Improving outcomes through Collaboration in OsteosarComa

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Please note: This trial protocol must not be applied to patients treated outside the ICONIC study. Cancer Research UK & UCL Cancer Trials Centre (UCL CTC) can only ensure that approved study investigators are provided with amendments to the protocol.

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TABLE OF CONTENTS

1. PROTOCOL SUMMARY

1.1. Summary of Study Design

Title:	Improving outcomes through Collaboration in OsteosarComa
Short Title/acronym:	ICONIC
Sponsor name & reference:	University College London Reference: 18/0248
Funder name & reference:	Bone Cancer Research Trust
ISRCTN/Clinicaltrials.gov no:	NCT04132895
Design:	Prospective observational cohort study.
Overall aim:	To improve treatment and outcomes for patients with osteosarcoma (OS) by establishing a clinically annotated cohort of newly diagnosed patients with OS with longitudinal collection of biospecimens.
Stage 1 primary objective:	To determine the feasibility of recruiting patients to a national study with high quality biospecimen collection.
Stage 2 main objectives:	To enrol a minimum of 160 patients to address the following questions: • What is the impact of variation in management of patients with OS across treatment sites in the UK? • Do tumour margin and response to chemotherapy predict local recurrence in OS? • What effects do tumour heterogeneity and clonal evolution have on chemotherapy response and patient outcome and can this be used to stratify patients for therapy? • Can formalin-fixed paraffin-embedded (FFPE) samples be used to validate whole genome sequencing (WGS) findings, identify patients for specific therapies and aid development of novel therapeutic trials? • Can circulating biomarkers be used to predict burden of disease, response to therapy and outcome? • What is the value of the Sarcoma Assessment Measure (SAM) for longitudinal assessment of patient quality of life and to identify predictors of variance in patient reported outcome measures (PROMs)? • What are the routes and timescales of diagnostic pathways for patients with OS? • Can this framework be used as a platform to develop a therapeutic clinical trial in OS and rapidly investigate novel agents in specific populations?

Target accrual:	***Stage 1:*** Recruitment rate of ≥ 5 patients per month when 15 sites are open ***Stage 2:*** Minimum 160 patients recruited over 3 years (including those in stage 1)
Eligibility criteria:	***Inclusion criteria*** • New histological diagnosis of osteosarcoma or in the absence of osteoid seen on biopsy, pathology and imaging supportive of a diagnosis of osteosarcoma • Written informed consent of patient and/or parent/legal guardian ***Exclusion criteria*** • Diagnosis more than four months prior to registration
Number of sites:	At least 15 sites representing oncology and surgical sites across the UK
Treatment summary:	• Treatment will be given as per usual standard of care. • No treatments are specified by this protocol.
Patient study activities:	***Clinical data collection*** Clinical data will be collected on a regular basis. Data to be collected include baseline characteristics, treatment details, results of key investigations (including blood tests and scans), and patient status (including performance status) and outcome. ***Tissue and blood collection*** • Tissue for fresh freezing according to local policy and FFPE will be collected during routine procedures at diagnosis, at surgery and at relapse if clinically indicated. Optional study specific biopsies will be requested at relapse in cases where a biopsy is not clinically indicated. • Blood samples will be collected on patients receiving chemotherapy and surgery at the same timepoints as tissue, with an additional sample presurgery and further optional samples. ***Patient Reported Outcome Measures (PROMs)*** Patients ≥13 years of age will be requested to complete a Sarcoma Assessment Method (SAM) questionnaire along with TESS, EORTC-QLQ-C30 and Global rating of change (GRC) at diagnosis, annually and at relapse. ***Prediagnostic data collection*** • Patients ≥13 years will be requested to complete a questionnaire at diagnosis detailing symptoms and routes to diagnosis.

	• For patients <13 years, the parents/legal guardian will be requested to complete the questionnaire at diagnosis detailing symptoms and routes to diagnosis.

Central laboratory analyses:	***Tissue***
	Frozen tumour tissue will be analysed as part of the 100,000 Genomes project, or through NHS England where possible for whole genome sequencing, further analyses may be undertaken including RNA and epigenetic analysis as part of Osteosarcoma Research Consortium.
	
	FFPE tissue will be used to:
	• Determine feasibility of DNA/RNA extraction for validation studies
	• Validate findings from WGS and other analyses including molecular targeted sequencing and copy number analysis
	
	Blood
	Blood samples will be collected to:
	• Investigate germline alterations
	• Evaluate two circulating tumour cell (CTC) isolation and characterisation methods:
	  • Parsortix™ and DEPArray™
	  • Flow cytometry using MT1-MMP Ab
	• Be stored for potential methylation profiling, genetic analysis of circulating tumour (ct)DNA and future research

Duration of recruitment:	3 years
Duration of follow up:	Patients will be followed up for minimum of 1 year
Definition of end of study:	The end of study will be declared four years after the start of recruitment

1.2. Study Summary

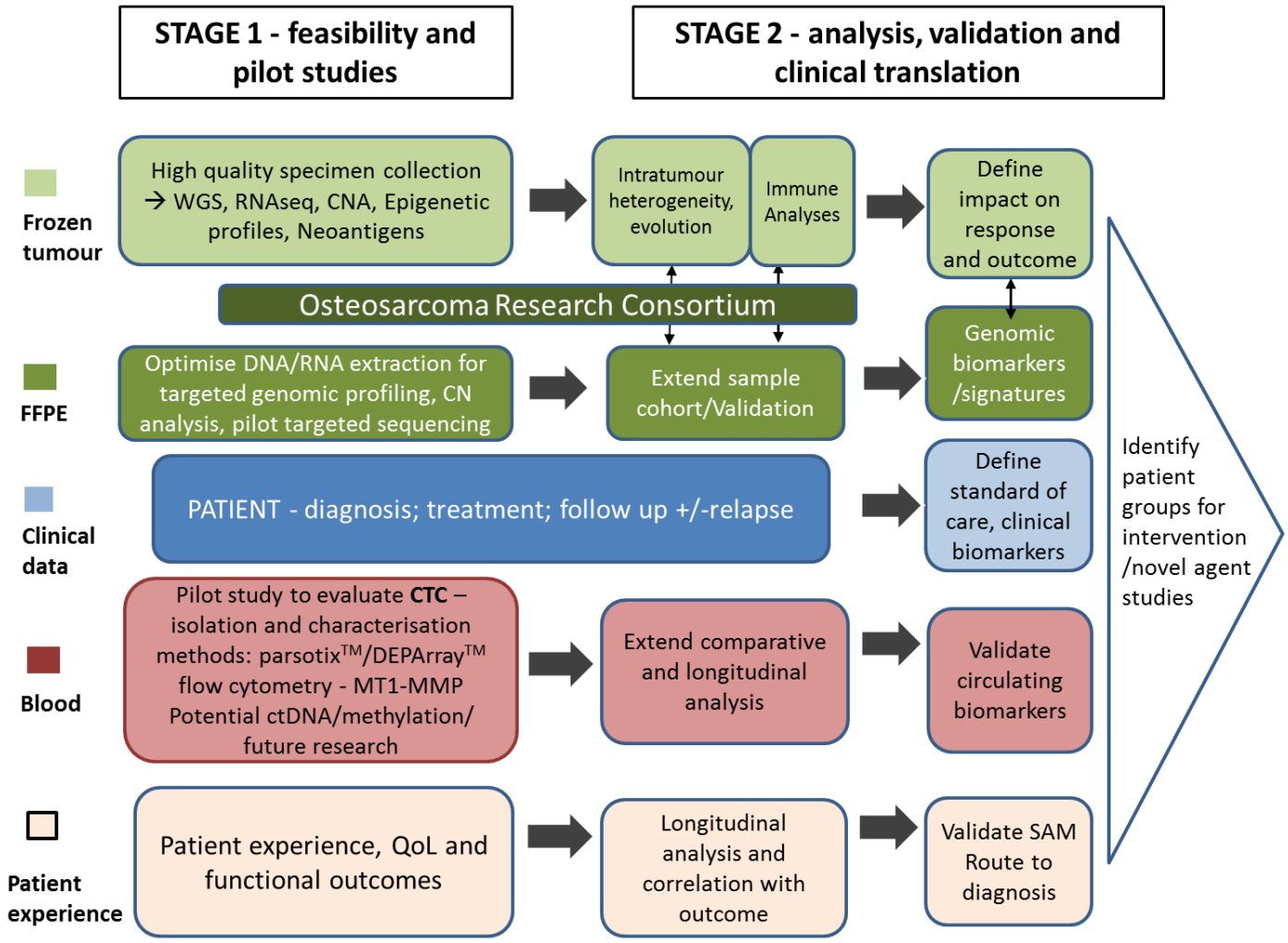

Figure 1: Summary Schema of ICONIC and output
FFPE = formalin-fixed paraffin-embedded,
WGS = whole genome sequencing,
CNA = copy number alterations,
CTC = circulating tumour cell,
PROM = patient reported outcome measures,
SAM = sarcoma assessment method.

2. BACKGROUND AND RATIONALE

Osteosarcoma (OS) is the most common bone sarcoma, accounting for approximately 30% of all bone sarcoma diagnoses. In England, it accounts for approximately 130 cases per year across all ages (Table 1). Age-specific overall survival incidence rates are generally bimodal, with the first peak in adolescence and a second peak in patients over 60 years of age. Treatment is multimodal, including multi-agent chemotherapy and surgery to the primary site and to metastases where appropriate. Outcome, however, has improved little over the past 25 years with 5-year survival rates remaining about 42% for all ages and stages in the UK. Five-year relative survival rates are significantly higher for patients <40 years of age (52%) compared with patients over 40 years (25%)[1]. Survival is also dependent on the primary site of disease, and is poor for patients with non-extremity tumours, at 36% for patients under the age of 40 years and as low as 6% for patients over 40 years. Outcome for patients with metastatic disease at diagnosis remains equally poor[2-5]. The use of chemotherapy and surgery varies according to factors such as age, primary site, stage and performance status with approximately two thirds currently receiving these treatments in England each year (Table 1).

Age (years)	< 40	%	40-59	%	60 +	%	Total No.	%
Total	**86**	**63**	**20**	**15**	**31**	**22**	**136**	**100**
Chemotherapy	70	82	13	65	6	19	89	66
Surgery	64	75	13	65	13	42	90	66

Table 1. Average number of patients diagnosed per year with OS receiving chemotherapy and/or surgery in England (2011-2015)[$]

[$](National Cancer Registration and Analysis Service, personal communication)

The most recent randomised trial in osteosarcoma, EURAMOS-1 (NCT00134030), was an excellent example of international collaboration to seek improved treatments for a rare cancer. Despite the participation of over 2000 patients, no changes to the standard of care for osteosarcoma resulted[6,7]. Thus, there continues to be an unmet need to identify new approaches, including biomarkers and novel, innovative and targeted therapeutic approaches if outcomes are to improve. This includes seeking improved systemic treatments and better approaches to management of the primary tumour. Failure of local control (local recurrence) is associated with high levels of morbidity and poor survival[8]. New imaging modalities such as CT-PET, MRI-PET and functional MRI (fMRI) could be useful to guide resection or detect local recurrence but there is insufficient evidence to identify a clear role for use in routine clinical practice[9]. Further prospective study of newly described prognostic systems and identification of other biomarkers of local failure are needed to assist in decision making about morbid surgery and to determine the role of technical advances in radiation oncology which may be of benefit to patients with OS[10].

OS clinical trials to date have focussed on a limited number of outcomes, usually in younger patients with localised extremity disease. Thus, addressing complex inter-related questions has not been possible and several sub-populations have been excluded from study so limiting opportunities to improve the standard of care. These include: those with widely metastatic disease; less common anatomic locations such as pelvis, spine and craniofacial bones; those arising on a background of skeletal dysplasia or underlying genetic predisposition; and finally, the 50% of OS arising in patients over 40 years. Little is currently known about factors influencing treatment decisions in this population and how consistently a standard of care is applied. The effect of treatment on quality of life (QOL), patient reported outcome and other

performance indicators is also not well described or understood. Overall, there is a need to broaden the ambition and scope of OS research while improving access for all patients.

