A prospective study of the genomic landscape of central nervous system disease secondary to breast cancer utilising cell-free DNA derived from cerebrospinal fluid (CSF).

PRIMROSE CSF Protocol

V2.0, 20/10/2020

Sponsor:
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ISRCTN: TBC
Sponsor Ref: UoL001567
Research Ethics Ref: TBC

Protocol Approval

I, the undersigned, hereby approve this clinical study protocol:

Authorised by Chief Investigator:

Signature: See accompanying email confirmation _____ **Date:** 21/10/2020 _____

Professor Carlo Palmieri
Chief Investigator

I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of Sponsor:

Signature: See accompanying email confirmation _____ **Date:** 20/10/2020 _____

Dr Neil French
Head of Clinical Operations
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Sponsor

I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of the Lead Statistician:

Signature: See accompanying email confirmation _____ **Date:** 21/10/2020 _____

Dr. Richard Jackson
Trial Statistician
Liverpool Clinical Trials Centre

General Information

This section describes the PRIMROSE CSF study. This study involves the conduct of a CSF extraction procedure which would **not** otherwise form part of the routine care of the patient.

This is a single-arm prospective study. This protocol defines the participant characteristics required for study entry. Participant recruitment will be undertaken in compliance with this document and applicable regulatory and governance requirements. Waivers to authorise non-compliance are not permitted.

PRIMROSE is an umbrella term used to identify a group of studies in patients with the diagnosis of CNS disease secondary to breast cancer. There are currently three separate studies/projects which come under the PRIMROSE header – the PRIMROSE Audit/Registry, PRIMROSE Tissue Study and PRIMROSE CSF Study (current protocol). These three projects are distinct from each other and there is no overlap in data or sample collection in the three studies. Should any patient who is enrolled onto the PRIMROSE CSF study (i.e. this protocol) they will not be entered in PRIMROSE Tissue or PRIMROSE Audit/Registry.

The protocol should not be used as an aide-memoir or guide for the treatment of other patients. Every care was taken in its drafting, but corrections or amendments may be necessary. Any amendments will be circulated to the investigators participating in the study, but sites entering patients for the first time are advised to contact the coordinating centre Liverpool Clinical Trial Centre to confirm they have the most up to date version. Clinical problems relating to this study should be referred to the relevant Chief Investigator, Professor Carlo Palmieri, via the CTC.

The template content structure is consistent with the SPIRIT (Standard Protocol Item: Recommendations for Interventional Trials 2013) and has regard for the Health Research Authority guidance. Regulatory and ethical compliance information is located in Section 15.

The Liverpool Clinical Trials Centre has achieved full registration by the UK Clinical Research Collaboration (www.ukcrc.org) as their standards and systems were assessed by an international review panel as reaching the highest quality. The Liverpool Clinical Trials Centre has a diverse trial portfolio underpinned by methodological rigour, a GCP compliant data management system, and quality management system.

Contact Details: Institutions

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Contact Details: Individuals

Individual Authorised to Sign the Protocol and Protocol Amendments on behalf of the Sponsor:	**Chief Investigator (CI):**	**Medical Expert who will Advise on Protocol Related Clinical Queries (in cases where the CI is unavailable to respond to urgent queries):**
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Translational Laboratory (for sample storage)	**Trial Coordinator**	**Senior Trial Coordinator**
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1. Table of Contents

2. Glossary

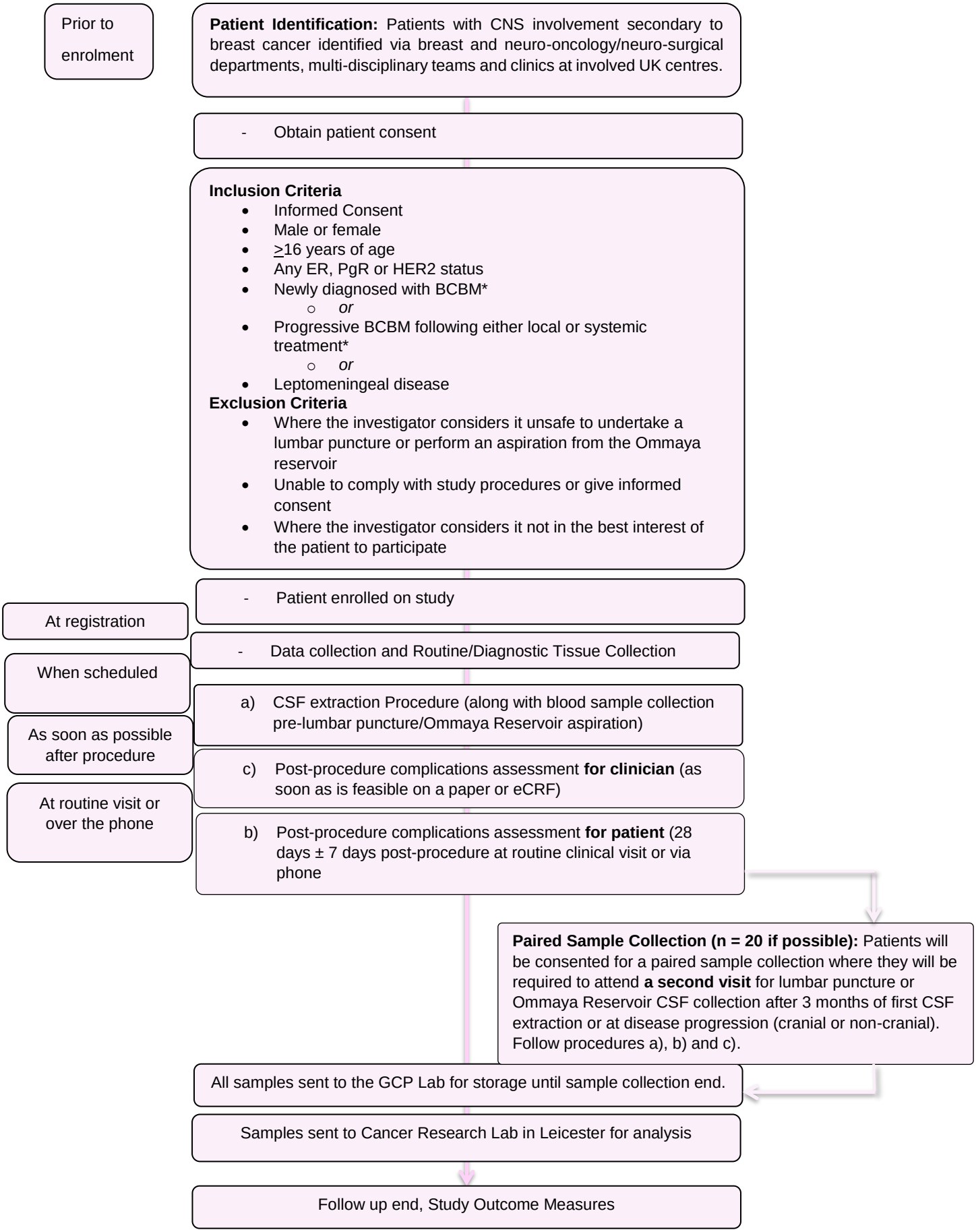
ABN	Association of British Neurologists
BCTRCG	Breast Cancer Trainees Research Collaborative Group
BNSU	British Neurological Surveillance Unit
CI	Chief Investigator
CM	Cerebral Metastases
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
cfDNA	Cell-Free DNA
CT	Computed Tomography
GCP	Good Clinical Practice
HER2	Human Epidermal Growth Factor Receptor 2
IDSMC	Independent Data Safety and Monitoring Committee
ISRCTN	International Standard Randomised Controlled Trials Number
LCTC	Liverpool Clinical Trials Centre
LM	Leptomeningeal Metastases
MBC	Metastatic Breast Cancer
MRI	Magnetic Resonance Imaging
NHS	National Health Service
PISC	Patient Information Sheet and Consent form
PND	Paraneoplastic Neurological Disorders
QA	Quality Assurance
QC	Quality Control
SRS	Stereotactic Radiosurgery
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

3. Protocol Overview

Full Title:	PRIMROSE CSF: A single arm prospective cohort sample collection study of the genomic landscape of central nervous system disease secondary to breast cancer utilising cell free DNA derived from cerebrospinal fluid.
Acronym:	PRIMROSE CSF Study
Target Population:	Breast cancer patients over 16 years old, male or female with central nervous disease.
Sample size:	Between 67 to 87 patients.
Inclusion Criteria:	1. Male or female 2. ≥16 years of age 3. Any ER, PgR or HER2 status 4. Newly diagnosed with BCBM* *or* Progressive BCBM following either local or systemic treatment* *or* Leptomeningeal disease 5. Informed Consent
Exclusion Criteria:	1. Where the investigator considers it unsafe to undertake a lumbar puncture or perform an aspiration from the Ommaya reservoir 2. Unable to comply with study procedures or give informed consent 3. Where the investigator considers it not in the best interest of the patient to participate
Study Centres and Distribution:	Open to all UK NHS centres involved in the medical or surgical care of patients with breast cancer
Patient Study Duration:	The patients will be followed up until death, withdrawal of consent or study closure, whichever is sooner.
Study Duration	2 years

Objectives	**Primary objectives** • To determine the feasibility of recruiting patients to undergo lumbar puncture or aspiration of Ommaya Reservoir to enable the collection of cerebrospinal fluid. • To determine the molecular aberrations, present within CSF cell-free DNA (cfDNA). **Secondary objectives** To determine • The proportion of cases with an actionable mutation based on CSF cfDNA findings using the TARGET (Tumour Alterations Relevant for Genomics-Driven Therapy) database. • The concordance between CSF cfDNA and brain metastases for detected mutations (applicable where brain metastasis has been resected). • The concordance between plasma cfDNA and brain metastases for detected mutations (applicable where brain metastasis has been resected). • The concordance between plasma cfDNA and CSF cfDNA for detected mutations. • The complications post-lumbar puncture or aspiration of CSF from an Ommaya reservoir (This will not apply to patients undergoing lumbar puncture at the time of neurosurgical procedure). **Exploratory objectives** • To compare the genomic landscape between paired CSF paired samples (i.e. where collected at different time points)

3.1. Schematic the Study



4. Roles and Responsibilities

Sponsor

The Sponsor is the University of Liverpool and is legally responsible for the study. They will formally delegate specific sponsoring roles to the Chief Investigator and Clinical Trials Centre.

