PRIMROSE TISSUE STUDY: A COLLECTION AND ANALYSIS OF TISSUE AND CSF SAMPLES FROM PATIENTS WITH CNS DISEASE SECONDARY TO BREAST CANCER.

PRIMROSE Tissue Protocol
V2.0, 20/10/2020

Study Sponsor:
Head of Clinical Operations
Clinical Directorate
4th Floor Thompson Yates Building
Faculty of Health and Life Sciences
University of Liverpool
Liverpool L69 3GB

ISRCTN:
Sponsor Ref: UoL001578
Funder Ref: N/A
Research Ethics Ref: TBC

Protocol Approval

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

Authorised by Chief Investigator:

See accompanying email confirmation
Signature: _____

23/10/2020
Date: _____

Carlo Palmieri

Chief Investigator

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

Authorised on behalf of Sponsor:

Signature: See accompanying email confirmation _____ **Date:** 23/10/2020 _____

Neil French

Head of Clinical Operations

University of Liverpool

Sponsor

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

Authorised on behalf of the Lead Statistician:

See accompanying email confirmation 21/10/2020

Signature: _____ **Date:** _____

Richard Jackson

Trial Statistician

CRUK Liverpool Clinical Trials Unit

General Information

This section describes the PRIMROSE Tissue Study. The study does not involve experimental treatment or placebo treatment.

This protocol defines the participant characteristics required for study entry. This is a single-arm prospective observational study. 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, PRIMROSE Tissue Study (current protocol) and PRIMROSE CSF Study. 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 Tissue study (i.e. this protocol) they will not be entered in PRIMROSE CSF 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 LCTC.

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

Sponsor:	**Trial Management, Monitoring and Analysis:**	**Statistics:**
Head of Clinical Operations Clinical Directorate 4th Floor Thompson Yates Building Faculty of Health and Life Sciences University of Liverpool Liverpool L69 3GB Email: sponsor@liverpool.ac.uk Tel: 0151 794 8739	Liverpool Clinical Trials Centre University of Liverpool 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL E-mail: primrose@liverpool.ac.uk Tel: 0151 794 2405	Dr Richard Jackson Liverpool Clinical Trials Centre University of Liverpool 1st Floor Block C Waterhouse Building 3 Brownlow Street Liverpool L69 3GL E-mail: r.j.jackson@liverpool.ac.uk Tel: 0151 794 8834
Translational Laboratory:		
Professor Bill Greenhalf GCP Laboratory Facility University of Liverpool William Henry Duncan Building 6 West Derby Street Liverpool L7 8TX E-mail: greenhaf@liverpool.ac.uk Tel: 0151 795 8030		

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):**
Ms. Charlotte Rawcliffe Liverpool Clinical Trials Centre, 1st Floor Block C, Waterhouse Building, 3 Brownlow Street, Liverpool, L69 3GL E-mail: charlotte.rawcliffe@liverpool.ac.uk Tel: 0151 794 8167	Professor Carlo Palmieri Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, Level 6, Duncan Building, Daulby Street, Liverpool, L69 3GA E-mail: c.palmieri@liv.ac.uk Tel: 0151 794 2405	Professor Michael Jenkinson The Walton Centre NHS Foundation Trust, Lower Lane, Fazakerley, Liverpool, L9 7LJ E-mail: michael.jenkinson@liv.ac.uk Tel: 0151 529 5683
Translational Laboratory (for sample storage)	**Trial Coordinator**	**Senior Trial Coordinator**
Dr Victoria Shaw GCP Laboratories, 1st Floor, William Henry Duncan Building, University of Liverpool, 6 West Derby Street, Liverpool, L7 8TX E-mail: torivick@liverpool.ac.uk Tel: 0151 795 8044	Andrea Newhouse Liverpool Clinical Trials Centre, 1st Floor Block C, Waterhouse Building, 3 Brownlow Street, Liverpool, L69 3GL E-mail: newhouse@liverpool.ac.uk Tel: 0151 794 2405	Helen Scott Liverpool Clinical Trials Centre, 1st Floor Block C, Waterhouse Building, 3 Brownlow Street, Liverpool, L69 3GL E-mail: hscott@liverpool.ac.uk Tel: 01515 794 8209
Breast Cancer Trainee Network Lead		
Dr Vinton Cheng Leeds Teaching Hospitals NHS Trust, St James' University Hospital, Beckett Street, Leeds, LS9 7TF E-mail:v.w.cheng@leeds.ac.uk Tel: 0755 730 726		

Contact Details: Coinvestigators

Co-investigator	**Co-investigator**	**Co-investigator**
Professor Michael Jenkinson The Walton Centre NHS Foundation Trust, Lower Lane, Fazakerley, Liverpool, L9 7LJ E-mail: michael.jenkinson@liv.ac.uk Tel: 0151 529 5683	Dr Iain MacPherson Wolfson Wohl Cancer Research Centre, Garscube Estate, Switchback Road, Glasgow, G61 1QU E-mail: iain.macpherson@glasgow.ac.uk Tel: 0141 330 4166	Professor Janet Brown Sheffield Teaching Hospitals NHS Foundation Trust, The University of Sheffield, Department of Oncology, Weston Park Hospital, Whitham Road, Sheffield, S10 S2J E-mail: j.e.brown@sheffield.ac.uk Tel: 0114 226 5208
Co-Investigator	**Co-Investigator**	**Co-Investigator**
Professor Jacqui Shaw Leicester Cancer Research Centre, Genetics and Genome Biology, Room 303, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, LE2 7LX E-mail: js39@leicester.ac.uk Tel: 0116 252 3148	Dr Ellen Copson Somers Cancer Sciences, Southampton General Hospital, Tremona Road, SO16 6YD E-mail: e.copson@soton.ac.uk Tel: 0238 120 5170	Dr Vinton Cheng Leeds Teaching Hospitals NHS Trust, St James' University Hospital, Beckett Street, Leeds, LS9 7TF E-mail:v.w.cheng@leeds.ac.uk Tel: 0755 730 726