2.1. Osteosarcoma biology and translational research

Therapeutic development in OS has been hindered by the heterogeneous nature of the disease and limited understanding of the molecular and cellular mechanisms that influence its pathogenesis and survival. Currently no molecular sub classification of OS exists and stratification of therapy based on genetic or molecular biomarkers is not possible. Research is under way to identify new biological insights but greater coordination and better linkage to clinical application will optimise the return and improve the delivery timelines of these efforts. In particular, access to systematically collected tissue linked to comprehensive clinical data for all OS is a pressing need.

2.2. Identification of genomic targets

Whole genome sequencing (WGS) and exome analysis is beginning to provide better insights into the genetic complexity and heterogeneity of OS. One such study, based on whole exome sequencing revealed a **BRCA-ness phenotype** that if validated and demonstrated to be functional could be used to identify patients for targeted therapy[11]. More recent data using whole genome analysis has however refuted this claim, with only one of 37 patients having an "HRD detect" phenotype, developed by the Sanger Institute as a measure of "BRCAness" thus further information is required to inform development of clinical trials[12]. The largest whole genome sequencing project to date, from the International Cancer Genome Consortium (ICGC), has identified distinct patterns of genomic rearrangement in OS but also recurrent mutation of **Insulin-like Growth Factor** (IGF) signalling genes, present in approximately 14% of patients[13]. IGFR inhibition has previously been investigated in sarcoma with a small proportion of patients benefitting, but no biomarker identifies those most likely to benefit and no studies are currently ongoing. **Fibroblast Growth Factor Receptor 1** (FGFR1) amplification appears to be related to chemoresistance in OS: 20% of patients with a poor histological response harbour the amplification[14]. These findings support further investigation in OS of these targets but prospective validation in diagnostic samples and correlation with outcome is now necessary to determine a role in stratification or therapy more clearly.

2.2.1. Intratumour heterogeneity and tumour evolution

Intratumour genetic heterogeneity, where different cells in the same tumour show distinct genomic profiles, is increasingly recognised as a cause of treatment failure, as it allows tumours to adapt and evolve to escape therapy[15]. A Cancer Research UK (CR UK) funded study in patients with primary lung cancer, TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx] (NCT01888601)) is studying the genomic landscape of lung cancer and how it changes through the disease course by taking multiple tumour and blood samples from patients at intervals from diagnosis to relapse[16]. This study has already significantly contributed to our understanding of cancer evolution, demonstrating that intratumour heterogeneity mediated through chromosome instability is associated with an increased risk of recurrence or death[16]. The study is also investigating the effects of heterogeneity on response to platinum chemotherapy and, more recently, enabled investigation into tumour interaction with the immune system with well publicised new discoveries that will support precision medicine[17]. This model is expected to be applicable to other cancer types, including OS, but requires significant investment.

Professor Flanagan is leading a project **'The Osteosarcoma Research Consortium (ORC)'** within UCL, funded by a generous donation from the Tom Prince Research Trust. This study is investigating multiple aspects of OS including genetic and epigenetic heterogeneity over space and time and has commenced

recruitment of patients from the London Sarcoma Service, The Robert Jones Agnes Hunt NHS Trust in Oswestry, and Ireland, and has aligned this study with the 100,000 Genomes project and ongoing sequencing through NHS England. The study includes WGS being performed on high quality patient samples including diagnostic biopsies, resection specimens (for multiple region WGS), and metastatic and relapse samples. Copy number alterations, RNA sequencing and processing of samples for epigenetic studies will be performed on the same samples in the Flanagan laboratory. Some tumour samples are also being prepared for single cell genomic analysis as a pilot project. The multi-'omic' analysis of the data will be undertaken by the research consortium comprising members of the Genomics England Clinical Interpretation Partnership (GeCIP) which at present includes Professor Campbell and Dr Bejhati – Wellcome Trust Sanger Institute (WTSI), Professor Stephan Beck and Dr Pillay – UCL Cancer Institute, Dr Van Loo – Francis Crick Institute, and clinicians involved in care of the patients. Data analysis of the osteosarcomas will be performed alongside that of the other 400-500 sarcomas submitted to the Genomics England 100,000 Genomes Project, the data of which became available in late 2018 with analysis expected to start in earnest in 2019. To date samples from 90 OS patients have been submitted for WGS and it is estimated that a further 50 patients will be recruited as part of this initiative which will provide the first detailed insights into OS heterogeneity and tumour evolution by 2020.

As part of the ORC, the immune landscape of OS is also being studied and specifically whether the genomic structural variation in OS is a source of neoantigens and therefore potential therapeutic targets. Associations will be sought between both the number and identity of neoantigens and phenotypic data available as well as their association with RNA expression profiles and host immune system response. This project will benefit from the CRUK Accelerator grant on Cancer ImmunoTherapy (CITA) in UCL working with Dr Herrero (Bill Lyons Informatics Centre) and Dr Quezeda, a world leading authority on immune checkpoint biology in cancer. This work is particularly relevant in view of disappointing initial results from checkpoint inhibitor trials in OS and will facilitate identification of patients for appropriate immune-based therapies.

Not all patients however have sufficient high quality frozen tissue for WGS, particularly from small diagnostic biopsies, and the above project is currently limited to patients recruited to the 100,000 Genomes project. Consistent coordinated collection of clinically annotated tumour samples within ICONIC will facilitate application for funding for analysis of additional samples and for validation studies on the basis of preliminary data. For patients without adequate frozen tumour samples, the potential to use formalin-fixed paraffin-embedded (FFPE) for targeted sequencing and validation studies, is desirable. Extraction of DNA/RNA from FFPE bone samples can be challenging but has been shown to be feasible as shown in our chondrosarcoma and chordoma genomic landscape manuscripts, although more samples could be made available if samples processing were optimised across the different centres[18,19].

2.2.2. MT1-MMP

MT1-MMP is under investigation as a potential biomarker and therapeutic target for OS. Preliminary analyses demonstrated that overexpression of MT1-MMP is present in OS preclinical models and patient tissues; that knockdown of MT1-MMP reduces invasive capability; and that immunohistochemical staining of MT1-MMP on OS biopsy specimens discriminates high grade from low grade OS (Kenny Rankin). His group in Newcastle are now currently investigating whether MT1-MMP can be used to detect circulating tumour cells (CTC) through a study involving three centres across the UK. This study has recruited 42 patients to date, and is now starting to optimise retention of potential CTC populations for genomic analysis. The clinical utility of this approach however, requires validation.

A novel molecule targeted to MT1-MMP, BT1718, has been developed by Bicycle Therapeutics in collaboration with Cancer Research UK, and a phase 1 clinical trial commenced in Feb 2018[20]. All solid cancers with overexpression of MT1-MMP are eligible for study entry, which represents an important

opportunity to enrol OS patients into a study that features companion diagnostics as the entry criteria. In addition, preclinical evaluation of an MT1-MMP activated theranostic has commenced in Newcastle University which will include *in vivo* evaluation of MRI enhancement in an orthotopic distal femur mouse bone sarcoma model. If successful this marker could also gain utility to monitor response to treatment. Samples from ICONIC can be used to validate the use of MT1-MMP to identify CTCs, as well as expression in tumour samples to determine suitability for upcoming clinical trials.

2.2.3. Circulating biomarkers

Measurement of circulating tumour cells (CTCs) and circulating tumour or cell free DNA (cfDNA: 'liquid biopsies') have found traction in many cancers, identifying patients with high risk disease, monitoring response to therapy, and detecting early relapsed disease. Isolation of CTCs in OS has been hampered by the lack of a specific antibody[21]. MT1-MMP offers one potential method; a different approach to isolating CTCs being optimised by Professor Heymann's group in Sheffield (Sarcoma Research Unit, European Associated Laboratory University of Sheffield/Inserm/University of Nantes) and funded by a BCRT Explorer Grant, uses the Parsortix™ System to enrich for CTCs, which are then isolated and captured using the DEPArray™ System[22]. The protocols are ready for clinical validation and a pilot study funded by Sarcoma UK to capture CTCs from patients with soft tissue sarcoma has begun recruiting (CIRCUS: A pilot study of CIRCUlating tumour cells in patients with soft tissue Sarcoma). Coordinated collection of blood samples from patients in ICONIC will enable this methodology to be validated efficiently in OS.

Professor Flanagan, in collaboration with the Wellcome Sanger Institute has been investigating the utility of cfDNA as a measure of burden of disease. The challenge to this methodology is that patient specific rearrangements identified in the primary tumour are required to quantify cfDNA in the blood. Results from this preliminary analysis are awaited. Another opportunity lies with the potential to use DNA methylation profiles in blood as biomarkers. The potential to collect serial blood samples for multiple analyses within the same cohort of patients provides the opportunity to compare different biomarker assays to better determine their clinical utility.

Patient serum biomarkers such as CRP, albumin and ALP, Hb and ANC:lymphocyte ratio, which are routinely collected, have been investigated as prognostic biomarkers. A novel composite score for sarcoma incorporating five biomarkers (Aarhus composite biomarker score: ACBS) has been shown to be associated with disease specific and overall survival in a retrospective analysis of patients with localised disease[23]. This is worthy of prospective validation in a wider cohort of patients.

2.3. Improving systemic therapy and introduction of new treatments

Improvements in systemic therapy for OS are needed urgently. The unmet needs which ICONIC addresses include: addressing variation in delivery of, and response to, standard therapies including chemotherapy and mifamurtide through evaluation of concordance with a national algorithm; identifying factors influencing decision making in specific subgroups such as those aged over 40 or with unresectable primary tumours; stimulating the development of new pilot therapeutic interventions in areas identified by analysis of clinical data on chemotherapy delivery and toxicity, especially for less common subgroups; promotion of access for individual patients to current trials through network impact; provision of the infrastructure shown to be necessary to provide a platform for new agent studies. Examples include: in another rare bone cancer, Ewing sarcoma, the novel multi-arm, multistage (MAMS) study: rEECur (ISRCTN36453794), supported by EU funding to rapidly evaluate treatments at recurrence and compare new agents to standard of care; in lung cancer, driven by the findings from TRACERx, DARWIN 1: Deciphering Afatinib Response and Resistance with INtratumour heterogeneity study (NCT02183883).

2.4. Patient Reported Outcome Measures

The introduction of patient reported outcome measures (PROMs) into clinical practice is known to improve patient-clinician communication and thus may impact on patient experiences and outcomes[24]. A sarcoma specific PROM, the Sarcoma Assessment Measure (SAM) has been developed for use in ICONIC with BCRT support. It covers the core domains of physical, emotional, social and financial wellbeing and sexuality so it is a comprehensive reflection of quality of life. The expected utility of SAM includes use as an endpoint in clinical trials as well as for comparing groups of patients over time. Validating the SAM as a longitudinal measure to detect changes in self-reported outcome over time linked to health status may extend its utility in informing clinicians of changes in health and as a secondary endpoint in future research evaluations.

2.5. Osteosarcoma and routes to diagnosis

Patients with OS often describe prolonged and complex pathways to diagnosis. How this impacts on outcomes and survival is not fully understood, however times to diagnosis have been shown to affect patient experience adversely in more common cancers[25]. The diagnostic experience of sarcoma patients has been shown to be inferior to those with other more common cancers: sarcoma patients are more likely to report multiple General Practitioner (GP) visits and be dissatisfied with the time taken to see a hospital doctor[26-28]. This requires further investigation and, in particular, examination of the role of the GP and secondary care professionals in the diagnosis of OS. Questionnaire data from patients and GPs together with clinical data will be triangulated to a provide comprehensive description of routes to diagnosis and key time intervals for patients with OS.

