part A will be performed when 11 relapses (confirmed on imaging) have been observed. This analysis will focus on miRNA sensitivity, feasibility, adherence and acceptability. If ≥10/11 relapses were identified by positive miRNA results, at the same time or prior to diagnosis on imaging, and there are no concerns based on secondary endpoints, the trial will proceed to Part B.

An updated analysis of the Part A cohort will take place when 21 relapses have accrued, to provide additional reassurance early on during Part B recruitment. A final analysis will take place when all Part A participants have been followed up for 36 months at which time 28 relapses are expected to have been observed. Further secondary endpoints, including specificity and lead time will be analysed at this time. Results of these analyses will be reviewed by the Independent Data Monitoring Committee (IDMC), in the first instance, and they will make recommendations about continuation of Part B. For continuation of Part B (and in accordance with the design, as described in 11.1.1), it is expected that at least 19 of the first 21 relapses and at least 26 of the first 28 relapses will be identified on miRNA (at the same time or before detection on imaging). However, IDMC recommendations will also consider secondary endpoint data available at this time.

11.3.2. Part A Analysis Sets

All Part A participants will be included in the analysis; however, analyses of specific endpoints will be dependent on availability of data. In particular, the estimation of sensitivity will include all patients with a confirmed relapse on imaging and a miRNA test performed within 2 weeks of detection on imaging. Seminoma and dysgerminoma patients will be analysed together, but tumour type-specific estimates for sensitivity, specificity and lead time will also be reported and considered when interpreting results. However, the dysgerminoma cohort is expected to be very small and this analysis will be considered exploratory.

11.3.3. Part A Analysis Plan

Part A analyses will be largely descriptive. Sensitivity of miRNA will be presented with a 60% exact binomial confidence interval (CI), reflecting the design; 95% CIs will also be presented.

Time from blood draw to miRNA results being available will be summarised (median, interquartile range, range), both overall and by centre, and the proportion of results available within 2 weeks will be presented with a 95% CI. Adherence to miRNA testing and surveillance imaging will be presented as number (proportion) for each time point and also summarised at the patient level (i.e. number missing at least one test or scan). MiRNA results at each time

point will be summarised (negative, positive, or indeterminate) as well as the numbers of samples that could not be analysed.

Acceptability questionnaires will be scored and analysed using the methods described by Sekhon et al[35]. Items assessing burden and opportunity costs will be reverse scored so that a higher score always indicates higher acceptability. For each patient, a single acceptability score will be computed as the total mean score for the seven items from the theoretical framework of acceptability; the general acceptability item and any trial-specific items will be reported separately. This will be a descriptive non-comparative analysis, reporting mean and standard deviation (or median and IQR if appropriate) for the composite acceptability score and, for individual items, the number and proportion of patients responding in each category. Responses to the general acceptability item will be correlated with individual items from the framework.

For the final analysis, specificity will additionally be presented with a 95% exact binomial confidence interval. Lead time (from detection on miRNA to detection on imaging) will be calculated considering miRNA results from additional samples, analysed retrospectively in relapsing patients. Central review of imaging for relapsing patients will be used to confirm the time that relapse detection was possible and will inform supplementary analyses considering the impact on estimates of sensitivity and specificity.

The Part A analysis will also incorporate comparison of patient and tumour characteristics of the seminoma cohort with the TRISST trial cohort to confirm the extent of heterogeneity and inform planned analyses in Part B.

11.4. Interim Analyses and Stopping Rules

An Independent Data Monitoring Committee (IDMC) will be formed and will confidentially review accumulating data on primary and secondary endpoints.

Prior to the primary analysis for Part A, relapses within the trial will be monitored on an ongoing basis by the IDMC. If, before 11 relapses detected on routine imaging have occurred, >1 relapse is not correctly identified on miRNA, and noting that participant safety is not compromised (all Part A participants have routine imaging), the oversight committees will consider the value of continued recruitment to support planned analyses of all Part A endpoints.

For each of the planned analyses in Part A (described in 11.2), the IDMC will review results and make recommendations to the Trial Steering Committee (TSC) regarding continuation of the trial, considering the pre-specified progression criteria outlined in 11.3.1.

During Part B, the IDMC will continue to monitor accumulating data at regular intervals (at least annually). Given the non-inferiority hypothesis, and that primary endpoint events will accrue slowly relative to recruitment timescales, early stopping on the basis of efficacy is not expected to be relevant and so no formal interim analysis or stopping rules are in place. However, at each review, the IDMC will consider the balance of risks and benefits in the accumulating data, as well as any emerging external evidence, in making recommendations to the TSC regarding continuation of the trial.