Funders

North West Cancer Research is the lead funder for this study. It is funding the feasibility study the collection of 40 CSF samples of CSF, storage at GCP Laboratories and analysis at the University of Leicester.

Daiichi Sankyo Europe GmBH is funding for 20 paired samples of CSF or up to 40 single CSF samples . The funding is for collection and storage at GCP Laboratories.

Make 2nds Count is funding for 7 samples of CSF. The funding is for collection, storage at GCP Laboratories and analysis at the University of Leicester.

A summary is provided below:

Funder	**Number of patients**	**Diagnosis: Histologically and/or cytologically confirmed breast cancer with CNS involvement, as defined as having the following:**
North West Cancer Research	40	Metastases to the brain parenchyma
Daiichi Sankyo	20 paired samples or up 40 single samples	Metastases to the brain parenchyma or Metastases to the leptomeninges or
Make 2nds Count	7	Metastases to the brain parenchyma or Metastases to the leptomeninges or

Chief Investigator

Professor Carlo Palmieri is the Chief Investigator for this study and is responsible for overall design and conduct of the study in collaboration with other members of the study team.

Principal Investigators/Co-Investigators

In each participating centre a principal investigator and co-investigators, part of breast and/or neuro-oncology/neurosurgical multidisciplinary teams, will be identified to be responsible for identification, recruitment, data collection and completion of CRFs, along with follow up of study patients and adherence to study protocol at site. They will also be responsible for safety reporting and processing any applicable safety information.

Clinical Trials Centre

The Liverpool Clinical Trials Centre at the University of Liverpool in collaboration with the Chief Investigator, will have overall management responsibility and will be responsible for study management activities including (but not limited to) study planning, budget administration, Trial Master File management, data management, statistical analysis and participating site coordination.

Oversight Committees

Trial Management Group (TMG)

A TMG will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical) and members of the LCTC. The TMG are responsible for monitoring all aspects of the progress and conduct of the study and will be responsible for the day-to-day running and management of the study. The group will work with research nurses and members of the Breast Cancer Trainees Research Collaborative Group to:

- Build the clinical network to maximise recruitment opportunities
- Ensure consistent identification and registration of eligible patients to the study
- Increase the study's exposure to trainees at all UK cancer and neurological units and centres.

The TMG will meet at least monthly at setup stage and then reduce to quarterly throughout the year unless more frequent meetings are required.

Trial Steering Committee (TSC)

The Trial Steering Committee will consist of an independent chairperson (i.e. the individual will not be involved in the development of the study and the hospital they work will not open as a site),

independent experts in the field of breast cancer brain metastases, independent statistician, patient representative and study coordinator. The role of the TSC is to provide overall supervision for the study, present and discuss any new presenting issues in the study and provide advice through its independent Chairman. The decision for the continuation of the study lies with the TSC and as such they will meet throughout the study (at least annually).

4.1 Protocol Contributors

Name	Affiliations	Contribution to protocol
Professor Carlo Palmieri	Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine Sherrington Liverpool L69 3GA	Drafted original grant application on which the protocol is based and Reviewed all sections. Signed off final protocol draft.
Professor Michael Jenkinson	The Walton Centre NHS Foundation Trust Lower Lane Fazakerley Liverpool L9 7LJ	Reviewed all section and provided feedback on Safety Reporting (List of expected events)
Dr Vinton Cheng	Leeds Teaching Hospitals NHS Trust, St James' University Hospital, Beckett Street, Leeds, LS9 7TF	Reviewed all sections and provided clinical insight
Ms Helen Scott	Liverpool Clinical Trials Centre 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Reviewed all sections
Andrea Newhouse	Liverpool Clinical Trials Centre 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Drafted and coordinated review process
Dr Richard Jackson	Liverpool Clinical Trials Centre 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool	Reviewed statistics section

	L69 3GL	
Dr Victoria Shaw	GCP Laboratories 1st Floor William Henry Duncan Building University of Liverpool 6 West Derby Street Liverpool L7 8TX	Reviewed samples, outcomes section
Ms Laura Marsh	Liverpool Clinical Trials Centre 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Reviewed Data section
Ms Charlotte Rawcliffe	Liverpool Clinical Trials Centre 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL	Reviewed all sections and signed off final protocol draft.

5. Background

5.1 Introduction

Breast cancer (BC) is the second most common solid malignancy to involve the central nervous system (CNS) and breast cancer brain metastasis (BCBM) is particular feature of HER2-positive and triple negative BC, with a 2 to 5-fold increased risk of developing brain metastasis (BM) compared to luminal A BC [1,2] , and 22-50% of patients with metastatic TNBC and HER2-positive BC will develop BM[2]. Recent clinical data has shown that BCBM is an increasing clinical problem in metastatic breast cancer (MBC)[3,4]. This is likely to be a reflection of women with MBC living longer with improvements in systemic therapy and increased detection with the use of brain MRI. Patients will often develop progressive BM in the setting of extracranial disease that is adequately controlled with existing chemotherapies or targeted therapies. BCBM causes significant morbidity and mortality[5,6] and despite this patients with BCBM have been disadvantaged by a relative lack of clinical research in this area, in fact such patients are often explicitly excluded from clinical trials. Progress is further hampered by the lack of annotated collection of biological material to underpin translational research in this area[7]. The current standard of care for newly diagnosed BM includes surgery, stereotactic radiosurgery (SRS), and/or whole brain radiotherapy (WBRT)[1]. On intracranial disease progression, systemic therapy is normally the standard of care if no further local therapy is possible[8]. However, the efficacy of systemic treatment is extremely limited and at present, no systemic therapies are specifically approved for the treatment of BM secondary to BC[8]. Therefore, CNS disease is a major clinical problem encountered by a significant number of women with MBC, and the lack of effective treatments makes this an area of unmet clinical need.

The advent of genomic medicine has enabled the characterisation of tumours both for the purposes of diagnostics and therapeutics. Notably, the advances in genomic medicine have helped dissect and understand the evolutionary process of cancer by comparing primary cancers with secondary cancers[9]. However, obtaining samples for genomic work in patients with CNS involvement secondary to breast cancer is difficult given the risks and complexity of neurosurgery. The procedure may not be always appropriate given the occurrence of multiple lesions while the increasing use of Stereotactic Radiosurgery (SRS) has reduced the need for surgical resection and so the availability of tissue. In addition, longitudinal tissue sampling during the course of the disease is not possible.
The importance of treating the metastases in the brain separately from any prior extracranial metastases has been demonstrated in a recent study of 86 paired primary and brain metastases of which 21 were primary breast cancer and their matched brain metastases[10]. This study

demonstrated that while the primary and brain metastases shared a common ancestor, both the primary tumour and the metastasis continued to evolve separately, reflected by the presence of distinct mutations so called ‘’private mutations’’[10]. Where the regional lymph nodes, or extracranial metastases were shown not to be reliable surrogates for the oncogenic alterations found in the brain metastases[10]. Inter- and intralesional sampling of the brain metastases demonstrated genetic homogeneity; with brain metastases samples sharing mutations that were not detected in the clinically sampled primary tumour, indicating that the subclones sampled in these lesions were more related to one another than to those detected in the primary tumour. In approximately half of cases, clinically informative alterations which were found in the brain metastases were not detected in the matched primary tumour [10]. These data therefore highlight the need to analyse the brain metastases as a distinct entity rather than relying solely on information from either the primary or the extra cranial systemic disease, as well as opportunities for innovating treatment with targeted agents based on information derived from the brain metastases.

Plasma cell-free circulating tumour DNA (cfDNA) is the fraction of the total cell-free DNA that is derived from tumour cells and can be defined by the presence of genomic alterations. cfDNA can be used as ‘liquid biopsy’ and in extracranial disease has been used to characterise tumours and allow patients and their cancers to be monitored, assess response to treatments and to develop clinical studies[11–13]. However, in the context of primary brain tumours and brain metastasis, plasma cfDNA has been shown to be either absent or present at low abundance [12].

Cerebrospinal Fluid (CSF) is in intimate contact with brain malignancies and two recent studies have shown that cfDNA can be isolated from CSF in patients with solid malignancies including breast cancer patients, highlighting its potential use in terms of treatment decision making[11,14]. In these studies of CSF cfDNA from patients with breast cancer with CNS involvement were analysed[11,14]. These studies demonstrated a number of important points related to CSF cfDNA:

(i) that it is representative of intracranial disease;
(ii) they showed that trunk mutations, present in all cancer cells, as well as private genomic alterations, present in just a subpopulation of cells or in specific metastatic lesions, can be identified in the CSF cfDNA;
(iii) that it can be used to identify actionable genomic alterations with potential therapeutic implications including those involving EGFR, ALK and HER2 and
(iv) that it can be used to identify mechanism of drug-resistance at disease progression to inhibitors of oncogenic kinases [11,14].

A recent case shows an ESR1 p.Y537N mutation in CSF cfDNA that was not detected in plasma cfDNA or CTCs taken on the same day, suggesting the presence of alternate dominant clones in blood and CSF (Figure 1 Prof J Shaw, unpublished data). Together, these data highlight the potential applications of CSF cfDNA in helping to diagnose and determine targeted therapy in patients with BCBM. No safety concerns have been reported to date with regard to undertaking lumbar puncture for CSF collection in the studies undertaken to date these have included primary as well as secondary CNS disease [14–23].