2.6. International perspective

EURAMOS-1 was a successful international randomised trial for osteosarcoma run by four study groups which sought to demonstrate survival benefits from interventions to change the standard of care. Other national study groups joined the EURAMOS core to develop successor trials but no testable question was identified and a recommendation was therefore made for national or cooperative study groups to work internally on hypothesis generating studies, some of which would then be suitable for future wider global evaluation. Although other European groups, notably In France and Italy, are establishing national translational networks for OS, in the UK, to date, no collaborative studies have been developed for OS which have a consistent approach to collection of samples with annotated clinical data. Networking and knowledge sharing between national groups is in operation, notably through forums such as the 4th European Bone Sarcoma Networking Meeting, London July 2017, and ongoing dialogue will be valuable going forward.

2.7. Benefit to Osteosarcoma Patients

This study will allow us to learn how to be more precise in selecting patients for different treatments, in order to reduce treatment morbidity and improve outcomes. ICONIC investigators will strive to provide an opportunity to all newly diagnosed patients with OS who enter this study to: donate tissue at diagnosis and beyond; contribute clinical information on treatment and outcomes, and participate in the longitudinal validation of a patient reported outcome measure. This will allow us to better understand OS biology to develop biomarkers and understand patient experience, which can be integrated with future treatment strategies.

We expect patients diagnosed with OS in the future to have the opportunity to take part in the hypothesis driven clinical trials driven by stratification created from the clinic-biological correlates exposed by the ICONIC investigations. Patient representatives from the NCRI Clinical Studies Group have contributed to the ideas underpinning ICONIC. We will extend patient involvement to encourage a new cadre of patient co-researchers to assist with priority setting and acceptability assessment.

3. STUDY DESIGN

This is a prospective observational cohort study in patients with osteosarcoma (OS). All newly diagnosed OS patients will be eligible to take part. Consenting patients will be recruited and followed up for at least one year.

The main aim of the study is to establish a platform which supports the recruitment of a consecutive cohort of newly diagnosed OS patients, whose clinical outcomes will be fully annotated and linked to their molecularly characterised biospecimens. The platform will support continuous collection, analysis and interrogation of data to generate hypothesis driven questions quickly for further evaluation. This project extends the Bone Cancer Research Trust tumour banking initiative with funding allocated to provide the infrastructure to optimise collection and storage of tissue samples in the 5 diagnostic and surgical bone tumour centres in England, and includes the development and validation of clinically relevant biomarkers to identify patients at high risk of relapse and patients suitable for therapeutic studies.

The study will be conducted in two stages. In **Stage 1**, we will establish the feasibility of patient recruitment and biospecimen collection, with initial biomarker validation in a subset of patients, as well as mechanisms for rapid interrogation of the clinical data**.** Subsequently, correlation with response to chemotherapy and outcome will help develop prognostic biomarkers and be an opportunity to identify patients for specific therapies (**Stage 2**). In the longer term, the study will maintain a platform for development of additional studies and development of therapeutic studies based on the findings from our initial work.

3.1. Study Objectives

3.1.1. Stage 1

To determine the feasibility of recruiting patients to a national study with high quality clinical data and biospecimen collection to address clinical and biological questions in osteosarcoma. Specific research objectives and associated analyses are detailed below.

Objectives	**Endpoint Analyses**
Recruitment	
1. Recruit patients across UK paediatric, TYA and adult centres to cohort study ➢ Open at least 15 sites representative across all age groups across UK ➢ Recruitment rate: 5 patients/month when 15 sites open	• Calculation of recruitment rate after opening of 15 sites • Tabulation of % patients recruited according to age and primary site

Objectives	Endpoint Analyses
Clinical data collection	
1. Determine patterns of treatment of patients across UK treatment sites ➢ Establish number and % of patients receiving systemic therapy across age cohorts ➢ Establish number and % patients undergoing surgery, ➢ Establish list of reasons for inoperability and their relative frequencies ➢ Establish current indications for RT and their relative frequencies	Tabulations of: • % patients with MDT documentation of rationale for planned treatment • % patients receiving chemotherapy and regimen • % patients undergoing surgery • % patients receiving radiotherapy and indications
2. Determine feasibility of surgical data capture on impact of surgery on complication rate and function ➢ Establish variation in reconstruction techniques ➢ Determine number and % patients with post-operative complications	Tabulations of: • possible reconstruction techniques and relative frequencies • % patients with post-operative complications
3. Imaging: Determine feasibility of establishment of an imaging repository to ➢ Evaluate conformance of primary tumour imaging and reporting of response to chemotherapy ➢ Evaluate variation in imaging modalities utilised for staging of OS patients	Tabulation • % of scans available for central radiology review • % of reports available for review • % patients staged with bone scan, PET/CT and/or WB–MRI
Biological data collection	
1. Sample collection of fresh frozen tissue across UK sites ➢ Collect diagnostic/resection specimens in 50% patients recruited	Calculation of • collection rate per site • number and % of metastatic/relapse specimens stored for future research • % of specimens sent for WGS
2. Determine quality of Formalin Fixed paraffin-embedded (FFPE) samples and feasibility of DNA/RNA extraction for validation studies ➢ Collect 80% of diagnostic/resection FFPE specimens in patients receiving chemotherapy and surgery	Calculation of collection rate of diagnostic/ resection FFPE specimens in patients treated with surgery and chemotherapy

Objectives	Endpoint Analyses
	• Audit to determine whether quality and quantity of RNA/DNA is sufficient for targeted sequencing and nanostring analysis
3. Evaluate whether the two methodologies of circulating tumour cell (CTC) detection can isolate and quantify CTCs in patients undergoing neo-adjuvant chemotherapy ➢ Collect pre and post neoadjuvant chemotherapy blood samples in 50 patients to evaluate isolation and quantification of CTC using DEPArray™ and flow cytometry	• Identification and quantification of CTC numbers at baseline (pretreatment) and after chemotherapy • Descriptive analysis of CTC numbers as histograms and ranges (min, max, IQR, median)
4. Collection of plasma for potential methylation/ctDNA/future research	• Calculation of collection rate per site
5. Evaluate collection of serum biomarkers for validation of prognostic scores ➢ Collect serum parameters in 90% patients	• Calculation of collection rate of serum parameters • Characterisation of distribution of serum biomarkers as histograms and ranges (min, max, IQR, median)
6. Determine feasibility of using standard pathology reporting to determine risk of local recurrence ➢ Collect pathology reports on 90% patients undergoing surgery	• Characterisation of tumour size, measurement of narrowest surgical margin status, pathological response to chemotherapy (% necrosis)
PROMs data collection	
1. Collect data for validation of a sarcoma assessment method (SAM) in patients undergoing treatment for OS ➢ Completion of SAM, TESS, EORTC QLQ-C30 and Global rating of change (GRC) in 60% of patients > 13 years at 2 time points	• Calculation of completion rate of SAM, TESS, EORTC QLQ-C30 and GRC. • Descriptive analyses of PROMs at both time points
Osteosarcoma and routes to diagnosis	
1. Determine feasibility of patients and GPs returning questionnaires ➢ All patients recruited given questionnaire ➢ GPs sent questionnaire	• % of patients returning questionnaires • % of GPs returning questionnaires

3.1.2. Stage 2

To enrol a minimum of 160 patients (including those enrolled in Stage 1) and to use the data and samples collected to begin to address the questions listed below. Note: following completion of stage 1 the research questions of stage 2 may be amended/adjusted to take into account previous findings:

- What is the impact of variation in management of patients with OS across treatment sites in the UK?
  - How are decisions reached for a tumour being inoperable? What criteria are used to determine inoperability? How do patients contribute to the final decision on surgical operability in terms of what surgery is acceptable?
  - What is the variation of use of chemotherapy across the UK and does it impact on outcome?
  - What are current indications for radiotherapy (RT) as part of local therapy, does use of RT with surgery improve local tumour control, and if so, which patients benefit?
- Do tumour margin and response to chemotherapy predict local recurrence in OS?
- What effects do tumour heterogeneity and clonal evolution have on chemotherapy response and patient outcome and can this be used to stratify patients for therapy?
- Can FFPE samples be used to validate WGS findings, identify patients for specific therapies and aid development of novel therapeutic trials?
- Can circulating biomarkers be used to predict burden of disease, response to therapy and outcome?
- What is the value of the Sarcoma Assessment Measure (SAM) for longitudinal assessment of patient quality of life? What are the routes and times to diagnosis for patients with OS, are there particular groups of patients at risk of prolonged/complex diagnostic routes, and what impact does this have on outcome?
- Can this framework be used as a platform to develop a therapeutic clinical trial in OS and rapidly investigate novel agents in specific populations?

3.2. Study Activation

UCL CTC will ensure that all study documentation has been reviewed and approved by all relevant bodies and that the following have been obtained prior to activating the study:

- Research Ethics Committee approval
- **'**Adoption' into NIHR portfolio
- NHS permission
- Adequate funding for central coordination
- Confirmation of sponsorship, and adequate insurance provision

4. SELECTION OF SITES/SITE INVESTIGATORS

4.1. Site Selection

In this protocol study 'site' refers to the hospital where study related activities are conducted.

Sites must be able to comply with:

- Clinical care, follow up schedules and all requirements of the study protocol
- Data collection requirements, including adherence to electronic data capture timelines as per section 11.4
- Sample collection, processing and storage requirements
- Monitoring requirements, as outlined in the protocol (section 14 and study monitoring plan)

4.1.1. Selection of Principal Investigator and other investigators at sites

Sites must appoint an appropriate Principal Investigator (PI), i.e. a health care professional authorised by the site to lead and coordinate the work of the study on behalf of the site. Coinvestigators must be trained and approved by the PI. All investigators must be medical doctors and have experience of treating osteosarcoma. The PI is responsible for the conduct of the study at their site and for ensuring that any amendments are implemented in a timely fashion. If a PI leaves/goes on a leave of absence, **UCL CTC must be informed promptly** and a new PI identified and appointed by the site.

4.1.2. Training requirements for site staff

All site staff must be appropriately qualified by education, training and experience to perform the study related duties allocated to them, which must be recorded on the site delegation log.

CVs for all staff must be kept up to date, signed and dated and copies held in the Investigator Site File (ISF). A current, signed copy of the CV with evidence of GCP training (or copy of GCP certificate) for the PI must be forwarded to UCL CTC upon request.

GCP training is required for all staff responsible for study activities. The frequency of repeat training may be dictated by the requirements of their employing institution, or 2 yearly where the institution has no policy, and more frequently when there have been updates to the legal or regulatory requirements for the conduct of clinical trials.

4.2. Site initiation and Activation

4.2.1. Site initiation

Before a site is activated, the UCL CTC trial team will arrange a site initiation with the site which the PI, and site research team must attend. The site will be trained in the day to day management of the study and essential documentation required for the study will be checked.

Site initiation will be performed for each site normally by teleconference. Re-initiating sites may be required where there has been a significant delay between initiation and enrolling the first patient, as per the monitoring plan.

4.2.2. Required documentation

The following documentation must be submitted by the site to UCL CTC prior to a site being activated by the UCL CTC trial team:

- Study specific Site Registration Form (identifying relevant local staff)
- Relevant institutional approvals
- A completed site delegation log that is initialled and dated by the PI (with **all** tasks and responsibilities delegated appropriately)
- Completed site contacts form (with contact information for all members of local staff)
- A signed and dated copy of the PI's current CV (with documented up to date GCP training, or copy of GCP training certificate)

In addition, the following agreements must be in place:

- a signed model Non-Commercial Agreement (mNCA) between the Sponsor and the relevant institution (usually an NHS Trust or Health Board)

4.2.3. Site activation letter

Once the UCL CTC trial team has received all required documentation and the site has been initiated, a site activation letter will be issued to the PI, at which point the site may start to approach patients.