12. TRIAL MANAGEMENT

12.1. Trial Management Group (TMG)

A Trial Management Group (TMG) will be set up and will include the Chief Investigator, ICR-CTSU Methodology Lead, Co-investigators and identified collaborators, the Trial Statistician and Clinical Trial Manager. Principal Investigators and key study personnel will be invited to join the TMG as appropriate to ensure representation from a range of sites and professional groups. Where possible, membership will include a lay/consumer representative. The TMG will meet at regular intervals, and at least annually. Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG have operational responsibility for the conduct of the trial. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

12.2. Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be set up and will comprise an independent Chairperson and at least two further independent members with clinical or statistical expertise (at least one member will be a statistician). The TSC will meet at regular intervals, and at least annually. The TSC will provide expert independent oversight of the trial on behalf of the Sponsor and funder(s). The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

12.3. Independent Data Monitoring Committee (IDMC)

An Independent Data Monitoring Committee (IDMC) will be set up to monitor the progress of the trial and will comprise a Chairperson and at least two further members with clinical or statistical expertise (at least one member will be a statistician). Membership of the IDMC will be proposed by the TMG and approved by the TSC.

The IDMC will meet in confidence at regular intervals, and at least annually. A summary of findings and any recommendations will be produced following each meeting. This summary will be submitted to the TMG, TSC and, if required, the main REC.

The IDMC will reserve the right to release any data on outcomes through the TSC to the TMG (and if appropriate to participants) if it determines at any stage that the combined evidence from this and other studies justifies it.

The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

13. RESEARCH GOVERNANCE

13.1. Sponsor Responsibilities

The Sponsor of this clinical trial is the Institute of Cancer Research (ICR). The Sponsor's Committee for Clinical Research conducted scientific peer review as part of the approval process.

13.2. Participating Site Responsibilities

Responsibilities delegated to participating sites are defined in an agreement between the Sponsor and the individual site. The Principal Investigator is responsible for the trial team and trial conduct at the participating site.

14. TRIAL ADMINISTRATION & LOGISTICS

14.1. Site Activation

Before activating the trial, participating sites are required confirm R&D capacity and capability and to sign an agreement accepting responsibility for all trial activity which takes place within their site.

Sites may commence recruitment once the site agreement has been signed by all required signatories, the required trial documentation is in place (as specified by ICR-CTSU) and a site initiation (visit or teleconference) has taken place. Site initiation visits will be conducted at sites where the Principal Investigator has requested one or where ICR-CTSU deems it is appropriate.

14.2. Data Acquisition

Electronic case report forms (eCRFs) will be used for the collection of trial data and will be purpose built within the GCP-compliant MEDRIO database. ICR-CTSU will provide guidance to sites to aid the completion of the eCRFs. The Trial Management Group reserves the right to amend or add to the eCRFs as appropriate. Such changes do not constitute a protocol amendment and revised or additional forms should be used by sites in accordance with the guidelines provided by ICR-CTSU.

14.3. Central Data Monitoring

Once data has been entered on the eCRF by the site personnel, ICR-CTSU will review it for compliance with the protocol, and for inconsistent or missing data. Should any missing data or data anomalies be found, queries will be raised for resolution by the site.

Any systematic inconsistencies identified through central data monitoring may trigger an on-site monitoring visit.

14.4. On-Site Monitoring

If a monitoring visit is required, ICR-CTSU will contact the site to arrange the visit. Once a date has been confirmed, the site should ensure that full participant's clinical notes, including electronic notes, of participants selected for source data verification are available for monitoring.

ICR-CTSU staff conducting on-site monitoring will review essential documentation and carry out source data verification to confirm compliance with the protocol. If any problems are identified during the course of the monitoring visit, ICR-CTSU will work with the Principal Investigator or delegated individual to resolve issues and determine appropriate action.

14.5. Completion of the Study and Definition of Study End Date

The study end date is deemed to be the date of last data capture.

14.6. Archiving

Essential trial documents should be retained according to local policy and for a sufficient period for possible inspection by the regulatory authorities (at least 5 years after the date of last data capture). Documents should be securely stored and access restricted to authorised personnel.

15. PARTICIPANT PROTECTION AND ETHICAL CONSIDERATIONS

15.1. Risk Assessment and Approval

This trial has been formally assessed for risk and approved by the Sponsor's Committee for Clinical Research.

15.2. Public and Patient Involvement

Patient advocate contributors were involved in protocol design including methodology, sample collection, participant information and consent forms and are represented on the TMG.