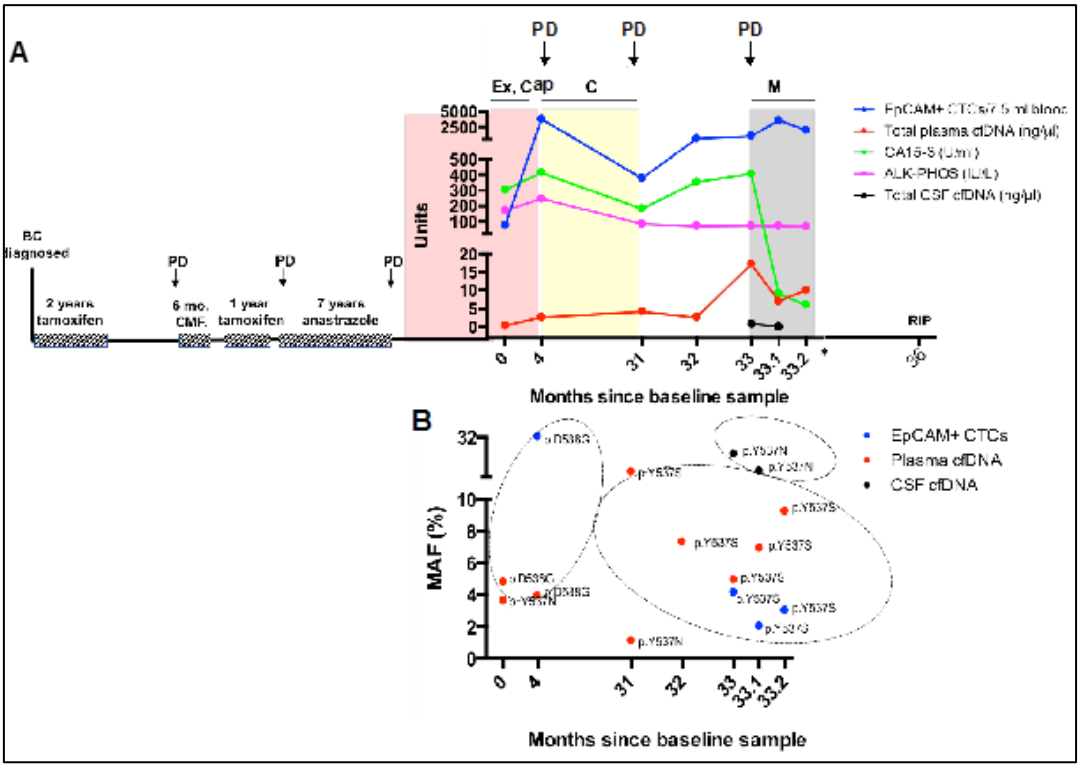


Figure 1. Mutational heterogeneity between blood and CSF of a patient with MBC. Seven blood samples and 2 CSF samples were collected over a 33-month period. (A) Treatment history and disease response, are shown with CTC counts, serum CA15-3 and ALK-PHOS plasma cfDNA and CSF cfDNA levels. Coloured shading: exemestane (Ex), capecitabine (Cap), multiple chemotherapy regimens (C), cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) and methotrexate (M) treatment. CTC counts and total plasma cfDNA levels were high throughout the monitoring period as were CA15-3 levels until the last 2 samples when a sharp drop was observed. (B) Seven serial cfDNA samples, 5 CTC samples and 2 CSF samples were analysed by NGS. Mutations detected at > 1% MAF are shown. Two ESR1 gene mutations were detected in the first plasma cfDNA sample;

p.D538G (MAF: 4.8%) and p.Y537N (MAF: 3.6%). Following 27 months of chemotherapy treatment, an ESR1 p.Y537S mutation was detected in plasma. The same mutation was detected one month later, and in all plasma and CTC samples analysed thereafter. ESR1 p.Y537N was detected in the plasma both at the beginning of the monitoring period and at month 31, at later stages this mutation was detected exclusively in CSF. *Note that the last 3 samples taken from this patient were collected one week apart

5.2 Rationale

Given the current paucity of research related to CNS disease secondary to breast cancer, the lack of CSF samples to analyse and the recent published data highlighting the need for therapeutic innovation in breast cancers with brain metastases we propose explore the feasibility of collecting CSF cfDNA within UK centres in patients with CNS involvement secondary to breast cancer. We intend to use the collected CSF cfDNA to interrogate and understand the molecular aberrations present within these samples with a view to developing stratified clinical trials. The utility of cfDNA in the CSF as a surrogate for genomic aberrations within brain metastasis will also be explored. This work will help improve our understanding of the underlying biology of CNS involvement secondary to breast cancer and the aberrations which underpin their establishment, development and progression.

5.3 Risks and Benefits

5.3.1 Potential Risks

This study involves collecting CSF via lumbar puncture (with atraumatic needle) or from aspiration of CSF from in situ Ommaya reservoirs form patients with CNS involvement secondary to breast cancer. For the majority of patients this will be an extra procedure, although in a small number of cases it will be performed as part of routine care. Potential risks and side effects of the procedures include infection (rare), nerve root irritation (in a minority of patients), headaches and back pain.

To minimise risk lumbar punctures will be performed using appropriate local anaesthesia by clinicians experienced in the procedure. Furthermore, atraumatic needles will be used which are associated with significantly less side effects than conventional needles for lumbar punctures. Where patients are undergoing a neurosurgical procedure, the lumbar puncture will be carried out once patient has been anaesthetised so reducing any side effects. Patients will be followed up to understand the side effects if any that occur post procedure.

5.3.2 Potential Benefits

It is likely that patients will not derive any direct benefit from this study. However, it is hoped that this study will provide evidence which will enable the development of future studies and treatments for patients with CNS disease secondary to breast cancer.

5.4 Aims and Objectives

5.4.1 Primary objectives

- To determine the feasibility of recruiting patients to undergo lumbar puncture or aspiration of Ommaya Reservoir to enable the collection of cerebrospinal fluid.
- To determine the molecular aberrations, present within CSF cell-free DNA (cfDNA).

5.4.2 Secondary objectives

To determine

- The proportion of cases with an actionable mutation based on CSF cfDNA findings using the TARGET (Tumour Alterations Relevant for Genomics-Driven Therapy) database.
- The concordance between CSF cfDNA and brain metastases for detected mutations (applicable where brain metastasis has been resected).
- The concordance between plasma cfDNA and brain metastases for detected mutations (applicable where brain metastasis has been resected).
- The concordance between plasma cfDNA and CSF cfDNA for detected mutations.
- The complications post-lumbar puncture or aspiration of CSF from an Ommaya reservoir (This will not apply to patients undergoing lumbar puncture at the time of neurosurgical procedure).

5.4.3 Exploratory objectives

- To compare the genomic landscape between paired CSF paired samples (where collected)

6. Study Design

This is a prospective cohort sample collection multicentre study. Patients with histological confirmed locally advanced or CNS disease secondary to breast cancer metastasis cancer who meet the entry criteria will be provided with details of the study and consented before registration. Patients entering the study will have CSF collected via a lumbar puncture (with atraumatic needles unless discussed with the chief investigator) or by aspiration from their Ommaya reservoir which is already in situ. This procedure will be in addition to standard of care treatments.

6.1 Study Setting

6.1.1 Selection of Participating Sites

This study has received support from several UK sites which were submitted with the grant application. Any UK centre with the appropriate capacity and resources to deliver CSF extraction procedures and process CSF and blood samples and where patients receive clinical care for breast cancer will be eligible to participate in the study.

A Delegation of Duties Log will be prepared for each site. This log will name the Principal Investigator, subinvestigator(s), trial/study coordinator(s), and all other clinical staff conducting activities for the study. New or replacement staff will be added and signed off as appropriate.

Logistically, we anticipate the majority of cases will be identified by surgeons, oncologists or neurologists either sitting within breast and neuro-oncology multidisciplinary team meetings or via cases seen in the outpatient or inpatient setting.

6.1.2 Selection of Principal Investigators

Clinical co-investigators, collaborators and trainee leads will be selected at each participating site.

All principal investigators will be required to demonstrate relevant experience and commitment during early phase feasibility assessment. All investigators must have the particular medical expertise necessary to conduct the study in accordance to the protocol. Written agreement to conduct research as such will be obtained prior to site initiation.

A suitable co-investigator should be identified at each site to deputise in case of PI absence.

7. Eligibility Criteria

The PRIMROSE CSF Study aims to recruit between 67 to 87 patients meeting the following criteria.

7.1 Inclusion Criteria

Patients eligible for the trial must comply with all the following at enrolment:

1. Male or female.
2. ≥16 years of age
3. Any ER, PgR or HER2 status
4. Newly diagnosed with BCBM*
   OR
   Progressive BCBM following either local or systemic treatment*
   OR
   Leptomeningeal disease
5. Informed Consent

*These patients will be recruited for the feasibility part of the study.

7.2 Exclusion Criteria

Any patient meeting any of the criteria listed below at baseline will be excluded from study participation:

1. Where the investigator considers it unsafe to undertake a lumbar puncture or perform an aspiration from the Ommaya reservoir.
2. Unable to comply with study procedures or give informed consent.
3. Where the investigator considers it not in the best interest of the patient to participate.

7.4 Co-enrolment Guidelines

Participants can be recruited into other trials during their participation in this study. This will be documented as part of the follow up process. Individuals who have participated in any other studies or trials of any medicinal product can also be enrolled onto this study if eligible.

8. Outcomes

8.1 Primary Outcomes

Objective	Outcome Measure
To describe the feasibility of recruiting patients to undergo lumbar puncture to enable collection of cerebrospinal fluid.	Quantitative measurement of study feasibility will be measures as the ability of the study to recruit patients and obtain clinical data for analysis. Specifically: RECRUITMENT • Recruitment rate: defined as the total number of patients randomised per month • Site opening: defined as the time take to open the target number of sites STUDY PROTOCOL • Patient adherence to protocol: to determine whether patient and /or care givers adhere to the conditions of the protocol, measured via the number of minor or major protocol deviations • Review of the practicality of delivering interventions SAMPLE SIZE INFORMATION • The estimation of quantities required for an accurate sample size calculation, such as standard deviation of the outcome measure DATA COLLECTION • The proportion of expected CRFs returned and the rate of missing key data items PATIENT ACCEPTABILITY • Estimate drop-out rates • Participation rates

To determine the molecular aberrations, present within CSF cell-free DNA (cfDNA).	This will be measured by quantifying the proportion of genomic aberrations in all collected types of cfDNA samples. The outcome will be percentage of specific of aberrations observed in samples as a proportion of all aberrations observed.