Following site activation, the PI is responsible for ensuring:

- adherence to the most recent version of the protocol and Laboratory Manual which provides detailed instructions on biological sample collection, processing, storage and shipping
- all relevant site staff are trained in the protocol requirements
- appropriate recruitment and medical care of patients in the study
- timely completion of eCRFs (including assessment of all adverse events related to study procedures)
- prompt notification and assessment of all serious adverse events related to study procedures

5. INFORMED CONSENT

Sites are responsible for assessing a patient's capacity to give informed consent.

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

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

The PI, or, where delegated by the PI, other appropriately trained site staff, are required to provide a full explanation of the study to each patient prior to study entry. During these discussions, the current approved patient information sheet for the study should be discussed with the patient.

Patients should be given adequate time to consider and discuss participation in the study. However, patients may consent on the same day they are given the information sheet if this is more convenient for them. In such cases, a member of the research team at the hospital must phone the patient in the following days to confirm that they are still willing to participate in the study.

Written informed consent on the current approved version of the consent form for the study must be obtained before any study specific procedures are conducted. The discussion and consent process must be documented in the patient notes.

Site staff are responsible for:

- ensuring that the current approved version of the patient information sheet and consent form are used
- ensuring that information on the consent form is complete and legible
- ensuring that the patient has initialled all relevant sections of the consent form and signed and dated the form
- ensuring that an appropriate member of staff has countersigned and dated the consent form to confirm that they provided information to the patient
- ensuring that an appropriate member of staff has made dated entries in the patient's medical notes relating to the informed consent process (i.e. information given, consent signed etc.)
- following registration, adding the patient's study number to all copies of the consent form, which should be filed in the patient's medical notes and investigator site file.
- following registration, giving the patient a copy of their signed consent form and patient information sheet

The right of the patient to refuse to participate in the study without giving reasons must be respected. All patients are free to withdraw at any time. Also refer to section 15 (withdrawal of patients).

5.1. Additional information for patients under 16

The person with parental responsibility or legal guardianship of the child must be informed of all aspects of the study. The child must also be informed about the study to the extent compatible with their understanding. The same information must be provided to children of all ages, however the level of detail is age appropriate.

Information sheets are available for the following age groups:

- 06 - 09 years old
- 10 - 12 years old
- 13 – 15 years old

There is also an information sheet available for parents/legal guardians.

The patient and parent/legal guardian should be given adequate time to consider and discuss participation in the study. However, the patient and parent/legal guardian may consent on the same day they are given the information sheet if this is more convenient for them. In such cases, a member of the research team at the hospital must phone the patient and parent/legal guardian in the following days to confirm that they are still willing to participate in the study.

Written informed consent on the current approved version of the consent form for the study must be obtained before any study specific procedures are conducted. If capable the child may give assent by signing and personally dating the informed consent form, in addition to the parent or legal guardian. The discussion and consent process must be documented in the patient notes.

A child's refusal to participate in the study must be respected.

6. SELECTION OF PATIENTS

6.1. Screening Log

A screening log must be maintained and appropriately filed at site. Sites should record all patients identified with newly diagnosed osteosarcoma and the reasons why they were not registered in the study if this is the case. The log must be sent to UCL CTC when requested.

6.2. Patient Eligibility

Queries in relation to the eligibility criteria should be addressed prior to registration. Patients are eligible for the study if all the inclusion criteria are met and none of the exclusion criteria apply.

Patient eligibility must be confirmed by an investigator who is suitably qualified and who has been allocated this duty, as documented on the site staff delegation log, prior to registering the patient. Confirmation of eligibility must be documented in the patient notes and on the registration CRF.

Patients must give written informed consent before any study specific investigations may be carried out. Refer to section 9.1 (Pre registration) for the list of assessments and procedures required to evaluate the suitability of patients prior to entry.

6.2.1. Inclusion criteria

- New histological diagnosis of osteosarcoma or in the absence of osteoid seen on biopsy, pathology and imaging supportive of a diagnosis of osteosarcoma. *(It is well recognised that some patients may present with features suggestive of osteosarcoma (under 40 years, radiological abnormality compatible) but in whom no osteoid is detected in needle biopsy. Although categorised as spindle cell tumour of bone, such patients are usually treated in an identical approach to osteosarcoma. A definite diagnosis of osteosarcoma is then often possible after surgery when the entire resection specimen is available.)*
- Written informed consent of patient and/or parent/legal guardian

6.2.2. Exclusion criteria

- Diagnosis more than four months prior to registration

7. REGISTRATION PROCEDURES

Patient registration will be performed via a remote electronic data capture system hosted by UCL CTC. Please refer to the registration instructions provided in the ICONIC Database User Manual for sites. Patients must be confirmed to be eligible and have given consent prior to registration.

Site staff responsible for patient registration must request access to the eCRF database by completing their contact details on the site database user access form and delegation log. Access to the database and instructions are provided by UCL CTC.

Note that patient initials, age at study entry, details of the Sarcoma MDT site and date of diagnosis are required to register a patient.

Upon registration a study number will be assigned to the patient. This will be in the registration confirmation email which is sent to the person registering the patient.

The study number must be recorded in the patient notes.

CONTACT DETAILS
ICONIC Trial Coordinator: 020 7679 9878 **ctc.iconic@ucl.ac.uk**

Once a patient has been registered onto the study they must be provided with the following:

- A copy of their signed consent form and patient information sheet.

8. STUDY TREATMENT

No treatments are specified by this protocol. Patients should be treated according to national guidelines including *UK guidelines for the management of bone sarcomas*[29] and applicable current and future NICE guidance.

9. STUDY ACTIVITIES

Please also see Schedule of Events table in Appendix 2

9.1. Pre registration

The following is required to evaluate the suitability of patients for the study:

- Histological confirmation of osteosarcoma, including date

The following patient information is also required in order to register the patient:

- Age at time of registration
- Sex

9.2. Baseline

9.2.1. Clinical Data

The following data should be collected from routine assessments carried out within 28 days of registration:

Medical History

- History of previous cancer
- Relevant previous medical conditions, procedures and treatments
- Current medical conditions, signs and symptoms
- Cancer signs and symptoms
- Duration of symptoms
- Causative risk factors (family history, previous radiotherapy, Paget's disease etc.)
- Smoking status

Baseline characteristics

- Physical examination (height and weight)
- Assessment of WHO performance status (patients ≥16) or Lansky performance status (see Appendix 3)
- Haematology: haemoglobin, ANC, lymphocytes, platelets
- Biochemistry: CRP, albumin, ALP, LDH, creatinine

Primary Tumour: Diagnostic imaging

- Date and type of scan and hospital where this was performed
- Site, size and location on bone of primary tumour
- Description of findings (intraarticular, pathological fracture, involvement of neurovascular bundle, presence of skip metastases etc.)

Primary tumour: Biopsy

- Date of biopsy and hospital where this was performed
- Tumour grade and histological subtype

Primary tumour: Surgery (if already performed)

- Date and type of surgery and hospital where this was performed
- Histological subtype
- Resection margins

Diagnostic staging: Imaging

- Date and type of imaging and hospital where this was performed
- Location, number and size of metastases, if applicable

Treatment plan

- Name and date of MDM where patient discussed
- Type of planned surgical operation or reason if surgery not to be performed
- Chemotherapy regimen, if planned or reason if no chemotherapy to be given
- Details on plans for radiotherapy or other care

- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research when applicable.

9.2.2. PROMs

Patients aged 13 or over should complete the PROMs including Sarcoma Assessment Measure (SAM), EORTC-QLQ-C30, TESS and GRC within 2 weeks after registration and prior to starting treatment (if possible).

9.2.3. Routes to Diagnosis

The routes to diagnosis questionnaire should be given to the patient or parent/legal guardian at baseline only. It takes around 20 minutes for the patient to complete and should be returned to site for data entry.

The GP information sheet and routes to diagnosis questionnaire should be sent (by email or post) to the patient's GP at baseline and should be returned to site for data entry. If not returned to the site within 4 weeks the GP should be sent one reminder.

- Patients aged 13 or over should complete the routes to diagnosis questionnaire within three months of registration.
- For patients aged less than 13, the parents or legal guardians should complete the routes to diagnosis questionnaire within three months of registration.

9.2.4. Research Samples

At study entry

- Blood sample for germline DNA (if not collected as part of local biobanking procedure)
- Surgery site to ship diagnostic FFPE tissue to RNOH (coordinated by UCL CTC)
- Collect information about frozen tumour samples (where sample stored, if taken or reason sample not taken)

Prior to start of chemotherapy

- Blood samples for CTCs (first 50 patients receiving chemotherapy)
- Plasma sample stored for ctDNA, DNA methylation profiling/future research (all patients who receive chemotherapy followed by surgery. NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected) **(optional)**

9.3. During Treatment

If patient does not receive treatment for any reason, skip to section 9.5 (Follow up).

The reason why no treatment was given should be documented.

9.3.1. Clinical Data

During treatment, data should be collected at the end of neoadjuvant chemotherapy, at surgery, at the end of adjuvant chemotherapy, after radiotherapy and at the end of treatment.

The following details should be collected at the end of neoadjuvant chemotherapy:

- Details of chemotherapy given, including drug doses, dates and reason(s) for reductions, delays or discontinuation
    - If neoadjuvant chemotherapy not given, reason should be documented
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research, when applicable
- Disease status (if assessment carried out)

The following details should be collected prior to surgery:

- WHO performance status (patients ≥ 16) or Lansky performance status
- Blood Biochemistry: CRP, albumin, ALP, LDH, creatinine
- Haematology: Hb, ANC, lymphocytes, platelets
- Pre-operative imaging
    - Date of MRI
    - Date of CT, if performed
    - Date and type of other imaging, if applicable
    - Disease status

The following details should be collected at surgery and following surgery:

- Date and time of surgery
- Hospital where surgery performed
- Type of surgery (amputation, limb sparing etc.)
- Details of surgical procedure (which anatomical structures removed, use of antibiotics, details on blood loss, whether central line in situ, type of reconstruction, surgical margin assessment etc.)
- ASA classification
- Post operative complications (wound complications, dehiscence, infection or haematoma requiring return to theatre, thromboembolism, deep implant infection etc.)

If surgery was planned but not done the reason should be documented.

Pathology

- Maximum dimension on pathology report
- Margins: bone margin, narrowest margin in mm
- Lymphovascular invasion
- Response to chemotherapy, % necrosis
- Was a fresh frozen specimen stored?
  - If so, where?
  - If not, why not?
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research, when applicable

The following details should be collected at the end of adjuvant chemotherapy:

- Details of chemotherapy given, including drug doses, dates and reason(s) for reductions, delays or discontinuation
- If adjuvant chemotherapy not given, reason
- Was mifamurtide given? If not, reason why
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture
- Disease status (if imaging assessment carried out)

The following details should be collected after radiotherapy (if given):

- Details of radiotherapy, including indication (primary, palliative or both), modality, technique, dates, doses and fractionation
- Adverse reactions: assessed only in relation to study procedures

If patients undergo thoracotomy at any time, please provide:

- Date, place of surgery;
- type of resection; side of resection; no of lesions resected;
- was metastatic disease verified histologically?
- no. of verified lesions; resection margins
- Was sample stored for research?
  - if so, where is it stored?