15.3. Ethics Approvals

The trial will not commence at any participating site until the required approvals are in place. ICR-CTSU, on behalf of the Sponsor, will ensure that the trial has received an independent favourable opinion from a research ethics committee (REC) for multi-centre trials, as well as HRA approval and relevant NHS Permissions. Before recruiting patients, the Principal Investigator at each site is responsible for obtaining local approvals.

15.4. Trial Conduct

This trial will be conducted according to the approved protocol and its amendments, supplementary guidance and manuals supplied by the Sponsor and in accordance with the UK Policy Framework for Health and Social Care and the principles of GCP.

15.5. Informed Consent

The Principal Investigator retains overall responsibility for the conduct of research at their site; this includes the taking of informed consent of participants. They must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to do so in accordance with the ethically approved protocol, principles of Good Clinical Practice and Declaration of Helsinki.

Potential participants (or their parent/guardian, where appropriate) should be asked to sign the appropriate version of the current ethics approved OTIS-S consent form at trial entry after receiving both verbal and written information about the trial, having been given sufficient time to consider this information. All consent forms must be countersigned by the Principal Investigator or a designated individual. A signature log of delegated responsibilities, listing the designated individuals and the circumstances under which they may countersign consent

forms, must be maintained at the participating site. This log, together with original copies of all signed informed consent forms, should be retained in the Site Investigator File and must be available for inspection. The current ethics approved OTIS-S participants information sheets should be provided in addition to any standard patient information sheets that are provided by the site and used in routine practice.

15.6. Participant Confidentiality

Participants will be asked to consent to their full name being collected at trial entry in addition to their date of birth, hospital number, postcode and NHS number or equivalent to allow linkage with routinely collected NHS data and ensure accuracy in handling biological samples. Additionally, participants will be asked to consent to their postal or email address to be collected for the purpose of the administration of the PRO questionnaires.

Each investigator should keep a separate log of all participants' Trial IDs, names, addresses and hospital numbers. The investigator must retain trial documents (e.g. participants' written consent forms) in strict confidence. The investigator must ensure the participants' confidentiality is maintained at all times.

Representatives of ICR-CTSU will require access to participants' hospital notes for quality assurance purposes. ICR-CTSU will maintain the confidentiality of participants at all times and will not reproduce or disclose any information by which participants could be identified.

15.7. Data Protection

All investigators and trial staff must comply with the Data Protection Act 2018 at all times.

15.8. Liability

Indemnity to meet the potential legal liability of investigators participating in this trial is provided by the usual NHS indemnity arrangements.

16. FINANCIAL MATTERS

This trial is investigator designed and led. ICR has received funding from Cancer Research UK for the central coordination of the trial. The trial meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England. The trial is part of the National Institute for Health Research Research Delivery Network (NIHR RDN) portfolio by virtue of its funding by an NIHR non-commercial partner. NIHR RDN resources should therefore be made available for the trial to cover UK specific research costs.

17. PUBLICATION POLICY

The main trial results will be published in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, consisting of members of the TMG. Participating clinicians may be selected to join the writing group on the basis of intellectual and time input. All participating clinicians will be acknowledged in the publication.

Any presentations and publications relating to the trial must be authorised by the TMG. Authorship of any secondary publications, e.g. those relating to sub-studies, will reflect

intellectual and time input into these studies. Authorship of all publication will usually be in accordance with ICMJE guidance.

No investigator may present or attempt to publish data relating to the OTIS-S trial without prior permission from the TMG.

It is an expectation that all publications relating to the trial are published as “open-access”.

A1. GLOSSARY

AE	Adverse Event
AFP	Alpha-Fetoprotein
AUC	Area Under Curve
CI	Chief Investigator
CT	Computed Tomography
eCRF	Electronic Case Report Form
DCF	Data Capture Form
FBC	Full Blood Count
HCG	Human Chorionic Gonadotropin
HR	Hazard Ratio
ICR	The Institute Of Cancer Research
IDMC	Independent Data Monitoring Committee
ICMJE	International Committee of Medical Journal Editors
LDH	Lactate dehydrogenase
LFT	Liver Function Test
MDT	Multi-disciplinary team
MiRNA	MicroRNA
MRI	Magnetic Resonance Imaging
PET-CT	Positron Emission Tomography and Computed Tomography
PI	Principal Investigator
PIS	Participant Information Sheet
PRO	Patient Reported Outcomes
R&D	Research and Development
RCT	Randomised controlled trial
RPLND	Retroperitoneal lymph node dissection
SAE	Serious Adverse Event
RU-SAE	Related and Unexpected Serious Adverse Event
TMG	Trial Management Group
TSC	Trial Steering Committee
U+E	Urea & Electrolytes
US	Ultrasound
WBC	White Blood Cells