8.2 Secondary Outcomes

Objectives	**Outcome Measure**
The proportion of cases with an actionable mutation based on CSF cfDNA findings using the TARGET (Tumour Alterations Relevant for Genomics-Driven Therapy) database	These will be determined by identifying and quantifying (as a percentage or proportion) actionable mutations found in CSF cfDNA that match a specific gene database, namely TARGET (Tumour Alterations Relevant to Genomics Driven Therapy) gene database.
• The concordance between CSF cfDNA and brain metastases for detected mutations • The concordance between plasma cfDNA and DNA from breast, extracranial and/or brain metastases for detected mutations (applicable for where brain metastasis has been resected) • The concordance between plasma cfDNA and CSF cfDNA for detected mutations.	This will be measured by quantifying the genomic aberrations in both the CSF cfDNA and plasma cfDNA or tissue sample (primary, extracranial and/or CNS metastasis). The level of agreement will be measures as the number of aberrations observed in both samples as a proportion of all aberrations observed.
To determine the complications post lumbar puncture or aspiration of CSF from an Ommaya reservoir (This will not apply to patients undergoing a lumbar	1 x Patient questionnaire to record complications post-procedure.

puncture at the time of a neurosurgical procedure).	Outcomes will be Percentage incidence, percentage severity experiences (i.e. headache, backache, pain down leg) and mean duration of experiences will be determined. 1 x Clinician questionnaire to record complications post-procedure. eCRF will be used to record the date, the ease of the procedure complications and the need for intravenous fluid and/or analgesia Outcomes will be percentage / proportion of patients over total recruited patients.

8.3 Exploratory Outcomes

Objective	**Outcome Measure**
To compare the genomic landscape between paired samples (where these are available)	This will be measured by quantifying the genomic aberrations in both the CSF cfDNA samples. The level of agreement will be measures as the number of aberrations observed in both samples as a proportion of all aberrations observed.

9. Participant Timelines and Assessments

9.1 Participant Identification

Patients will be identified by the oncologist, neurologist, neuro-oncologist or clinical staff in direct care of the patient. Once a site has been greenlighted, the necessary research team members will be made aware of the inclusion criteria of the study.

9.2 Informed Consent

Informed consent is a process initiated prior to an individual agreeing to participate in the study and continues throughout the individual's participation. Informed consent is required for all patients participating in this study. In obtaining and documenting informed consent the investigator should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Discussion and explanation of the study, using the Patient Information Sheet should be provided to patients by staff with appropriate experience. An appropriate Patient Information Sheet and Consent Form, describing in detail the study procedures and risks will be approved by an ethics committee and the patient will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the patient and answer any queries that arise. A contact point where further information about the study may be obtained will be provided to the patient. If a potential participant does want to withdraw from the study, they do not have to give a reason.

Permission will be sought, and all patients will be required to give informed consent prior to any study procedures. Patients will be approached during their first or second clinic visit/appointment. Patients will be given time to consider the study, and if they wish to take part will be asked to sign the relevant consent form.

Consent will be obtained to take part in the study and to collect the following samples:

- Collection of available and future routinely collected Formalin Fixed Paraffin Embedded (FFPE) samples of primary breast cancer (from diagnostic biopsies), non-CNS metastasis (from diagnostic biopsies of brain metastases – where available) and brain metastasis (where available)
- Collection of Blood and Cerebrospinal Fluid sample[1]
- Collection of a repeat Blood and Cerebrospinal Fluid sample can occur at the time of disease progression or if patient is well and responding to treatment at 3 months after the initial procedure.

[1]Patients having surgical resection of brain metastasis can have their lumbar puncture under general anaesthesia at start of procedure (where safe to do so). In addition, CSF can be collected at time of insertion of collection of Ommaya reservoir.

Informed consent should be re-affirmed throughout the study and all discussions and consent should be documented appropriately. The original signed document will be retained in the trial site's Investigator Site File (ISF) and copies will be made:

- One copy provided to the patient for their information,
- One copy transferred to the CTC via portal upload or encrypted email
- One copy filed in the participant's medical records paper/electronic.

N.B. Details of the consent process (date, persons involved, version and type of information sheet and consent form used) must also be recorded directly into the participant's medical records.

9.3 Eligibility Assessment and Confirmation

Eligibility should be confirmed by an appropriately qualified member of the research team who is named on the delegation log. This must not occur until informed consent is documented. Eligibility Criteria are described in detail in Section 7.

Eligibility confirmation must be documented in the participant's medical notes and then on the relevant study CRF.

9.4 Patient Recruitment, Screening and Enrolment

Potential study participants will be identified from patients who meet the inclusion criteria.

Patients will be provided with a patient information sheet and the accompanied informed consent form during clinic visits or by post/email following appropriate discussion with a member of the research team. The clinical research team will be available in person or by phone to discuss information relating to the study to interested patients.

After sufficient time for review and consideration of the trial, patients will return to the clinic to address any questions and will be asked to provide informed written consent. **Please note that in exceptional circumstances (i.e. when a patient lives far away from site or when the patient shows full understanding of the study and is agreeable to take part in the study) consent may be taken within the first visit.**

Investigators should complete eligibility screening based on inclusion/exclusion criteria outlined in section 7 of this protocol. During the screening visit the following reviews/investigations will take place to ensure eligibility of patients to participate into the study (see Section 7 for full eligibility criteria)

After a patient has been screened, their details MUST be entered onto the eCRF database by the clinical study staff to register the patient and generate a Study ID for patient.

Participants will be registered via a secure (24-hour) web-based eCRF system controlled centrally by the LCTC. A personal login username and password provided by the LCTC will be required to access the registration system. Designated research staff will be issued with their personal login and password upon completion of training in the use of the system. This training will be coordinated by the LCTC. A Work Instruction will also be provided to sites to support the registration process.

When the system requirements (i.e. consent, eligibility) are confirmed the participant registration and a unique study number will be displayed on a secure webpage. An automated email confirmation will be sent to the authorised Principal Investigator and PRIMROSE team.

PRIMROSE Registration Details:

Web access: https://www.lcturedcap.org.uk/redcap/

Tel: 0151 794 2405

Email: primrose@liverpool.ac.uk

(*Note that LCTC is open between 0900-1700, Monday – Friday, excluding public holidays)*

In the event of a registration system failure, the centre should contact the coordinating team at the LCTC (Monday to Friday between 9:00 to 17:00 excluding bank holidays) to try to resolve the problem. If the problem cannot be resolved the coordinating LCTC will perform central registration and provide the details over the phone.

To enable monitoring of the consent process and registration of patient, the following study documents should be forwarded to the LCTC by the Investigator/research site team within 2 days of a patient registration:

- A copy of the Signed Consent Form signed by both the patient and the investigator

The documents should be uploaded to the PORTAL/emailed to the LCTC on Monday - Friday from 09:00 to 17:00 GMT, email: primrose@liverpool.ac.uk. Prior to sending documents, site staff should telephone 0151 794 2405 to inform the LCTC staff of the incoming registration.

The patient's GP and referring consultant will be informed of patient's decision to participate in the study.

9.5 Assessments and Follow-up

Once the patient has been registered on the PRIMROSE CSF study, the patient will be scheduled in for a lumbar puncture (with atraumatic needles) or aspiration of CSF from Ommaya Reservoir procedure as soon as is reasonably possible or at the time of neurosurgery if this planned or has been scheduled as part of routine care.

The schedule of assessments is outlined below. The following research data and samples will be collected from the patient:

9.5.1 Schedule of Study Procedures

	Screening & Registration (1st or 2nd visit)	**First CSF Sample collection Visit***	**2nd CSF Sample Collection Visit***	**Disease Progression OR Event driven Follow-up (e.g. Neurosurgery or relapse)****
Procedures				
Communication of study information & PIS	**X**			
Informed Consent	**X**			
Check of Eligibility Criteria	**X**			
Confirmation of ER, PgR, HER2 status (primary breast cancer)***	**X**			
Data Collection				
Demographic Information	**X**			
Clinico-pathological information	**X**			
Prior Cancer Therapy (top line)	**X**			
Collection of data: At study entry and then subsequently when there is disease progression, treatment changes and	**X**			**X**

death				
Diagnostic Biopsies				
Requesting and posting of FFPE Tissue: i) Collection of Primary Breast Biopsy and/or ii) Collection of non-CNS recurrent/metastatic tissue and/or ii) Collection of CNS Biopsy	**X**			**X**
Blood and CSF				
Collection of blood samples: *1 x 10ml EDTA blood tube* *1 x 10ml SERUM tube* ***(prior to lumbar puncture with atraumatic needle)***		**X**	**X**	
Lumbar Puncture *Collection of:* *1 x 10ml CSF tube* *1 x 3ml - 5ml CSF tube*		**X**	**X**	
Questionnaire (Completed on patient's discharge)				

Clinical complications questionnaire (clinical staff to complete on eCRF)		**X**	**X**	
Questionnaire (28 days ± 7 days post-lumbar puncture)				
Patient complications questionnaire (patient to complete via phone or at routine visit)		**X**	**X**	

*For initial and sequential CSF sample (at disease progression or if patient is well 3 months after first CSF extraction). This will be mentioned on the Patient Information Sheet and Consent Form.

**Data will be collected for all patients if there are changes in their progression, treatment or in the event of death up until study closure or patient withdrawal.

***ER, PgR and HER2 status of all CNS and non-CNS metastatic (where available) collected will documented.

10. Procedures for all patients

10.1 Screening and Enrolment

Screening and Enrolment will be completed as detailed in Section 9, 9.4.

10.2 After Enrolment

Following patient enrolment, the following forms should be completed on the database using existing medical records/patient medical notes:

i. Demographic Information
ii. Clinico-pathological information
iii. Prior Cancer Therapy

The diagnostic FFPE blocks available for the patient should be requested from pathology as soon as possible after the patient is enrolled. The sample information should be logged on the database and the sample sent to the GCP Laboratories using the sample kits provided.