9.3.2. Research Samples

- Blood samples for CTCs (at least 50 patients receiving chemotherapy) during neoadjuvant chemotherapy (just prior to surgery)
  - For patients receiving MAP chemotherapy collect 3 - 0 days prior to cycle 2 day 21 methotrexate (approximately week 8)
  - For patients receiving AP or other chemotherapy, collect pre cycle 3 (ideally up to 7 days prior/ 14 days post cycle 2)
- Plasma sample for potential ctDNA, methylation profiles and storage for future research (all patients who receive chemotherapy followed by surgery) **(optional)**
  - For patients receiving MAP chemotherapy collect 3 - 0 days prior to cycle 2 day 21 methotrexate (approximately week 8)
  - For patients receiving AP chemotherapy collect 3 – 0 days prior to cycle 3 (approximately week 6)

9.4. End of treatment

9.4.1. Clinical data

The following details should be collected at the end of treatment:

- Total number of cycles of chemotherapy and doses
- Total number of mifamurtide cycles
- If terminated early, provide reason
- Disease status
- Imaging performed, date, modality, result
- Blood Biochemistry: CRP, albumin, ALP, LDH, creatinine
- Haematology: Hb, ANC, lymphocytes, platelets
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research.

9.4.2. PROMs

- Patients aged 13 or over should complete the PROMs (SAM, EORTC QLQ-C30, TESS, & GRC) at the end of treatment.

9.4.3. Research Samples

- Plasma sample for ctDNA, methylation profiles and storage for future research (all patients who receive chemotherapy followed by surgery) **(optional)**
- Surgery site to ship FFPE tissue from the surgical resection to RNOH (coordinated by UCL CTC)

9.5. Follow up

9.5.1. Clinical Data

Follow up visits should take place, at least annually, according to routine practice, on all patients including those who did not receive treatment. The following details should be collected:

- Disease status
- Survival status
- Details on any post-operative complications (e.g. deep implant infection)
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research, when applicable

9.5.2. PROMs

- Patients aged 13 or over should complete the PROMs including SAM, EORTC-QLQ-C30, TESS, GRC annually from registration until relapse.

9.5.3. Research Samples

- Plasma sample for ctDNA, methylation profiles and storage for future research every 6 months (all patients who receive chemotherapy followed by surgery) **(optional)**

9.6. Relapse (local recurrence or metastases)

9.6.1. Clinical Data

The following data should be collected on confirmation of relapse (local recurrence or metastases), and at every subsequent relapse:

- Site(s) of relapse (local, distant etc.)
- Treatment received (surgery for local recurrence or metastases, systemic therapy, radiotherapy etc.)
- Adverse reactions: assessed only in relation to study procedures, e.g. venepuncture, biopsy for research.

9.6.2. Research Samples

- Blood samples for CTCs (at least 50 patients receiving chemotherapy, and only if the first two samples have been collected)
- Plasma sample for ctDNA, methylation profiles and storage for future research (all patients who receive chemotherapy followed by surgery) **(optional)**
- Tumour tissue collected as part of standard of care, or for research (optional, FFPE and fresh frozen according to local policy, if possible, and stored locally at surgical site: collection coordinated by UCL CTC)

10. EXPLORATORY BIOLOGICAL STUDIES

The following sections provide an overview of sample collection. For details of sample collection, processing and shipping, refer to the **ICONIC Laboratory Manual**.

10.1. Archival FFPE tumour tissue samples

FFPE samples should be obtained for all patients and shipped to the Royal National Orthopaedic Hospital (RNOH). These will be used to optimise processes to enable DNA/RNA extraction from samples to perform molecular targeted sequencing and copy number (CN) analysis, and to validate findings from reported and ongoing whole genome sequencing (WGS) studies.

The collection of samples will be coordinated centrally by UCL CTC.

10.1.1. Collection time points:

The following samples will be collected:

- Archival FFPE tissue blocks from previous biopsy or surgery
- FFPE tissue blocks from routine surgery taking place after registration

10.1.2. Processing

All samples should be fixed and embedded according to local practice.

For routine samples, sites should obtain at least one and ideally two FFPE tumour tissue blocks from a previous biopsy or surgery.

Review the pathology report and select blocks as follows:

- Biopsy cores: Choose cores that have the highest tumour tissue content.
- Surgical resection tissue: Choose blocks containing the most tumour tissue.

10.1.3. Shipping

Archival samples should be shipped as soon as possible after registration, ideally within 4 weeks, but not later than 12 weeks after registration. All other FFPE samples should be shipped within 8 weeks after the procedure.

10.1.4. Return

All FFPE samples will be stored at RNOH. During the Study sites may request return of routine samples if required for clinical purposes but would be requested to return the samples to RNOH after this.

At the end of the Study all archival FFPE samples will be returned to site.

Samples taken for research (i.e. those not clinically indicated) will not be returned to sites.

10.2. Fresh Frozen tumour specimens

Information will be captured about routine collection of frozen tumour specimens, at baseline, surgical resection of primary tumour or metastases and on relapse/local recurrence.

The collection of samples will be coordinated centrally by UCL CTC.

10.3. New biopsy

A new biopsy, FFPE and fresh frozen according to local policy if possible, should be obtained at relapse/resection of local recurrence or metastases for the study if not done as part of standard of care. This is optional for patient.

For new biopsies for research, two cores should be obtained. One core should be fresh frozen in liquid nitrogen according to local policy and one core should be formalin fixed paraffin embedded.

If it is not possible to obtain two cores, then one is acceptable and should be fresh frozen according to local policy.

- The fresh frozen sample should be stored at site at -80°C until requested for analysis.

10.4. Blood samples for Circulating Tumour Cells

Blood samples will be collected for analysis of circulating tumour cells (CTCs). Two samples will be collected at each time point.

One sample will be shipped to the University of Sheffield where a new method for isolating CTCs is being optimised. The procedure uses the Parsortix™ System to enrich for CTCs, which are then isolated and captured using the DEPArray™ System[22]. The protocols are ready for clinical validation and a pilot study funded by Sarcoma UK to capture CTCs from patients with soft tissue sarcoma has begun recruiting. Blood samples from patients in ICONIC will enable this methodology to be validated in OS.

The second sample will be shipped to Newcastle University. These samples will be used for ongoing validation of the MT1-MMP method of CTC isolation through the use of flow cytometry and subsequent genetic characterisation.

10.4.1. Collection time points

Samples will be collected for at least 50 patients with both pre and post chemotherapy samples. **Samples should not be collected if there is no plan for the patient to have chemotherapy.**

Samples should be collected at the following timepoints (at the same time as routine bloods are taken):

- Baseline (prior to start of chemotherapy)
- Pre cycle 2 day 21 methotrexate (MAP patients) or pre cycle 3 (AP or other chemotherapy patients) (ideally up to 7 days prior/14 days post cycle 2)
- At relapse (local recurrence or metastases) and only the if first two samples have been collected

10.4.2. Processing and shipping

Whole blood should be collected into 2 x 10 mL Streck tubes and inverted 10 times to mix. Samples must be shipped on the day they are collected, using the shipping materials provided. One sample should be shipped to the University of Sheffield and the other to Newcastle University.

10.5. Blood samples for ctDNA, methylation profiles and future research

Blood samples for future research including measurement of cell free DNA and analysis of methylation profiles may be collected from all patients who receive chemotherapy followed by surgery.

These samples are optional.

10.5.1. Collection time points

Samples should be collected at the following timepoints (at the same time that routine bloods are taken):

- Baseline (prior to start of chemotherapy. NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected)
- Pre cycle 2 day 21 methotrexate (MAP patients) or pre cycle 3 (AP or other chemotherapy patients) (ideally up to 7 days prior/14 days post cycle 2)End of treatment
- Every 6 months during follow up
- At relapse (local recurrence or metastases)

10.5.2. Processing and shipping

Whole blood should be collected into 2 x 10 mL BD Vacutainer® PAXgene ccfDNA blood collection tubes and inverted 10 times to mix. Samples must be shipped to RNOH on the day they are collected, using the shipping materials provided.

10.6. Blood samples for germline DNA

Blood samples for germline DNA will be collected from all patients and used for WGS and targeted sequencing.

This sample should be taken only if a sample has not already been taken as part of local biobanking policy. If it is unknown whether a sample has been collected it should be collected for ICONIC.

10.6.1. Collection time points

One sample should be collected at baseline (study entry) and can be collected at the same time as routine bloods are taken.

10.6.2. Processing and shipping

Whole blood should be collected into a 10 mL BD Vacutainer® PAXgene ccfDNA blood collection tube and inverted 10 times to mix. Samples must be shipped to RNOH on the day they are collected, using the shipping materials provided.

11. DATA MANAGEMENT AND DATA HANDLING GUIDELINES

Data will be collected from sites using an eCRF (electronic case report form) created and maintained by UCL CTC. Data must be accurately entered into the eCRFs and must be verifiable from source data at site. Examples of source documents are hospital records which include patient notes, laboratory and other clinical reports, etc.

11.1. Entering data into the eCRF

The eCRF must be completed by staff who are listed on the site staff delegation log and authorised by the PI to perform this duty. Each authorised staff member will have their own unique login details for the eCRF. They must never be shared among staff as the eCRF audit trail will record all entries/changes made by each user. The PI is responsible for the accuracy of all data reported in the eCRF. The use of abbreviations and acronyms should be avoided.

11.2. Corrections to eCRF Forms

Corrections can be made to data on the eCRF where necessary, the eCRF audit trail will record the original data, the change made, the user making the change and the date and time.

11.3. Missing Data

To avoid the need for unnecessary data queries, fields should not be left blank on the eCRF. If data is unavailable, please refer to the ICONIC Database Manual for sites for information on how to indicate that data is “Not Done”, “Not Applicable”, “Not Available” or “Not Known” (only use if every effort has been made to obtain the data).

11.4. Timelines for Data Entry

The relevant eCRF forms must be completed as soon as possible after a patient’s visit. Eligibility and registration forms must be completed for a patient to be registered onto the study. All other forms must be completed within 7 days of the patient being seen.

Sites who persistently do not enter data within the required timelines may be suspended from recruiting further patients into the study by UCL CTC and subjected to a ‘triggered’ monitoring visit. See section 14.2 (‘Triggered’ On Site Monitoring) for details.

11.5. Data Queries

Data entered onto the eCRF will be subject to some basic checks at the time of entry, and any discrepancies will be flagged to the user in the form of a warning. The data can be corrected immediately, or where this is not possible, the warning can be saved and the data amended at a later stage.

Further data review will be carried out at UCL CTC and queries raised where necessary. Further guidance on the process for handling data queries can be found in the ICONIC Database Manual for sites.

12. SAFETY REPORTING

12.1. Definitions

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

This is a low risk prospective observational cohort study.

Adverse Reactions (AR)
All untoward and unintended events causally related to a 'Study Procedure'; where a causal relationship between a 'Study Procedure' and an event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Serious Adverse Reactions (SAR)
SARs are an adverse reaction that meets any of the following criteria:

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

Related & Unexpected SARs
A serious adverse reaction, the nature or severity of which **is not consistent** with the applicable Study Procedure.

Study Procedure means the blood sampling procedure and/or biopsy procedure for the purposes of obtaining research samples within the study.

12.2. Serious Adverse Reactions (SARs)

SARs are not anticipated in this study however SARs that are attributable to the study procedures will be reported immediately to the UCL CTC and documented on the study database.

12.3.1 Reporting of Serious Adverse Reactions (SARs)

As this is an observational cohort study where patients follow their normal clinical pathways, and the study introduces two procedural interventions (blood sampling and biopsies), the PI, or other delegated site investigator should monitor each participant at each visit and only report to UCL CTC events that are serious and related (i.e. a SAR) to the study procedure.

Causality
The PI, or other delegated site investigator, must perform an evaluation of causality for each SAR.
UCL CTC will consider events evaluated as related to be adverse reactions.