A2. HISTORY OF PROTOCOL AMENDMENTS

PROTOCOL VERSION AND DATE	SUMMARY OF CHANGES
v.1.1	**Format, punctuation and spelling correction.**
V 1.2	**Administrative changes (addition of logo, trial identifiers), minor clarifications**

18. REFERENCES

1. Cancer Research UK. Testicular cancer incidence statistics (2016-2018). https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/testicular-cancer/incidence (accessed June 2024).
2. Horwich A, Shipley J, Huddart R. Testicular germ-cell cancer. *Lancet* 2006; **367**(9512): 754-65.
3. Smith HO, Berwick M, Verschraegen CF, et al. Incidence and survival rates for female malignant germ cell tumors. *Obstet Gynecol* 2006; **107**(5): 1075-85.
4. Berney DM, Stoneham S, Arora R, Shamash J, Lockley M. Ovarian germ cell tumour classification: views from the testis. *Histopathology* 2020; **76**(1): 25-36.
5. Bloom JC, Loehr AR, Schimenti JC, Weiss RS. Germline genome protection: implications for gamete quality and germ cell tumorigenesis. *Andrology* 2019; **7**(4): 516-26.
6. Shah R, Xia C, Krailo M, et al. Is carboplatin-based chemotherapy as effective as cisplatin-based chemotherapy in the treatment of advanced-stage dysgerminoma in children, adolescents and young adults? *Gynecol Oncol* 2018; **150**(2): 253-60.
7. Beyer J, Collette L, Sauve N, et al. Survival and New Prognosticators in Metastatic Seminoma: Results From the IGCCCG-Update Consortium. *J Clin Oncol* 2021; **39**(14): 1553-62.
8. Singhera M, Lees K, Huddart R, Horwich A. Minimizing toxicity in early-stage testicular cancer treatment. *Expert Rev Anticancer Ther* 2012; **12**(2): 185-93.
9. Kerns SL, Fung C, Monahan PO, et al. Cumulative Burden of Morbidity Among Testicular Cancer Survivors After Standard Cisplatin-Based Chemotherapy: A Multi-Institutional Study. *J Clin Oncol* 2018; **36**(15): 1505-12.
10. Murray MJ, Huddart RA, Coleman N. The present and future of serum diagnostic tests for testicular germ cell tumours. *Nat Rev Urol* 2016; **13**(12): 715-25.
11. Chung P, Warde P. Stage I Seminoma: Adjuvant Treatment is Effective but is it Necessary? *JNCI: Journal of the National Cancer Institute* 2011; **103**(3): 194-6.
12. Ray-Coquard I, Morice P, Lorusso D, et al. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; **29**(Suppl 4): iv1-iv18.
13. Sessa C, Schneider DT, Planchamp F, et al. ESGO-SIOPE guidelines for the management of adolescents and young adults with non-epithelial ovarian cancers. *Lancet Oncol* 2020; **21**(7): e360-e8.
14. Fern LA, Greenwood M, Smith S, et al. Pre-Implementation Assessment of the Acceptability of Using Circulating microRNAs for Follow-Up of Malignant Germ-Cell Tumors. *Clinical Genitourinary Cancer* 2021; **19**(5): 381-7.
15. Berrington de Gonzalez A, Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. *Lancet* 2004; **363**(9406): 345-51.
16. Joffe JK, Cafferty FH, Murphy L, et al. Imaging Modality and Frequency in Surveillance of Stage I Seminoma Testicular Cancer: Results From a Randomized, Phase III, Noninferiority Trial (TRISST). *J Clin Oncol* 2022; **40**(22): 2468-78.
17. Aldiss S, Fern LA, Phillips RS, et al. Research priorities for young people with cancer: a UK priority setting partnership with the James Lind Alliance. *BMJ Open* 2019; **9**(8): e028119.
18. Charytonowicz D, Aubrey H, Bell C, et al. Cost Analysis of Noninvasive Blood-Based MicroRNA Testing Versus CT Scans for Follow-up in Patients With Testicular Germ-Cell Tumors. *Clin Genitourin Cancer* 2019; **17**(4): e733-e44.
19. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**(7043): 834-8.
20. Palmer RD, Murray MJ, Saini HK, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer Res* 2010; **70**(7): 2911-23.