10.3 First Lumbar Puncture / Ommaya Reservoir Aspiration Visit

The following procedures will occur at this visit:

- Blood sample collection (*1 x 10ml EDTA blood tube and 1 x 10ml SERUM tube)* – **this should be collected immediately before the CSF extraction procedure**
- CSF extraction (*1 x 10ml CSF tube and 1 x 3ml - 5ml CSF tube)* – **this should be carried out via Lumbar Puncture or Ommaya Reservoir aspiration as per standard procedure (atraumatic needles should be used unless discussed with PI & CI)**
- Clinical complications questionnaire (completed by clinical staff via eCRF)

10.4 Follow-up - 28 days ± 7 days post-procedure (after CSF collection)

This visit can be carried out either at a routine visit or via phone call.

The following procedures will occur at this visit

- Completion of the patient complications questionnaire.

The clinician should read the questions to the patient and record the patients answers either directly into the eCRF or onto the paper worksheet. If the answers are recorded on the paper worksheet, these should be transferred onto the database. The worksheet does not need to be sent to the LCTC but should be kept in the patient notes.

The worksheet can be downloaded from the LCTC portal.

10.5 Disease Progression / Event driven Follow-up

If there are no changes in patient's condition, disease or treatment then there is no requirement to provide follow-up data.

In the event of patient disease progression, treatment changes or in the event of death, the following forms should be completed on the database using existing medical records/patient medical notes:

i. Disease progression
ii. Treatment changes
iii. Death data (cause and date)

In the event of new tissue samples being collected either at the point of neurosurgery or when there is a change in patient's condition, disease or treatment, the FFPE blocks available for the patient should be requested from pathology. The sample information should be logged on the database and the sample(s) sent to the GCP Laboratories using the sample kits provided.

10.6 Second Lumbar Puncture / Ommaya Reservoir Aspiration Visit

Where a patient is undergoing a second CSF collection, this should be carried out either at disease progression or if the patient remains well – after 3 months of the first CSF extraction.

The following procedures should occur at this visit:

- Blood sample collection (*1 x 10ml EDTA blood tube and 1 x 10ml SERUM tube)* – **this should be collected immediately before the CSF extraction procedure**
- CSF extraction (*1 x 10ml CSF tube and 1 x 3ml - 5ml CSF tube)* – **this should be carried out via Lumbar Puncture or Ommaya Reservoir aspiration as per standard procedure (atraumatic needles should be used unless discussed with PI & CI)**
- Clinical complications questionnaire (completed by clinical staff via eCRF)

Following this visit, the Follow-up visit should take place as per Section 10.4

10.7 Participant Discontinuation/Withdrawal

In consenting to the trial, participants agree to all trial activities including sample collection and data collection. Every effort should be made to facilitate the completion of these for every recruited participant. If it is not possible to complete these activities (or it is deemed inappropriate) the reasons why should be documented.

Patients may be withdrawn from the study for any of the following reasons:

- Patient (or their designated legal representative, where applicable) withdraws consent.
- Where the investigator deems it not in the patient's best interest to continue on the study
- Death
- Any change in the patient's condition which results in contraindication to collection procedures or causes the patient to fall under the exclusion criteria

Data to be collected at the time of discontinuation includes the date of discontinuation and reason for discontinuation.

If a patient wishes to withdraw, the value of existing samples should be explained, and permission sought to continue retaining and using these samples. Generally, samples will be retained unless it is specifically requested for them not to be. Upon withdrawal, the LCTC and TMG should be informed in writing immediately (ideally via E-mail) and no further samples or data should be collected.

Permission will be sought to use all samples collected thus far in the case of withdrawal. However, patients have the right to request that any samples which have not yet already been used in research to be disposed of. If a patient explicitly states that this is their wish, then the LCTC should be informed immediately in writing and the samples will be destroyed.

In some cases, it will be impossible to destroy the samples without affecting other samples as they will have been irreversibly linked to others (such as in cases of tissue microarrays being produced or plasma samples being pooled). In these cases, the sample itself will not be destroyed; however, information linking the sample to any patient identifiers will be destroyed, deleted or censored as appropriate to eliminate the link and make the sample unidentifiable.

10.8 Patient Transfer

If a participant moves from the area, every effort should be made for the participant to be followed-up at another participating trial centre and for this trial centre to take over responsibility for the participant.
A copy of the participant eCRFs should be provided to the new site. The participant remains the responsibility of the original site until the new site PI has signed the Transfer Form.

10.9 Loss to Follow-up

A participant will be considered lost to follow up if they fail to return for scheduled visits and cannot be contacted by the site research team.

If a participant fails to attend/facilitate a required study visit the following actions must be taken:

- Site will attempt to contact the participant and reschedule the missed visit and advise the participant on the importance of maintaining the assigned visit schedule.
- Before a participant is deemed to be lost to follow up, site research staff will make every effort to regain contact with the participant (i.e. 3 telephone calls and, if necessary, a headed letter to last known address). These efforts should be recorded in the patient medical notes.
- If the participant continues to be unreachable, they should be considered withdrawn from the study with a primary reason of lost to follow up and this should be recorded on the appropriate CRF.

10.10 End of Study

The end of the study is defined to be the date on which data for all participants has been analysed. Prior to analysis start, data entry privileges will be withdrawn from the study database. The study may be closed prematurely on the recommendation of the Trial Steering Committee (TSC).

Site and study closure activities will be centrally coordinated and conducted in accordance with LCTC processes regardless of whether the study closes as planned or prematurely. This includes activities such as:

- End of Trial notification to REC
- Checking that all site data entered onto the study database, discrepancies raised, and satisfactory responses received

- Quality Control checks of the Investigator Site Files and Trial Master File as appropriate.

11. Samples

All PRIMROSE CSF and blood samples should be collected, processed, stored and shipped according to the PRIMROSE CSF Study Laboratory Manual.

Sample kits will be provided, and postage costs will be covered by the trial.

11.1 Formalin Fixed Paraffin Embedded (FFPE) Tissue Samples

The FFPE block from the diagnostic biopsy (breast cancer, brain metastases biopsy if available or non-cranial biopsy if available) will be collected for patients enrolled on the PRIMROSE CSF Study. This will be requested immediately after registration confirmation and sent to the GCP Laboratory Facility as soon as possible.

These samples should be shipped to the GCP Lab according to the PRIMROSE Laboratory Manual.

11.2 Blood (EDTA & Serum) Samples

The following blood samples will be taken at the first CSF Extraction visit for all patients, and at the second CSF Extraction visit for patients undergoing a 2nd CSF extraction:

- *1 x 10ml EDTA blood sample*
- *1 x 10ml SERUM blood sample*

These samples will be processed at each trial site, stored at -80 °C and transferred in batches to the GCP Laboratory Facility. Samples will then be stored under appropriate conditions for up to 15 years from study start.

11.3 Fresh CSF Sample

All patients will have a CSF sample collected at the First CSF Extraction Visit. Some patients will also have a 2nd CSF sample collected at the Second CSF Extraction Visit.
The following CSF sample will be collected at these visits:

- *1 x 10ml CSF sample*
- *1 x 3ml - 5ml CSF sample*

This should be carried out via Lumbar Puncture or Ommaya Reservoir aspiration as per standard procedure (atraumatic needles should be used unless discussed with PI & CI)

This sample should be processed, stored at -80^{o}C and transferred in batches to the GCP Lab according to the PRIMROSE CSF Study Laboratory Manual.

All samples will be stored until analysis under appropriate conditions for up to 15 years from study start. Following analysis, all samples will be transferred to the LCTC Post-Trial Tissue Bank.

12 Safety Reporting

Safety reporting in clinical trials/studies is a legal and ethical requirement and it is imperative that all applicable requirements detailed here are followed during the study.

All adverse Events (AEs) related to PRIMROSE CSF Study should be reported to LCTC within the given timelines in this protocol and appropriately assessed and recorded on forms provided on the LCTC portal. **IMPORTANT: For any Serious Adverse Events (SAEs) please fill in the appropriate forms and send to the following email address: lctcsafe@liverpool.ac.uk immediately after filling in.**

The reporting procedures detailed in this section should be followed. Any questions concerning AE reporting should be directed to the LCTC in the first instance via phone and/or email (primrose@liverpool.ac.uk).

The active monitoring period for this study is from the date of procedure to the date of post-procedure questionnaire.

12.1 Terms and Definition

Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the study procedures.

Related Adverse Event (Related AE)

An AE which resulted from administration of any of the research procedures – i.e. assessed as “probably”, “possibly” or “almost certainly” related to the trial procedures (see Section 12.4)

Related Unexpected Adverse Event (RUAE)

A Related AE which is not expected, i.e. not consistent with the known effects of the study procedures (see Section 12.5)

Serious Adverse Event (SAE)

An adverse event which meets the definition of “serious” (see Section 12.2)

Related Serious Adverse Event (Related SAE)

A SAE which is assessed to be “probably”, “possibly” or “almost certainly” related to the trial procedures (see Section 12.4)

Related Unexpected Serious Adverse Event (RUSAE)

A Related SAE which is not expected, i.e. not consistent with the known effects of the trial procedures (see Section 12.5)

12.2 Assessment of Seriousness

The assessment of seriousness of safety events should be performed by an appropriately delegated, medically qualified member of the site research team.

A safety event (whether or not assessed as related to the study) is assessed as serious if:

- Results in death;
- Is life-threatening (i.e. the investigator considers the event places the subject at immediate risk of death from the experience as it occurred (this does not include an adverse experience that, had it occurred in a more severe form, might have cause death);
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation);
- Results in persistent or significant disability or incapacity (substantial disruption of one’s ability to conduct normal life functions);
- Consists of a congenital anomaly or birth defect (in offspring of study participants, or their partners, regardless of time of diagnosis), or
- Is otherwise considered medically significant by the investigator.

12.3 Severity of Adverse Events

All adverse events should be assessed for severity. The assignment of the severity/grading should be made by the investigator responsible for the care of the participant using the definitions in the table below:

The following table will be utilised to classify severity.

Severity	Description
Mild	Does not interfere with routine activities.
Moderate	Interferes with routine activities.
Severe	Impossible to perform routine activities.
Life-Threatening	
Death	

N.B. A distinction is drawn between serious and severe AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in Section 12.2. Hence, a severe safety event need not necessarily be a “serious” safety event.