- Related (reasonable possibility) to a study procedure
- Not related (no reasonable possibility) to a study procedure

Severity
Severity of each event must be determined by using the Common Terminology Criteria for Adverse Events (CTCAE) v5 as a guideline, wherever possible. The criteria are available online at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

In those cases where the CTCAE criteria do not apply, severity should be coded according to the following criteria:

1 = Mild (awareness of sign or symptom, but easily tolerated)
2 = Moderate (discomfort enough to cause interference with normal daily activities)
3 = Severe (inability to perform normal daily activities)
4 = Life threatening (immediate risk of death from the reaction as it occurred)
5 = Fatal (the event resulted in death)

For the purposes of the study, SARs of all grades should be reported.

All SARs that occur between the start of the first study procedure and 15 days post the last study procedure must be submitted electronically within **24 hours** of observing or notification/occurrence of the event, using the study specific SAR Report.

All sections on the SAR Report must be completed. If the SAR report **is not sent within 24 hours** to UCL CTC, the circumstances that led to this must be detailed in the SAR Report to avoid unnecessary queries.

Completed SAR Reports must be submitted to UCL CTC within 24 hours of becoming aware of the event

Email: CTC.iconic@ucl.ac.uk
[Back up option: Fax number +44 (0)20 7679 9871]

SAR Follow Up Reports

All SARs must be followed up until resolution and until there are no further queries.

Sites must ensure any new and relevant information is provided promptly. If the reaction term changes or a new reaction is added, the causality must be re-assessed by an Investigator.

SAR Processing at UCL CTC

On receipt of the SAR Report, UCL CTC will check for legibility, completeness, accuracy and consistency. There are no expected SARs for the study, therefore all will be considered Related and Unexpected Serious Adverse Reactions.

The CI, or their delegate (e.g. a clinical member of the TMG) will be contacted to review the SAR and to perform an evaluation of causality on behalf of UCL CTC.

12.3. Related and Unexpected Serious Adverse Reaction

If the event is evaluated as a related and unexpected Serious Adverse Reaction, UCL CTC will submit a report to the REC within the required timeline. For this study, all SARs will be considered as unexpected.

Wherever possible, evaluations of causal relationship by both the site and the Sponsor's clinical reviewer will be reported.

13. INCIDENT REPORTING AND SERIOUS BREACHES

13.1. Incident Reporting

Organisations must notify UCL CTC of all deviations from the protocol or GCP immediately. An incident report may be requested and will be provided, but an equivalent document (e.g. Trust Incident form) is acceptable where already completed.

If site staff are unsure whether a certain occurrence constitutes a deviation from the protocol or GCP, the UCL CTC trial team can be contacted immediately to discuss.

UCL CTC will use an organisation's history of non-compliance to make decisions on future collaborations.

UCL CTC will assess all incidents to see if they meet the definition of a serious breach.

13.2. Serious Breaches

A "serious breach" is defined as a breach of the protocol or of the conditions or principles of Good Clinical Practice (or equivalent standards for conduct of non-CTIMPs) which is likely to affect to a significant degree the safety or physical or mental integrity of the study subjects, or the scientific value of the research.

Systematic or persistent non-compliance by a site with the principles of GCP and/or the protocol, occurring on study within the specified timeframe, may be deemed a serious breach.

In cases where a serious breach has been identified, UCL CTC will inform the REC within 7 calendar days of becoming aware of the breach.

14. STUDY MONITORING AND OVERSIGHT

Participating sites and PIs must agree to allow study related on site monitoring, Sponsor audits and regulatory inspections by providing direct access to source data/documents as required. Patients are informed of this in the patient information sheet and are asked to consent to their medical notes being reviewed by appropriate individuals on the consent form.

UCL CTC will determine the appropriate level and nature of monitoring required for the study. Risk will be assessed on an ongoing basis and adjustments made accordingly.

14.1. Centralised Monitoring

Sites will be requested to submit screening logs and staff delegation logs to UCL CTC at the frequency detailed in the trial monitoring plan, or on request, and these will be checked for consistency and completeness. Also refer to sections 4.2.2 (Required documentation) and 6.1 (Screening Log).

Ensuring patient eligibility is the responsibility of the PI or other delegated Investigator(s). Also refer to section 7.1 (Registration Procedures).

Sites will be required to complete information about the patient's informed consent process on the eCRF when registering the patient. Details of the versions of informed consent form/patient information sheet used, patient completion of the consent form, the name of the person taking consent etc., will be recorded and are subject to review by UCL CTC as part of patient eligibility. Also refer to section 5 (Informed consent).

Sites will be requested to conduct quality control checks of documentation held within the Investigator Site File at the frequency detailed in the trial monitoring plan. Checklists detailing the current version and date of version controlled documents will be provided for this purpose.

Data received at UCL CTC will be subject to review in accordance with section 11.5 (Data Queries).

Sites will be requested to conduct quality control checks of documentation held within the Investigator Site File at the frequency detailed in the trial monitoring plan. Checklists detailing the current version/date of version controlled documents will be provided for this purpose.

Where centralised monitoring of data and/or documentation submitted by sites indicates that a patient may have been placed at risk, the matter will be raised urgently with site staff and escalated as appropriate (refer to section 13 (Incident reporting and serious breaches) and 14.2 ('Triggered' On Site Monitoring) for further details).

14.2. 'Triggered' On Site Monitoring

On site monitoring visits may be scheduled where there is evidence or suspicion of non-compliance at a site with important aspect(s) of the study protocol/GCP requirements. Sites will be sent a letter in advance outlining the reason(s) for the visit and confirming when it will take place. The letter will include a list of the documents that are to be reviewed, interviews that will be conducted, planned inspections of the facilities and who will be performing the visit.

Following a monitoring visit, the Trial Monitor/Trial Coordinator will provide a follow up email to the site, which will summarise the documents reviewed and a statement of findings, incidents, deficiencies, conclusions, actions taken and/or actions required. The PI at each site will be responsible for ensuring that monitoring findings are addressed in a timely manner, and by the deadline specified.

UCL CTC will assess whether it is appropriate for the site to continue participation in the study and whether the incident(s) constitute a serious breach. Refer to section 13 (Incident reporting and serious breaches) for details.

14.3. Oversight Committees

14.3.1. Trial Management Group (TMG)

The TMG will include the Chief Investigator, clinicians and experts from relevant specialities and ICONIC study staff from UCL CTC (see page 2). The TMG will be responsible for overseeing the study. The group will meet regularly once a year in person and will send updates to PIs (via newsletters or at Investigator meetings) and to the NCRI Sarcoma Clinical Studies Group.

The TMG will review substantial amendments to the protocol prior to submission to the REC. All PIs will be kept informed of substantial amendments through their nominated responsible individual and are responsible for their prompt implementation.

A TMG charter, which outlines the responsibilities for the ICONIC study, must be signed by all members of the committee before the first meeting is held.

14.3.2. Trial Steering Committee (TSC)

The role of the TSC is to provide overall supervision of the study. The TSC acts on behalf of the funder and the Sponsor.

The ICONIC study will be reviewed by an established UCL CTC TSC that has oversight of a number of trials. All members have signed a TSC charter.

14.3.3. Role of UCL CTC

UCL CTC will be responsible for the day to day coordination and management of the study and will act as custodian of the data generated in the study (on behalf of UCL).

15. WITHDRAWAL OF PATIENTS

In consenting to the study, patients are consenting to assessments, collection of biological samples, follow up and data collection.

15.1. Future Data Collection

If a patient explicitly states they do not wish to contribute further data to the study their decision must be respected and recorded on the relevant CRF. In this event data due up to the date of withdrawal must be submitted but no further data sent to UCL CTC.

15.2. Losses to Follow Up

If a patient moves from the area, every effort should be made for the patient to be followed up at another participating study site and for this new site to take over the responsibility for the patient. Details of participating study sites can be obtained from the UCL CTC trial team, who must be informed of the transfer of care and follow up arrangements.

If it is not possible to transfer to a participating site, the registering site remains responsible for submission of forms.

If a patient is lost to follow up at a site every effort should be made by the site to contact the patient's GP to obtain information on the patient's status.

15.3. Loss of Capacity

Patients who lose capacity during the study would continue in the study for the purposes of data collection. If the patient regained capacity, an Investigator would discuss with the patient their continued participation in the study and together the patient and Investigator would decide what action, if any, to take.

16. STUDY CLOSURE

16.1. End of Study

For regulatory purposes the end of the study will be defined as 4 years after the start of recruitment at which point the 'declaration of end of study' form will be submitted to the Ethics Committee, as required.

Following this, UCL CTC will advise sites on the procedure for closing the study at the site.

Once the end of study has been declared, no more prospective patient data will be collected but sites must cooperate with any data queries regarding existing data to allow for analysis and publication of results.

16.2. Archiving of Study Documentation

At the end of the study, UCL CTC will archive securely all centrally held study related documentation for a minimum of 5 years. Arrangements for confidential destruction will then be made. It is the responsibility of PIs to ensure data and all essential documents relating to the study held at site are retained securely for a minimum of 5 years after the end of the study, and in accordance with national legislation.

Essential documents are those which enable both the conduct of the study and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.

UCL CTC will notify sites when study documentation held at sites may be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

16.3. Early Discontinuation of Study

The study may be stopped before completion as an Urgent Safety Measure on the recommendation of the TSC or IDMC (see section 14.3.2 Trial Steering Committee (TSC)). Sites will be informed in writing by UCL CTC of reasons for early closure and the actions to be taken with regards the treatment and follow up of patients.

16.4. Withdrawal from Study Participation by a Site

Should a site choose to close to recruitment the PI must inform UCL CTC in writing. Follow up as per protocol must continue for any patients recruited into the study at that site and other responsibilities continue as per the mNCA.

17. STATISTICS

This is a prospective observational cohort study of patients of all ages with histopathologically confirmed OS.

17.1. Sample Size as feasibility endpoint

One of the objectives of Stage 1 is to assess the feasibility of recruiting to this cohort. Our assumption is that 15 sites will be opened during the course of the first 11 months of recruitment. Once all sites are opened (from month 12 onwards), we estimate that the average recruitment rate will be 5 patients/ month.

Since recruitment is expected to last for 3 years in total (Stage 1 + Stage 2), the total expected sample size will be at least 160 patients (assuming that our recruitment assumptions are verified in practice).

At the end of Stage 1, the average recruitment rate (from month 12 onwards) will be calculated. The total sample size will then be re-estimated at the end of Stage 1 given the findings. However, a total sample size of 160 patients will be assumed for the rest of this section.

Specific analyses in dataset of patients treated with surgery and chemotherapy

Based on data from England (2011-2015), 66% of patients receive surgery and 66% receive chemotherapy (see Section 2). For this section, we assume that 60% of patients receive both treatments. The size of the full dataset of patients treated with both modalities will be:

- 86 patients for serum biomarkers analysis, assuming routine blood results in $\geq$ 90% patients;
- 50 patients for exploratory ctDNA/CTC analyses (based on funding secured for analysis)

Power of analyses

This section corresponds to the analyses based on blood results.

For serum biomarkers, 86 patients provide 80% power (with 80% two-sided confidence interval) to detect a risk ratio of 0.55 for response to treatment between the first half and the second half of the distribution of serum biomarkers (based on the median), assuming a good histological response rate of 50% for patients in the first half.

For ctDNA/CTCs, 50 patients provide 80% power (with 80% two-sided confidence interval) to detect a risk ratio of 0.40 for response to treatment between patients with detectable and undetectable ctDNA, assuming a good histological response rate of 50% for patients with undetectable ctDNA.

The software used to do the power calculations was EPI INFO (version 7.2.2.6).

17.2. Statistical analysis

17.2.1. Stage 1

Descriptive analyses and tabulations will be performed to answer Stage 1's research questions, as detailed in the table below.