21. Murray MJ, Halsall DJ, Hook CE, Williams DM, Nicholson JC, Coleman N. Identification of microRNAs From the miR-371~373 and miR-302 clusters as potential serum biomarkers of malignant germ cell tumors. *Am J Clin Pathol* 2011; **135**(1): 119-25.
22. Murray MJ, Bell E, Raby KL, et al. A pipeline to quantify serum and cerebrospinal fluid microRNAs for diagnosis and detection of relapse in paediatric malignant germ-cell tumours. *Br J Cancer* 2016; **114**(2): 151-62.
23. Murray MJ, Ajithkumar T, Harris F, et al. Clinical utility of circulating miR-371a-3p for the management of patients with intracranial malignant germ cell tumors. *Neurooncol Adv* 2020; **2**(1): vdaa048.
24. Leao R, Albersen M, Looijenga LHJ, et al. Circulating MicroRNAs, the Next-Generation Serum Biomarkers in Testicular Germ Cell Tumours: A Systematic Review. *Eur Urol* 2021; **80**(4): 456-66.
25. Murray MJ, Coleman N. Can circulating microRNAs solve clinical dilemmas in testicular germ cell malignancy? *Nat Rev Urol* 2019; **16**(9): 505-6.
26. Murray MJ, Coleman N. MicroRNA Dysregulation in Malignant Germ Cell Tumors: More Than a Biomarker? *J Clin Oncol* 2019; **37**(16): 1432-5.
27. Murray MJ, Scarpini CG, Coleman N. Serum tumour markers for testicular cancer recurrence. *Nat Rev Urol* 2023.
28. Dieckmann KP, Radtke A, Geczi L, et al. Serum Levels of MicroRNA-371a-3p (M371 Test) as a New Biomarker of Testicular Germ Cell Tumors: Results of a Prospective Multicentric Study. *J Clin Oncol* 2019; **37**(16): 1412-23.
29. Piao J, Lafin JT, Scarpini CG, et al. A Multi-institutional Pooled Analysis Demonstrates That Circulating miR-371a-3p Alone is Sufficient for Testicular Malignant Germ Cell Tumor Diagnosis. *Clin Genitourin Cancer* 2021; **19**(6): 469-79.
30. Fankhauser CD, Christiansen AJ, Rothermundt C, et al. Detection of recurrences using serum miR-371a-3p during active surveillance in men with stage I testicular germ cell tumours. *Br J Cancer* 2022; **126**(8): 1140-4.
31. Lafin JT, Scarpini CG, Amini A, et al. Refining the serum miR-371a-3p test for viable germ cell tumor detection. *Sci Rep* 2023; **13**(1): 10558.
32. Billingham L, Malottki K, Steven N. Research methods to change clinical practice for patients with rare cancers. *Lancet Oncol* 2016; **17**(2): e70-e80.
33. Mackay EK, Springford A. Evaluating treatments in rare indications warrants a Bayesian approach. *Front Pharmacol* 2023; **14**: 1249611.
34. Richeldi L, Azuma A, Cottin V, et al. Trial of a Preferential Phosphodiesterase 4B Inhibitor for Idiopathic Pulmonary Fibrosis. *N Engl J Med* 2022; **386**(23): 2178-87.
35. Sekhon M, Cartwright M, Francis JJ. Development of a theory-informed questionnaire to assess the acceptability of healthcare interventions. *BMC Health Serv Res* 2022; **22**(1): 279.
36. Sekhon M, Cartwright M, Francis JJ. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Serv Res* 2017; **17**(1): 88.
37. A'Hern RP. Sample size tables for exact single-stage phase II designs. *Stat Med* 2001; **20**(6): 859-66.
38. Patterson DM, Murugaesu N, Holden L, Seckl MJ, Rustin GJ. A review of the close surveillance policy for stage I female germ cell tumors of the ovary and other sites. *Int J Gynecol Cancer* 2008; **18**(1): 43-50.
39. Boormans JL, Sylvester R, Anson-Cartwright L, et al. Prognostic Factor Risk Groups for Clinical Stage I Seminoma: An Individual Patient Data Analysis by the European Association of Urology Testicular Cancer Guidelines Panel and Guidelines Office. *Eur Urol Oncol* 2024; **7**(3): 537-43.
40. Neuenschwander B, Capkun-Niggli G, Branson M, Spiegelhalter DJ. Summarizing historical information on controls in clinical trials. *Clin Trials* 2010; **7**(1): 5-18.

41. Humphris GM, Watson E, Sharpe M, Ozakinci G. Unidimensional scales for fears of cancer recurrence and their psychometric properties: the FCR4 and FCR7. *Health Qual Life Outcomes* 2018; **16**(1): 30.
42. van Leeuwen M, Kieffer JM, Young TE, et al. Phase III study of the European Organisation for Research and Treatment of Cancer Quality of Life cancer survivorship core questionnaire. *J Cancer Surviv* 2023; **17**(4): 1111-30.