12.4 Assessment of Causality

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below:

Definitions of Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship. N.B. An alternative cause for the AE should be given
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after the study procedures). There is another reasonable explanation for the event (e.g. the participant’s clinical condition, other concomitant treatment).
Possibly	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after the study procedures). However, the influence of other factors may have contributed to the event (e.g. the participant’s clinical condition, concomitant treatments).

Probably	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Almost certainly	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Events that are assessed as being possibly, probably or almost certainly related will be reported as having a reasonable possibility of being related, and events assessed as unrelated or unlikely will be reported as having no reasonable possibility of being related.

If any doubt about the causality exists, the local investigator should inform the LCTC who will notify the Chief Investigator, Professor Carlo Palmieri. In the case of discrepant views on causality between the treating investigator and others, the opinion of the treating investigator will never be downgraded, and the REC will be informed of both points of view.

12.5 Assessment of Expectedness

The CI or Clinical Coordinator (CC) for the PRIMROSE CSF study is responsible for determining whether a safety event is expected or unexpected. The CI/CC cannot assess their own patients. There is no requirement for a reporting investigator to make an assessment of expectedness.

An event will be considered unexpected if it is not listed within the current Expected Events list for the study at the time of the event's onset. The expected events list can be found in the table below. The nature, severity, or frequency of the event should be considered – if this is not consistent with that described for the type of event in the protocol the event should be assessed as unexpected. The information to be used for expectedness assessment for the study is below[22].

12.5.1 Reference Safety Information used to Assess Expectedness

This study involves collecting CSF via lumbar puncture (with atraumatic needles) or from aspiration of CSF from in situ Ommaya reservoirs form patients with CNS involvement secondary to breast cancer. For the majority of patients this will be an extra procedure, although in a small number of cases it will be performed at disease progression or following continued patient response (i.e. if disease is not progressing). Potential risks and side effects of the procedure include infection (rare), nerve root irritation (in a minority of patients), headaches, nausea/vomiting and back pain.

To minimise risk lumbar punctures will be performed using appropriate local anaesthesia by clinicians experienced in the procedure. Furthermore, atraumatic needles will be used which are associated with significantly less side effects than conventional needles for lumbar punctures. Where patients are undergoing a neurosurgical procedure, the lumbar puncture will be carried out once patient has been anaesthetised so reducing any side effects. Patients will be followed up to understand the side effects if any that occur post procedure[22].

The information to be used for expectedness assessment for PRIMROSE CSF Study is below:

Event	**Lumbar Puncture or Ommaya Reservoir aspiration**	**<Incidence/prevalence/frequency of event>***
Back Pain at the time of injection (Bleeding, swelling and bruising may occur)	**Lumbar Puncture**	Common
Post-procedure headache (can be associated with nausea and vomiting)	**Lumbar Puncture**	Common, possibly begins immediately or a few days after procedure and usually lasts less than 1 week
Persisting back pain	**Lumbar Puncture**	Very rare
Nerve root irritation (tingling or pain down the back of your legs)	**Lumbar Puncture**	Rare
Brainstem herniation	**Lumbar Puncture**	Very very rare
Post-procedure infections[a]	**Ommaya Reservoir aspiration**	Very very rare
*These side-effects are listed on the following sites: https://www.mayoclinic.org/tests-procedures/lumbar-puncture/about/pac-20394631 https://www.nhs.uk/conditions/lumbar-puncture/ [a]Please see section 7; 7.2 Exclusion Criteria. Where the investigator deems it unsafe to perform CSF extraction due to high risk of infection, the procedure will not be conducted.		

12.6 Time Period for Recording Adverse Events

All adverse Events (AEs) should be reported from the time of first lumbar puncture until the follow up visit (28 days +/- 7 days post-procedure). For patients undergoing a second lumbar puncture, AEs should also be reported from the time of the second lumbar puncture until the follow-up visit (28 days +/- 7 days post-procedure). Depending on the nature of the event the reporting procedures

below should be followed. Any questions concerning AE reporting should be directed to the LCTC in the first instance.

12.7 Notes on Safety Event Recording

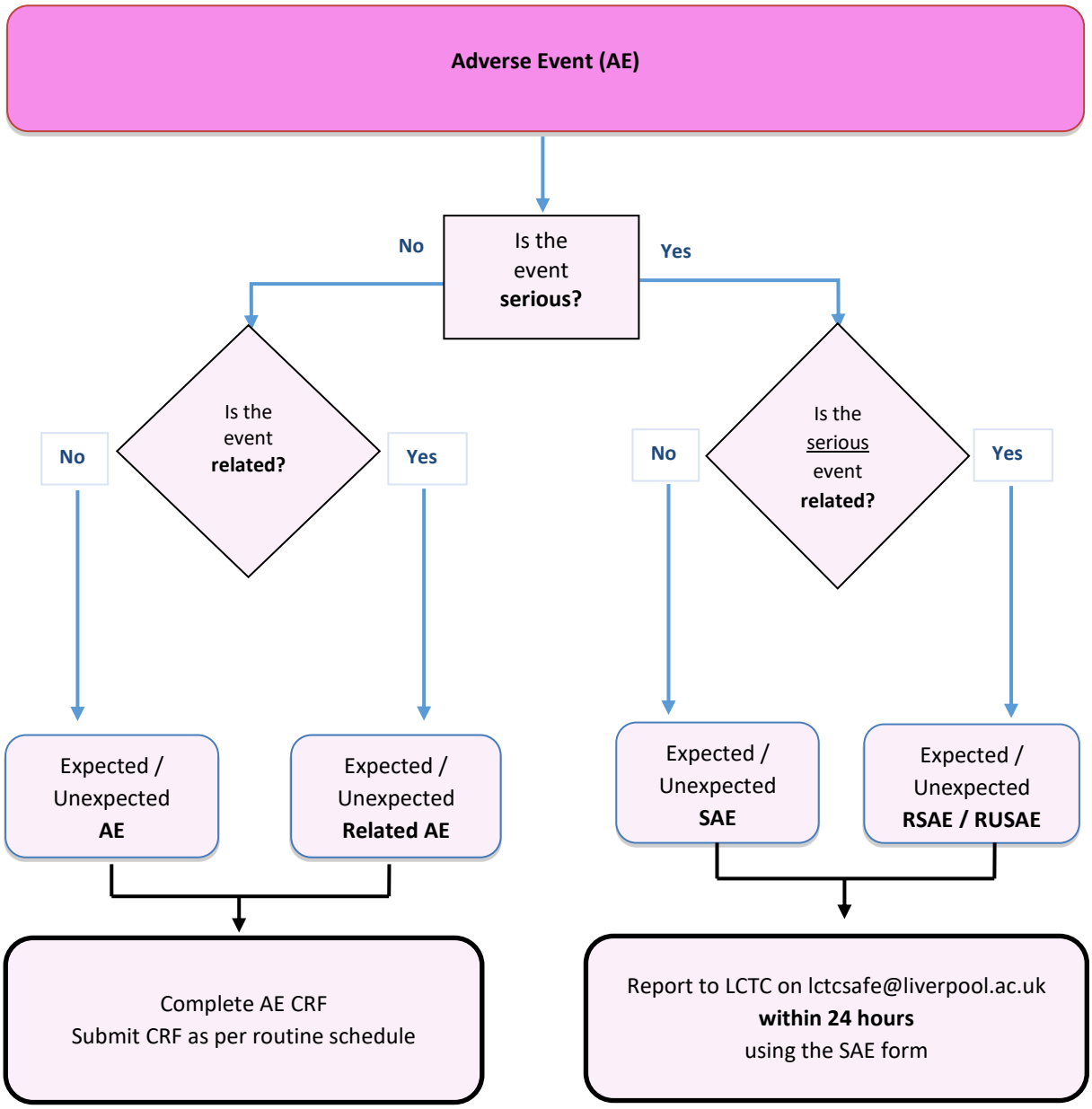
It is anticipated that as lumbar puncture (with atraumatic needles) or aspiration from Ommaya Reservoir procedures carry a moderate to low risk, they will only be recommended in patients with CNS involvement secondary to breast cancer where risks-benefit ratio is favourable. For those patients, who are undergoing the procedure any safety event will be recorded if the procedure was conducted for the purposes of the study. The relevant Safety Event form will be provided for completion and the event should also be recorded in the patient notes.

IMPORTANT: Any safety events occurring after the end of the “active monitoring” period defined in Section 12.6 which meet the definition of serious should be recorded as per the guidance here if it is related to the PRIMROSE CSF Study.

12.8 Reporting Procedures

All safety events which are recorded for the study should be reported following the procedures detailed below. The occurrence of a safety event may come to the attention of research staff during routine study visits, from the participant’s notes, directly from the participant or by other means. Note that reporting procedures vary dependent on the nature of the incident (i.e. “serious” events are to be reported to LCTC in an expedited manner). Any questions concerning adverse event reporting should be directed to the LCTC in the first instance. A flowchart is given below to aid in determining reporting procedures for different types of adverse events.

Flowchart for Site Reporting Requirements of Adverse Events



12.8.1 Reporting Safety Events to the LCTC

All safety events (whether or not assessed as serious / related / expected) should be recorded on an Adverse Event Form; multiple events can be recorded on one form.

Safety events which are assessed as "serious" must **also** be recorded in more detail on Serious Safety Event Forms; a single form is used for each individual event (i.e. a single diagnosis), though multiple symptoms can be recorded. Each SAE should have a corresponding record on the participant's AE form. Where additional information is received by site after initial submission to LCTC, this should be provided on a follow-up form within 5 days. Serious Safety Event Forms collect data regarding the nature of event, date of onset, severity, corrective therapies given, outcome and causality; all serious events reported to LCTC will be reviewed by the Chief Investigator or Clinical Coordinator and assessed for causality and expectedness.

12.8.2 Follow-up After Adverse Events

All reportable adverse events should be followed until satisfactory resolution or until the investigator responsible for the care of the participant deems the event to be chronic or the patient to be stable.