Research question	Analysis
Recruitment objective	
1. Feasibility of recruiting patients across UK paediatric, TYA and adult centres to cohort study	• Recruitment rate after opening of 15 sites • % patients recruited according to age and primary site
Clinical objectives	
2. Determine patterns of treatment of patients across UK treatment sites to inform stage 2 objectives and future studies	• % patients with MDT documentation of rationale for planned treatment • % patients receiving chemotherapy and regimen • % patients undergoing surgery • % patients receiving radiotherapy and indications
3. Determine feasibility of surgical data capture to inform future studies	• List of possible reconstruction techniques and their relative frequencies • % patients with post-operative complications
4. Imaging: • determine primary tumour imaging and reporting pre and post chemotherapy • Determine variation in imaging modalities used for staging of OS patients to inform future studies	• % patients staged with bone scan, PET/CT and/or WB–MRI
Biological objectives	
1. Determine feasibility of fresh frozen tissue sample collection across sites across the UK	• Collection rate per site • Number and % of metastatic/relapse specimens stored for future research • % sent for WGS
2. Determine quality of Formalin Fixed paraffin-embedded (FFPE) samples and feasibility of DNA/RNA extraction for validation studies	• Collection rate of diagnostic/resection FFPE specimens in patients treated with surgery and chemotherapy • Audit to determine whether quality and quantity of RNA/DNA sufficient for targeted sequencing and nanostring analysis
3. Evaluate whether two methodologies of circulating tumour cell (CTC) detection can isolate and quantify CTCs in patients undergoing neo-adjuvant chemotherapy	• Identification and quantification of CTC numbers at baseline (pre-treatment) and changes to CTC numbers in response to chemotherapy • Descriptive analysis of CTC numbers as histograms and ranges (min, max, IQR, median)

Research question	Analysis
4. Collection of plasma for potential methylation/ctDNA/future research	• Collection rate per site
5. Evaluate collection of serum biomarkers for validation of prognostic scores	• Collection rate of serum parameters • Characterisation of distribution of serum biomarkers as histograms and ranges (min, max, IQR, median)
6. Determine feasibility of using standard pathology reporting to assess margin status for risk of local recurrence	• Collection rate of pathology reports in patients undergoing surgery • Characterisation of tumour size, assessment of resection margins, % necrosis
Patient Reported Outcome Measures	
1. Determine feasibility of data collection for validation of a sarcoma assessment method (SAM) in patients undergoing treatment for OS	• Completion rate of SAM, TESS, EORTC-QLQ-C30 and GRC • Descriptive analyses of PROMs

17.2.2. Stage 2

The analyses within Stage 2 may be updated based on findings of Stage 1. Nonetheless, the following is foreseen.

Pattern of treatment:

Tabulations will be provided to describe current patterns of treatment for surgery, chemotherapy and radiotherapy:

- Surgery: how are decisions reached for a tumour being inoperable? Do patients contribute to the final decision?
- Chemotherapy: what are the variations in use of chemotherapy?
- Radiotherapy (RT): what are current indications for RT?

Effect of treatment and precision medicine

The analyses of survival (overall survival, event free survival and progression free survival) will be descriptive, based on Kaplan-Meier analysis and Cox regression.

The analyses will investigate how survival depends on:

- baseline characteristics (age, performance status, grade, stage);
- patterns of treatment;
- tumour heterogeneity and clonal evolution;
- serum biomics and ctDNA/CTCs.

17.3. Transition between Stage 1 and Stage 2

At the end of Stage 1, the analyses described in section 17.2.1 will be performed. The analyses proposed for Stage 2 will be reviewed during the transition and may be altered based on findings.

18. ETHICAL CONSIDERATIONS

In conducting the study, the Sponsor, UCL CTC and sites shall comply with all relevant guidance, laws and statutes, as amended from time to time, applicable to the performance of clinical trials including, but not limited to:

- the principles of Good Clinical Practice
- Human Rights Act 1998
- Data Protection Act 2018 , and General Data Protection Regulation (EU)2016/679 (GDPR)
- Freedom of Information Act 2000
- Human Tissue Act 2004
- Mental Capacity Act 2005
- UK Policy Framework for Health and Social Care Research

18.1. Ethical Approval

The study will be conducted in accordance with the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (1996 version) and in accordance with the terms and conditions of the ethical approval given to the study.

The study has received a favourable opinion from the London – Camden and King's Cross Research Ethics Committee (REC) and Health Research Authority (HRA) approval for conduct in the UK.

UCL CTC will submit Annual Progress Reports to the REC, commencing one year from the date of ethical approval for the trial.

18.2. Site Approvals

Evidence of assessment of capability and capacity by the Trust/Health Board R&D for a trial site must be provided to UCL CTC. Sites will only be activated when all necessary local approvals for the study have been obtained.

18.3. Protocol Amendments

UCL CTC will be responsible for gaining ethical approval, for amendments made to the protocol and other study related documents. Once approved, UCL CTC will ensure that all amended documents are distributed to sites as appropriate.

Site staff will be responsible for acknowledging receipt of documents and for implementing all amendments promptly.

18.4. Patient Confidentiality & Data Protection

Patient identifiable data, including initials, age and gender will be collected by UCL CTC. UCL CTC will preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified. Data will be stored in a secure manner and UCL CTC trials are registered in accordance with the Data Protection Act 2018 and GDPR, with the Data Protection Officer at UCL.

19. SPONSORSHIP AND INDEMNITY

19.1. Sponsor Details

Sponsor Name:	University College London
Address:	Joint Research Office Gower Street London WC1E 6BT
Contact:	Director of Research Support
Tel:	020 3447 9995/2178 (unit admin)
Fax:	020 3447 9937

19.2. Indemnity

University College London holds insurance against claims from participants for injury caused by their participation in the study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the study. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise. Participants may also be able to claim compensation for injury caused by participation in this clinical study without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office. Hospitals selected to participate in this study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

20. FUNDING

The Bone Cancer Research Trust (BCRT) is supporting the central coordination of the study through UCL CTC.

Research A costs will be reimbursed to sites as per the finance section of the mNCA.

21. PUBLICATION POLICY

All publications and presentations relating to the study should be authorised by the TMG. The TMG will form the basis of the writing committee and advise on the nature of the publications. All collaborators who have actively contributed to the study will be named authors on all main study papers and anyone else who has had a significant input into the conduct, analysis and interpretation of the study.

Specialist papers focusing on a particular aspect of translational research may not require all collaborators to be authors. Data from all sites will be analysed together and published as soon as possible after endpoints have been reached. Participating sites may not publish study results prior to the first publication by the TMG or without prior written consent from the TMG.

The Chief Investigator will make the final decision on authorship. The study data is owned by the Sponsor. The ClinicalTrials.gov number (NCT04132895) should be quoted in any publications resulting from this study.

22. REFERENCES

1. Whelan J, McTiernan A, Cooper N, Wong Yuen K, Francis M, Vernon S Strauss SJ. Incidence and survival of malignant bone sarcomas in England 1979-2007. Int J Cancer. 2012;131(4):E508-E517.
2. Matsuo T, Sugita T, Sato K, et al. Clinical outcomes of 54 pelvic osteosarcomas registered by Japanese musculoskeletal oncology group. Oncology. 2005; 68:375-81
3. Ozaki T, Flege S, Liljenqvist U, et al. Osteosarcoma of the spine: experience of the Cooperative Osteosarcoma Study Group. Cancer 2002; 94: 1069-77.
4. Kager L, Zoubek A, Pötschger U, et al. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. J Clin Oncol. 2003; 21: 2011-8.
5. Bacci G, Briccoli A, Ferrari S,et al. Neoadjuvant chemotherapy for osteosarcoma of the extremities with synchronous lung metastases: treatment with cisplatin, adriamycin and high dose of methotrexate and ifosfamide. Oncol Rep 2000: 2:339-46
6. Bielack SS, Smeland S, Whelan JS, et al. Methotrexate, Doxorubicin, and Cisplatin (MAP) Plus Maintenance Pegylated Interferon Alfa-2b Versus MAP Alone in Patients With Resectable High-Grade Osteosarcoma and Good Histologic Response to Preoperative MAP: First Results of the EURAMOS-1 Good Response Randomized Controlled Trial.J Clin Oncol. 2015 Jul 10;33(20):2279-87
7. Marina N, Smeland S, Bielack B, et al. Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. Lancet Oncology 2016, 17:1396-408
8. Grimer RJ, Sommerville S, Warnock D, Carter S, Tillman R, Abudu A, et al. Management and outcome after local recurrence of osteosarcoma. Eur J Cancer, 2005;41(4):578–583.
9. Sharp SE, Shulkin BL, Gelfand MJ, McCarville MB. FDG PET/CT appearance of local osteosarcoma recurrences in pediatric patients. Pediatr Radiol 2017
10. Jeys, L, Thorne CJ, Parry M, et al. A Novel System for the Surgical Staging of Primary High-grade Osteosarcoma: The Birmingham Classificati A Novel System for the Surgical Staging of Primary High-grade Osteosarcoma: The Birmingham Classification Clin Orthop Relat Res, 2017.
11. Kovac, Blattmann C, Ribi S, et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency Nat Communications, 2015, Dec 3;6:8940.
12. Davies H, Glodzik D, Morganella S, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures.Nat Med. 2017, 23(4):517-525
13. Fernanda Amary, et al. Fibroblastic growth factor receptor 1 amplification in osteosarcoma is associated with poor response to neo-adjuvant chemotherapy. Cancer Med. 2014 3(4):980-7
14. Behjati S, Tarpey P, et al. Recurrent mutation of IGF signalling genes and distinct patterns of genomic rearrangement in osteosarcoma. Nature Communications, 2017, 8(15936):1-8
15. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell. 2017;168(4):613-628.
16. Jamal-Hanjani M, Wilson GA, McGranahan N, et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. N Engl J Med. 2017;376(22):2109-2121.
17. McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016, 351(6280): 1463-9.
18. Tarpey PS et al, Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. Nat Genet. 2013 Aug;45(8):923-6. doi: 10.1038/ng.266
19. Tarpey PS, et al. The driver landscape of sporadic chordoma. Nat Commun. 2017 Oct 12;8(1):890.
20. Webpage : https://clinicaltrials.gov/ct2/show/NCT03486730 accessed 12/02/2019ax

21. Tellez-Gabriel M, Brown HK, Young R, Heymann MF, Heymann D. The Challenges of Detecting Circulating Tumor Cells in Sarcoma. Front Oncol. 2016, 6:202.
22. Gabriel MT, Calleja LR, Chalopin A, Ory B, Heymann D. Circulating Tumor Cells: A Review of Non-EpCAM-Based Approaches for Cell Enrichment and Isolation. Clin Chem. 2016 ;62(4):571-81.
23. Aggerholm-Pedersen N, Maretty-Kongstad K, Keller J, et al, The Prognostic Value of Serum Biomarkers in Localized Bone Sarcoma. Transl Oncol. 2016 9(4):322-8.
24. Kotronoulas G, Kearney N, Maguire et al. What is the value of the routine use of patient-reported outcome measures toward improvement of patient outcomes, processes of care and health service outcomes in cancer care? A systematic review of controlled trials. J Clin Oncol, 2014, 32(14): 1480-1501.
25. Mendonca SC, Abel GA, Saunders CL, Wardle J, Lyratzopoulos G. Pre-referral general practitioner consultations and subsequent experience of cancer care: evidence from the English Cancer Patient Experience Survey. Eur J Cancer Care (Engl). 2016 May;25(3):478-90. doi: 10.1111/ecc.12353. Epub 2015 Jul 30.
26. Gerrand C, Francis M, Dennis N, Charman J, Lawrence G, Evans T, Grimer R. Routes to diagnosis for sarcoma - Describing the sarcoma patient journey.Eur J Surg Oncol. 2015 Oct;41(10):1393-9. doi: 10.1016/j.ejso.2015.07.009. Epub 2015 Jul 29.
27. Mendonca SC, Abel GA, Lyratzopoulos G. Pre-referral GP consultations in patients subsequently diagnosed with rarer cancers: a study of patient-reported data. Br J Gen Pract. 2016 Mar;66(644): e171-81. doi: 10.3399/bjgp16X683977.
28. Department of Health. National Cancer Patient Experience Survey 2014: National Report. https://www.quality-health.co.uk/resources/surveys/national-cancer-experience-survey/2014-national-cancer-patient-experience-survey/2014-national-cancer-patient-experience-survey-national-reports/688-2013-national-cancer-patient-experience-survey-national-report-pdf/file accessed 21/11/2018
29. Gerrand C., Athanasou N., Brennan B., et al. British Sarcoma Group (2016) UK guidelines for the management of bone sarcomas. Clinical Sarcoma Research 6: 7, doi: 10.1186/s13569-016-0047-1