When reporting "serious" safety events the investigator responsible for the care of the participant should apply the following criteria to provide information relating to event outcomes:

- resolved
- resolved with sequelae (specifying with additional narrative)
- not resolved/ongoing
- ongoing at final follow-up
- fatal or unknown.

12.9 Reporting of Pregnancy

Pregnant women will not be excluded from this study. The study has no interventions which will affect any pregnancy. A pregnancy report form will be filled out if a participant is pregnant or becomes pregnant for the purposes of recording the event. Pregnancies will not be routinely followed up. Additional information can be provided at the discretion of the local PI.

12.10 Notification of Deaths

If the research team become aware of the death of a participant (whether related to the study or not) this should be notified to the LCTC preferably using email and recorded on eCRF within 24 hours of becoming aware.

12.11 Investigator Reporting Responsibilities

The PI is responsible for ensuring that all safety events requiring recording on this study which the local research team becomes aware of are reported to LCTC. It is the responsibility of the PI or Coinvestigator as recorded on the Delegation Log (medically qualified person) to assess the seriousness and causality of events. When documenting adverse events, the correct medical terminology **must** be used in accordance with CTCAE v5.0.

All safety events must be recorded on an AE form and transferred to LCTC **within seven days of the site team becoming aware of the event**.

Safety events which meet the definition of "serious" must be reported in more detail to the LCTC on an SAE form or by remote data entry and reported **immediately and in no circumstances later than 24 hours from becoming aware** where they will be appropriately processed.

The SAE form should be completed by an appropriately delegated member of the research team; the assessments of seriousness and causality must be performed by an appropriately medically qualified investigator. Minimum reporting information must be provided in initial reports for all studies.

N.B. In the absence of a delegated medically qualified investigator the form should be completed and signed by an alternative member of the research site trial team and submitted to the LCTC. As soon as possible thereafter the responsible investigator should check the SAE form, make amendments as appropriate, sign and re-send to the LCTC. The initial report shall be followed by detailed follow-up reports as appropriate.

Safety events should be reported to the site R&D team in accordance with local policy.

12.11.1 Reporting an Initial or Follow-up SAE

The investigator should ensure the actions below are completed for all reportable SAEs:

1) Research sites should telephone the appropriate trial co-ordinator / data manager on telephone number **0151 794 2405** to advise that an SAE report has been submitted as soon as possible.
2) **The SAE form should be transferred securely to PRIMROSE inbox (lctcsafe@liverpool.ac.uk) (within 24 hours) to the LCTC.**
3) The responsible investigator must notify their R&D department of the event (as per standard local governance procedures).
4) The patient must be identified by trial number, age or month and year of birth and initials **only**. The patient's name **should not** be used on any correspondence.
5) SAEs must be subsequently followed up in line with the processes below:
    - Follow up must continue until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised. N.B. Follow-up may continue after completion of protocol treatment if necessary.
    - Follow-up information is noted on a new SAE form to be transferred securely to the LCTC as soon as more information becomes available
    - Tick the appropriate box on the new SAE form to identify the type of report; this is dependent on resolution status of the SAE e.g. follow-up / final.
6) Extra, annotated information and/or copies of anonymised test results may be provided separately.

In the event of a problem with sending SAEs to the PRIMROSE email account, the site staff should contact the LCTC to arrange for another secure method of transfer.

Patient safety incidents that take place in the course of research should be reported to the National Reporting and Learning System (NRLS) by each participating NHS Trust in accordance with local reporting procedures.

12.12 LCTC Responsibilities

The trial Sponsor, The University of Liverpool, have delegated to LCTC the duty of onward reporting of safety events to REC. SOPs will be followed to ensure appropriate reporting as detailed below.

All "serious" safety events will be forwarded to the Chief Investigator or Clinical Coordinator by LCTC within 24 hours of receiving the minimum information from site. The CI or Clinical Coordinator will

review information provided by site and for all events assessed as “related” will provide an assessment of “expectedness”.

Safety events which are assessed as “serious”, “related” and “unexpected” (Section 12.5) will be onward reported by LCTC to the ethics committee **within 15 days** of the LCTC first becoming aware of the event.

Additionally, RUSAEs will be reported to the trial Sponsor(s) and Principal Investigators of participating sites.

A list of all safety events recorded for the trial will also be reported annually by LCTC to the ethics committee and Independent Data Safety & Monitoring Committee.

Any concerns raised by the TSC or inconsistencies regarding safety reporting noted at a given site may prompt additional training at sites, with the potential for the LCTC to carry out site visits if there is suspicion of unreported AEs / ARs and SARs / SAEs in patient case notes. Additional training will also be provided if there are unacceptable delays in safety reporting timelines.

12.13 Safety Reports

Safety reports will be generated during the course of the trial which allows for monitoring of safety event including reporting rates and safety events by site. The LCTC will send Annual Progress Reports (APRs) containing a list of all SAEs and SARs to the TSC and main REC. If any safety reports identify issues that have implications for the safety of trial participants, the PIs at all institutions participating in the trial will be notified.

12.14 Urgent Safety Measures (USMs)

An urgent safety measure (USM) is a procedure to protect clinical study participants from any immediate hazard to their health and safety but has not previously been defined by the protocol. It can be put in place prior to authorisation by the REC.

LCTC will notify the REC immediately and, in any event, within 3 days that such a measure has been taken and the reasons why it has been taken. The initial notification to the REC will be by telephone (ideally within 24 hours) and a notice in writing will be sent within 3 days, setting out the reasons for the USM and the plan for further action. After discussion with the REC, further action will be agreed,

which may include submission of a substantial amendment, a temporary halt, or permanent termination of the trial.

Following notification, if a substantial amendment is required this must be submitted as soon as possible to the REC. If the study is temporarily halted it may not recommence until authorised to do so by the REC. If the study is permanently terminated before the date specified for its conclusion (in the original applications to REC), the Sponsor should notify the REC within 15 days of the date of termination by submitting the formal End of Trial Notification.

12.15 Contact details and Out-of-hours Medical Cover

This study is moderate risk as it involves procedures above routine care. Emergency and out-of-hours medical care will be in line with usual NHS arrangements and local standard practice; no special provision is required for participants. During office hours, the CI or delegate are able to provide medical advice in relation to participation using the contact details listed at the beginning of this document.

13 Statistical Considerations

13.1 Introduction

Details are provided on the statistical principles to be adhered to for the duration of the study including details on study design and an overview of analytical techniques.

13.2 Sample Size Estimation and Recruitment

As there are no formal hypothesis being tested, no power calculations are presented. Instead, sample size calculations are presented basis of what can reasonable recruitment estimates over the study period. The study will recruit for 36 months. With 7 sites recruiting at an expected average rate of 0.3 then a target of 70 patients is set. This is sufficient to estimate a binary endpoint with a conservative standard error of 6%.

13.3 Outcome Measures

Aside from recruitment, feasibility measures will also be taken on the rate of patients recruited to those eligible, the ability of patients and clinicians to adhere to study protocol (measure via the number of major/minor deviations) and the ability of the study to collect data (measured as the number of complete CRFs returned and metrics on the QC and data query issues).

13.4 Feasibility Success Criteria

The success of the study to demonstrate feasibility is primarily based on the ability of the study to recruit patients. If the observed recruitment rate is ≥ 80% of this target (≥ 32 patients) it will be deemed that feasibility will have been satisfied. If between 50 – 80% (20 – 31 patients) are recruited, further studies will only be deemed feasible if actions can be taken to increase recruitment. If < 50% (19 patients or fewer) are recruited the study will have failed the feasibility criteria.

13.5 Methods of Analysis

All analyses will be exploratory in nature with descriptive statistics and graphical representations used to communicate all data collected. Continuous data will be expressed as median (IQR) (Range) and categorical data will be expressed as frequencies of counts with associated percentages. Estimated rates statistics will be represented alongside 95% confidence intervals.

14 Data Management and Study Monitoring

For the PRIMROSE CSF Study, the responsibilities for Data Management and monitoring are delegated to LCTC. Separate Data Management and Trial Monitoring Plans have been developed which provide detail regarding the internal processes that will be conducted at the LCTC throughout the trial. Justification for the level of monitoring is provided within those documents and the trial-specific risk assessment. All data will be managed as per local LCTC processes and in line with all relevant regulatory, ethical and legal obligations.

14.1 Source documents

The eCRF will be considered the source document for data where no prior record exists and which is recorded directly in the bespoke CRF. A source document list will be produced for each site to be kept in the ISF and provide detail of what constitutes specific source data.

Date(s) of informed consent processes should be added to the patient's medical record chronologically.

14.2 Data collection method

Data are to be entered into the study eCRF database by members of the research team at site. Training will be provided prior to any data entry, access and data protection. Relevant manuals on data entry will be provided.

14.3 Monitoring

Monitoring is conducted to ensure protection of patients participating in the trial and all aspects of the trial (procedures, laboratory, trial intervention administration and data collection) are of high quality and conducted in accordance with sponsor and regulatory requirements.

A detailed Trial Monitoring Plan will be developed and agreed by the TMG and CI to describe who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted. This will be dependent on the documented risk assessment of the trial which determines the level and type of monitoring required for specific hazards. All processes may be subject to monitoring, e.g. enrolment, consent, adherence to trial interventions, accuracy and timeliness of data collection etc.

Trial Oversight Committees related to the monitoring of the trial are detailed in Roles and Responsibilities see Section 4.

14.4 Central Monitoring

There are a number of monitoring features in place at the LCTC to ensure reliability and validity of the data, to be detailed in the study monitoring plan. Data will be entered into a validated database and during data processing there will be checks for missing or unusual values (range checks) and for consistency within participants over time. Data queries will be raised for any suspect data. Data query forms will be produced at the LCTC from the study database and sent either electronically or through the post to a named individual (as listed on the site delegation log). Sites will respond the queries (either on the form, or directly on eCRF) providing an explanation/resolution to the discrepancies and make the appropriate corrections on the database. If the query form was used to respond, this will be returned to the LCTC, where it will be filed.

Site monitoring visits may be 'triggered' in response to concerns regarding study conduct, participant recruitment, outlier data or other factors as appropriate.

14.5 Clinical Site Monitoring

On site monitoring visits will not be carried out routinely, these will only be performed as part of this study if triggered during central monitoring, or if there are other concerns raised by the CI/TMG (please see monitoring plan for details of triggered monitoring).

In agreeing to participate in this study, a PI grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation. The purposes of site monitoring visits include, but are not limited to:

- assessing compliance with the study protocol;
- discussing any emerging problems that may have been identified prior to the visit;
- checking eCRF and query completion practices.

14.6 Risk Assessment

A detailed Risk Assessment will be developed and agreed by the Study Coordinator, Chief Investigator, Sponsor, Study Statistician and LCTC Operational Director. In conducting this risk assessment, the contributors will consider potential patient, organisational and study hazards, the likelihood of their occurrence and resulting impact should they occur.

The outcome of the risk assessment is categorised into three groups:

- Type A = Comparable to the risk of standard medical care.
- Type B = Somewhat higher than the risk of standard medical care.
- Type C = Markedly higher than the risk of standard medical care.

The PRIMROSE CSF Study is categorised as a Type B study.

14.7 Confidentiality

This trial will collect personal data (e.g. participant names), including special category personal data (i.e. participant medical information) and this will be handled in accordance with all applicable data protection legislation. Data (including special category) will only be collected, used and stored if necessary, for the trial (e.g. evidencing provision of consent, for data management and central monitoring, statistical analysis, regulatory reporting, etc.). At all times, this data will be handled confidentially and securely.

CRFs will be labelled with a unique trial ID number. Verification that appropriate informed consent is obtained will be enabled by the provision of copies of participant's signed informed consent forms being supplied to the LCTC by recruiting sites. This transfer of identifiable data is disclosed in the PISC.
N.B. Consent forms must be transferred separately to any other trial documentation to ensure the pseudonymisation of special category data is maintained.

Site-specific study-related information will be stored securely and confidentially at sites and all local relevant data protection policies will be adhered to.

The LCTC as part of The University of Liverpool will preserve the confidentiality of participants taking part in the study. The University of Liverpool, LCTC is registered as a Data Controller with the Information Commissioners Office.

Breaches of data protection principles or regulations identified by LCTC will be notified promptly to the study Sponsor and The University of Liverpool's Data Protection Officer and appropriate processes followed.

14.8 Quality Assurance and Control

To assure protocol compliance, ethical standards, regulatory compliance and data quality, as a minimum, the following will occur as part of quality assurance:

- The PI and other key staff from each centre will attend initiation training, which will incorporate elements of study-specific training necessary to fulfil the requirements of the protocol.
- The TMG will determine the minimum key staff required to be recorded on the delegation log in order for the centre to be eligible to be initiated.
- The TC at the LCTC will verify appropriate approvals are in place prior to initiation of a centre and the relevant personnel have attended the study specific training.
- A greenlight checklist will verify all approvals are in place prior to study initiation at LCTC and the individual centre.
- The study will be conducted in accordance with procedures identified in the protocol.
- Independent members of the TSC will provide independent oversight of this study (see Section 4)
- The TMG will monitor screening between centres and compliance with the protocol.
- Data quality checks and monitoring procedures will be undertaken in line with the study Data Management Plan.

14.9 Records Retention

The retention period for the PRIMROSE data and tissue and information is 10 years from the official End of study date.

While the current study plans to run for three years, data collection will be capped at 87 patients.

The PI at each investigational site must make arrangements to store the essential study documents (as defined by GCP guidelines) including the Investigator Site File for the full length of the study's retention period and will arrange for destruction at the end of this period as instructed by the LCTC.

The PI is also responsible for archiving all relevant source documents so that the study data can be compared against source data after completion of the study (e.g. in case of inspection from authorities). They must ensure the continued storage of the documents, even if they, for example, leave the clinic/practice or retire before the end of required storage period. Delegation of responsibility for this must be documented in writing.

All other persons and organisations involved in the study will be responsible for storing and archiving the parts of the TMF relevant to their delegated duties.

The LCTC undertakes to archive as per their contractual requirements; documents will be archived in compliance with the principles of GCP. All eCRFs and study data will be archived onto an appropriate media for long term accessible storage. Hard copies of data will be boxed and transferred to secure premises where unique reference numbers are applied to enable confidentiality, tracking and retrieval.

15 Regulatory and Ethical Considerations

15.1 Statement of Compliance

This study is designed to comply with the guidelines of Good Clinical Practice (GCP) and will be managed in compliance with the protocol and LCTC Standard Operating Procedures. In addition, sample collection and storage will be in compliance with the Human Tissue Act 2004. Data collection and retention will be in compliance with the General Data Protection Regulation (2016/679).

15.2 Ethical Considerations

The trial will abide by the principles of the World Medical Association Declaration of Helsinki and has been designed to be as pragmatic as possible. The protocol has undergone ethical review by an independent Research Ethics Committee and has received a favourable opinion.

15.3 Approvals

The protocol, PISC and any proposed public-facing material will be submitted to an appropriate Research Ethics Committee (REC), Health Research Authority (HRA) and host institution(s) for written approval.

Any substantial amendments to the original approved documents will be submitted and, where necessary, approved by the above parties before use.

15.4 Protocol Deviation and Serious Breaches

Deviations from, breaches or violations of, or non-compliance to either the protocol, the conditions or principles of GCP, are handled based on their nature and severity.

15.4.1 Non-Serious breaches

Protocol deviations and other non-serious breaches of GCP etc. will be managed according to local site and LCTC procedures as appropriate. They will be reported to study oversight committees.

15.4.2 15.4.2 Serious breaches

A breach of the protocol or GCP is 'serious' if it meets the definition of being "likely to affect to a significant degree the safety or physical or mental integrity of the study participants, or the scientific value of the study". This assessment can only be determined by the Sponsor.

If any persons involved in the conduct of the study become aware of a potential serious breach, they must immediately report this to the LCTC who will in turn notify the Sponsor. The Sponsor will assess the breach and determine if it meets the criteria of a 'serious' breach.

The Sponsor may seek advice from medical expert members of the TMG and/or of the independent oversight committees (TSC) in determining whether or not the breach is likely to affect to a significant degree the safety, physical or mental integrity of participants.

The Sponsor may seek advice from the Study Statistician in determining whether or not the breach is likely to significantly affect the scientific value of the study. However, the Sponsor retains responsibility for the assessment of whether or not a breach meets the definition of 'serious'.

Breaches confirmed as 'serious' will be notified to the TMG and TSC at their next meeting.

Any requests for additional information from the Sponsor, TMG or TSC, will be promptly actioned by the relevant member(s) of the research team and open communication will be maintained to ensure appropriate corrective actions are taken and documented.

Incidents of protocol non-compliance will be recorded as protocol deviations, the incidence of which are monitored and reported to trial oversight committees.

16 Indemnity

The University of Liverpool holds insurance against claims from participants for harm caused by their participation in this clinical study. However, the treating hospital continues to have a duty of care to the participant and the Sponsor does not accept liability for any breach in the hospital's duty of care, or any negligence of the part of hospital employees. In these cases, clinical negligence indemnification will rest with the participating NHS Trust or Trusts under standard NHS arrangements.

17 Publication and Dissemination

17.1 Publication Policy

The results from different participating sites will be analysed together and published in the name of the study as soon as possible, on behalf of all collaborators, maintaining participant confidentiality at all times. Individual clinicians must undertake not to submit any part of their individual data for publication without the prior consent of the Trial Management Group.

The TMG will form the basis of the writing committee and will advise on the nature of publications. The manuscript will be prepared by a writing group, appointed from amongst the TMG. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals will be respected.

The members of the TSC will be listed with their affiliations in the Acknowledgements/Appendix of the main publication. Any publications arising from this research will be reviewed appropriately prior to publication.

17.2 Authorship

Contributors to all four of (i) the design, conduct, data analysis and interpretation, (ii) writing, (iii) manuscript approval and (iv) accountability for the integrity of the work will, depending on their contribution and journal requirements, be included by name at the manuscript head or listed at the end in a by-line as members of the PRIMROSE Consortium which will also be named at the manuscript head.

Citable collaborators will have been required to make considerable contribution to the study. These will include Unit leads and any other team members (including consultant surgeons, oncologists, radiologists, neurosurgeons or pathologists, clinical nurse specialists, trainees, research nurses or students) who have recruited at least two to four patients to the study. Recruitment in this context includes the submission of at least ONE completed data set. Judgement may be used to determine participation according to local centre practice. Unit leads will be asked to provide details of their local team and whether individuals fulfil the criteria for citable or acknowledged collaborator status.

17.3 Dissemination to Key Stakeholders

On completion of the research, a Final Study Report will be prepared and submitted to REC. This will also be submitted to North West Cancer Research, Daiichi Sankyo Europe GmBH and Make

Seconds Count in accordance with the stipulated guidance in the grant letter. The results will be published regardless of the magnitude or direction of effect.

17.4 Data Sharing

At the end of this study, after the primary results have been published, the anonymised individual participant data (IPD) and associated documentation (e.g. protocol, statistical analysis plan) will be prepared in order to be shared with external researchers. All requests for access to the IPD will be reviewed by an internal committee at the LCTC and discussed with the Chief Investigator in accordance with the LCTC policy on data sharing. As this is a register intended to function as a databank, the appropriate ethical processes will be followed should external researchers request use of the data. While there is no evidence that the genomic analysis would improve care, the results of analysis can be shared with clinical team at the end of all analysis.

18 Chronology of Protocol Amendments

18.1 Version 2.0 (20/10/2020)

Summary of Amendments from Protocol V1.0 to Protocol V2.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
Page 1 and Page 6	**Sponsor Address**	Changed from Waterhouse address to Head of Clinical Operations Clinical Directorate 4th Floor Thompson Yates Building Faculty of Health and Life Sciences University of Liverpool Liverpool L69 3GB
Page 3	**Sponsor Contact**	Changed from Alex Astor to Neil French (AA now left). Changed on 20/10/2020 on Sponsor's request pre authorisation.

18.2 Version 1.0 (18/09/2020)

Original Approved version

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