APPENDIX 1: ABBREVIATIONS

ACBS	Aarhus composite biomarker score
AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AP	Doxorubicin/Cisplatin chemotherapy
AR	Adverse Reaction
ASA classification	American Society of Anesthesiologists classification of fitness for surgery system classification
AST	Aspartate aminotransferase
BCRT	The Bone Cancer Research Trust
BRCA	BReast CAncer susceptibility gene
CNA	Copy Number Alterations
ccfDNA	Circulating cell free DNA
cfDNA	Cell free DNA
CI	Chief Investigator
CITA	CRUK Accelerator grant on Cancer ImmunoTherapy
CM	Centralised monitoring
CN	Copy number analysis
CRP	C-reactive protein
CR UK	Cancer Research UK
CT	Computerised Tomography
CTC	Circulating tumour cells
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumour DNA
DARWIN 1	Deciphering Afatinib Response and Resistance with INtratumour heterogeneity study
DEPArray™	DEPArray enables manipulation and recovery of rare cells by combining image-based cell selection with DEP movement single cell sorter
DPA	Data Protection Act
eCRF	Electronic Case Report Form
EDTA	Ethylene Diamine Tetra Acetate
EORTC QLQ-C30	European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30
EURAMOS-1	A randomized trial of the European and American Osteosarcoma Study Group to optimize treatment strategies for resectable osteosarcoma based on histological response to pre-operative chemotherapy
FFPE	Formalin fixed paraffin embedded blocks
FGFR1	Fibroblast Growth Factor Receptor 1
fMRI	Functional MRI
GCP	Good clinical practice
GDPR	EU General Data Protection Regulation 2016
GeCIP	Genomics England Clinical Interpretation Partnership
GRC	Global rating of change Scale
Hb	Haemoglobin
HRA	Health Research Authority
HRD	Homologous Recombination Deficiency
ICH GCP	International Conference of Harmonisation-Good Clinical Practice
ICONIC	Improving outcome through Collaboration in OsteosarComa
ICGC	International Cancer Genome Consortium
IDMC	Independent data monitoring committee
IGF(R)	Insulin-like Growth Factor (receptor)
ISRCTN	International Standard Randomised Controlled Trial Number

IV	Intravenous
LDH	Lactate dehydrogenase
LN	Lymph node
MAMS	Multiarm, multistage study
MAP	High dose methotrexate, doxorubicin, cisplatin
MDM	Medical decision making
MDT	Multi Disciplinary team
MT1-MMP	Membrane type 1 metalloprotease enzyme contributing to angiogenesis.
mNCA	Model Non-Commercial Agreement
MRI	Magnetic Resonance Image
NCRI	National Cancer Research Institute
NICE	National institute for health and care excellence
NIHR	National institute for health research
Non CTIMP	Clinical Trial not involving an Investigational Medicinal Product
ORC	Osteosarcoma Research Consortium
OS	Osteosarcoma
PET/CT	Positron Emission Tomography - Computed Tomography scan
PI	Principal Investigator
PIS	Patient information sheet
PROM	Patient Reported Outcome Measure
QoL	Quality of life
R&D	Research & development
rEECur	An international randomised controlled trial of chemotherapy for the treatment of recurrent and primary refractory Ewing sarcoma.
REC	Research Ethics Committee
RNOH	Royal National Orthopaedic Hospital
RT	Radiotherapy
RTD	Routes to Diagnosis
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAM	Sarcoma Assessment Measure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TESS	Toronto Extremity Salvage Score of function
TMF	Trial Master File
TMG	Trial Management Group
TRACERx	TRAcking non-small cell lung Cancer Evolution through therapy [Rx]
TSC	Trial Steering Committee
TYA	Teenage & young adult cancers
UCL	University College London
UCL CTC	CR UK and UCL Cancer Trials Centre
WB MRI	Whole body MRI
WGS	Whole genome sequencing
WTSI	Wellcome Trust Sanger Institute

APPENDIX 2: QUICK REFERENCE GUIDE TO PATIENT VISITS

SCHEDULE: PATIENTS NOT RECEIVING CHEMOTHERAPY	Pre-registration	Within 28d after registration & before treatment starts	Surgery	End of Treatment	Follow up	Relapse (local recurrence or metastases)
Histological confirmation of osteosarcoma	X					
Informed consent	X					
Relevant Medical History		X				
Cancer signs & symptoms		X				
Smoking status		X				
WHO/Lansky PS[A]		X	X			
Haematology & Biochemistry[B]		X	X			
Data collected on primary tumour diagnostic imaging		X				
Data collected on primary tumour biopsy/surgery		X				
Data collected on diagnostic imaging		X				
Data collected on treatment plan		X				
PROMs[C]		X		X	X[D]	
Diagnostic pathway questionnaire		X[E]				
Blood sample for germline DNA[F]		X				
Ship FFPE tumour tissue to RNOH		X[G]	X[H]			
Assess Adverse Reactions (in relation to study procedures)		X	X	X	X	X
Pre-operative imaging			X			
Data collected on surgery			X			
Data collected on radiotherapy (if given)				X		
Survival status					X	
Data collected on relapse (site, treatment etc.)						X[I]
Tumour tissue						X[J]

A WHO PS for patients ≥16 years; Lansky PS for patients <16 years
B Haematology: Hb, ANC, lymphocytes, platelets; Biochemistry: CRP, albumin, ALP, LDH
C Comprising of: Sarcoma Assessment Measure (SAM), EORTC QLQ-C30, Toronto Extremity Salvage Score (TESS) and Global rating of change scale (GRCS); aged 13 years and over
D Annually (time from registration)
E To be completed within 3 months of registration
F If not collected as part of local biobanking procedure
G Diagnostic archival tissue - shipped as soon as possible after registration, ideally within 4 weeks, not later than 12 weeks
H Ship within 8 weeks after the procedure
I First and any subsequent relapses
J Collected as part of standard of care, or (optional) for research. For new biopsies for research, two cores should be obtained. One fresh frozen in liquid nitrogen according to local policy and one FFPE. If not possible to obtain two cores, then one should be fresh frozen according to local policy.

SCHEDULE: PATIENTS RECEIVING CHEMOTHERAPY	Pre-registration	Baseline	During Treatment			End of Treatment	Follow up	Relapse (local recurrence or	
		Within 28d after registration & before treatment	Neoadjuvant chemotherapy (every cycle)	Surgery	Adjuvant chemotherapy (every cycle)				
Histological confirmation of osteosarcoma	X								A WHO PS for patients ≥16 years; Lansky PS for patients <16 years
Informed consent	X								B Haematology: Hb, ANC, lymphocytes, platelets; Biochemistry: CRP, albumin, ALP, LDH
Relevant Medical History		X							C Comprising of: Sarcoma Assessment Measure (SAM), EORTC QLQ-C30, Toronto Extremity Salvage Score (TESS) and Global rating of change scale (GRCS); aged 13 years and over
Cancer signs & symptoms		X							D Annually (time from registration)
Smoking status		X							E To be completed within 3 months of registration
WHO/Lansky PS[A]		X	X	X	X				F If not collected as part of local biobanking procedure,
Haematology & Biochemistry[B]		X		X					G Diagnostic archival tissue - shipped as soon as possible after registration, ideally within 4 weeks, not later than 12 weeks
Data collected on primary tumour diagnostic imaging		X							H Ship within 8 weeks after the procedure
Data collected on primary tumour biopsy/surgery		X							I First 50 patients receiving chemotherapy only
Data collected on diagnostic imaging		X							J If MAP chemo: collect 3-0 days prior to C2D21 methotrexate; If AP or other chemo: collect pre cycle 3 (ideally up to 3-7 days prior/14 days post cycle 2)
Data collected on treatment plan		X							K Only if first two samples have been collected
PROMs[C]		X				X	X[D]		L NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected
Diagnostic pathway questionnaire		X[E]							M Prior to surgery
Blood sample for germline DNA[F]		X							N Collected every 6 months during follow up
Ship FFPE tumour tissue to RNOH		X[G]		X[H]					O First and any subsequent relapses
Blood sample for CTCs[I]		X	X[J]					X[K]	P Collected as part of standard of care, or (optional) for research. For new biopsies for research, two cores should be obtained. One fresh frozen in liquid nitrogen according to local policy and one FFPE. If not possible to obtain two cores, then one should be fresh frozen according to local policy.
Plasma sample for future research (optional for all patients)[L]		X	X[J]	X[M]		X	X[N]	X	
Data collected on chemo regimen (dose & dates)			X		X				
Assess Adverse Reactions (in relation to study procedures)		X	X	X	X	X	X	X	
Disease assessment (if performed)			X		X	X	X		
Pre-operative imaging				X					
Data collected on surgery				X					
Data collected on chemotherapy given						X			
Data collected on radiotherapy (if given)						X			
Survival status							X		
Data collected on relapse (site, treatment etc.)								X[O]	
Tumour tissue								X[P]	

APPENDIX 3: PERFORMANCE STATUS

WHO Performance Status

Grade	Description
0	Able to carry out all normal activity without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work
2	Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair

Lansky Performance Status

	Lansky score (Age: <16 years)
100	Fully active, normal.
90	Minor restrictions in physically strenuous activity.
80	Active, but tires more quickly.
70	Both greater restriction of, and less time spent in, active play.
60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
40	Mostly in bed; participates in quiet activities.
30	In bed; needs assistance even for quiet play.
20	Often sleeping; play entirely limited to very passive activity.
10	No play does not get out of bed. Moribund.
0	Unresponsive. Dead.

APPENDIX 4: PROTOCOL VERSION HISTORY

Protocol:		**Amendments:**		
Version no.	**Date**	**Amendment no.**	**Protocol Section (no./title)**	**Summary of main changes from previous version.**
1	07/03/2019	n/a	n/a	n/a
2	23/01/2020	2	Throughout	References to *snap frozen* changed to *fresh frozen according to local policy*. Administrative changes - general updates, minor clarifications, correction of typos/formatting errors
			1.1, 6.2.2	Change exclusion criteria from: • Diagnosis more than *three* months prior to registration To: • Diagnosis more than *four* months prior to registration
			9.2.3	Request that the early diagnosis questionnaire should be completed within 3 months of diagnosis changed to within 3 months of registration. Parent or guardian should complete the Early Diagnosis Questionnaire for patients under the age of 13.

			9.2.4	Added: NB if patient has started chemotherapy prior to study entry this baseline sample may still be collected)
			10.1.4	First paragraph changed from: All FFPE samples will be stored at RNOH. Sites may request return of routine samples if required for clinical purposes, but would be requested to return the samples to RNOH. To: All FFPE samples will be stored at RNOH. *During the Study* sites may request return of routine samples if required for clinical purposes but would be requested to return the samples to RNOH after this. *At the end of the Study all archival FFPE samples will be returned to site.*
			10.3	Changed to: A new biopsy, FFPE and snap fresh frozen according to local policy if possible, should be obtained at relapse/resection of local recurrence or metastases for the study if *not* done as part of standard of care. This is optional for patient.