Additional Contacts:

The contact details for the trial oversight committee members and participating centres are detailed in documents supplementary to the protocol and stored in the Trial Master File

Contents

1. Glossary

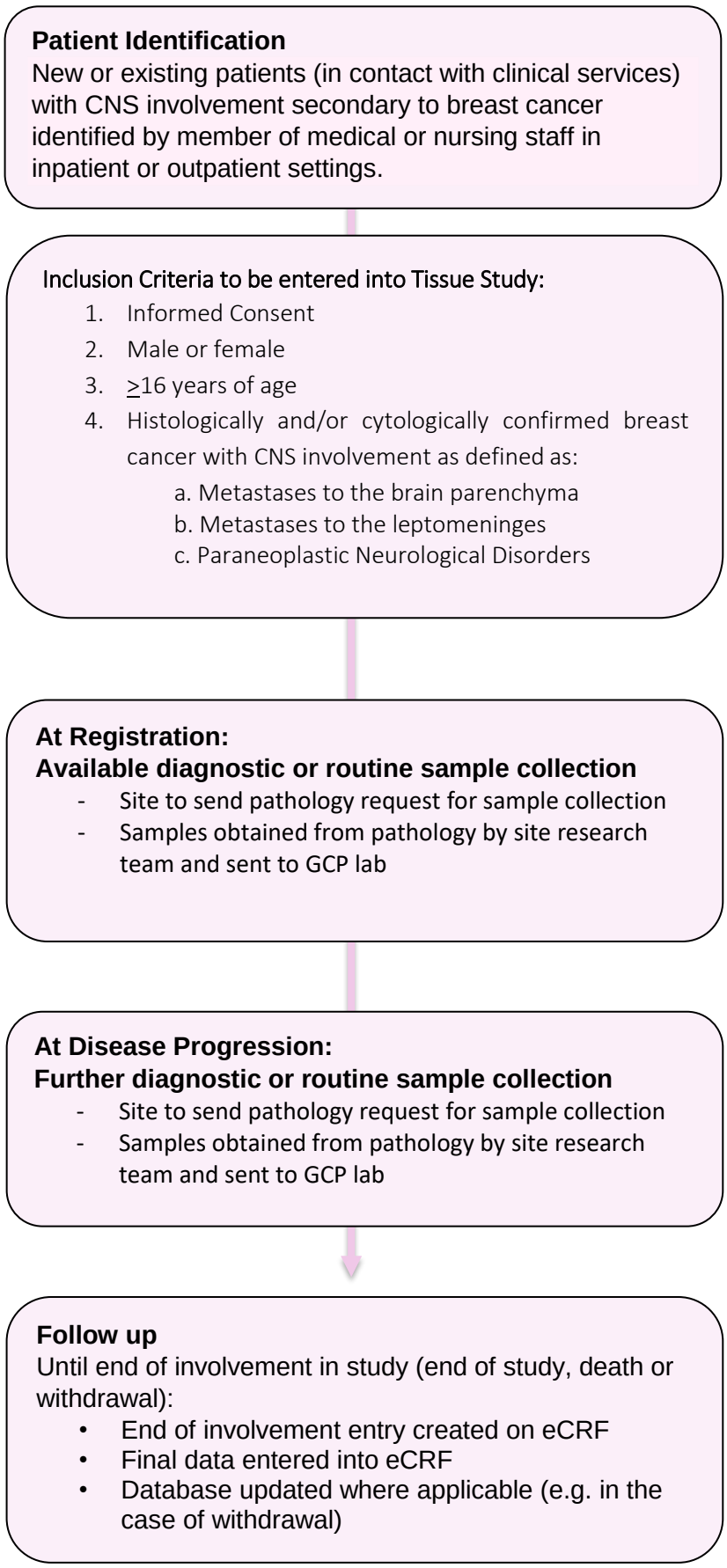
ABN	Association of British Neurologists
BCTRCG	Breast Cancer Trainees Research Collaborative Group
BNSU	British Neurological Surveillance Unit
CI	Chief Investigator
CM	Cerebral Metastases
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Computed Tomography
GCP	Good Clinical Practice
HER2	Human Epidermal Growth Factor Receptor 2
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
PND	Paraneoplastic Neurological Disorders
QA	Quality Assurance
QC	Quality Control
ECRF	Electronic Clinical Record Form
SRS	Stereotactic Radiosurgery
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

2. Protocol Overview

Full Title:	PRIMROSE Tissue Study: A collection and analysis of tissue and CSF from patients with CNS disease secondary to breast cancer.
Acronym:	PRIMROSE Tissue Study
Target Population:	Patients over 16 years old, male or female with CNS disease secondary to breast cancer in the UK.
Sample size:	300 patients (up to 600 paired [primary breast + CNS disease tissue] samples)
Inclusion Criteria:	1. Male or female. 2. ≥16 years of age 3. Histologically and/or cytologically confirmed breast cancer with Central Nervous System (CNS) involvement as defined as having one or more of the following: a. Metastases to the brain parenchyma b. Metastases to the leptomeninges c. Paraneoplastic Neurological Disorders 4. FFPE of biopsied or resected primary breast cancer, non-CNS metastasis and/ or brain metastasis is available or will be available. 5. Informed consent
Exclusion Criteria:	Unable to comply with study procedures or give informed consent
Study Centres and Distribution:	Open to all UK NHS centres involved in the care of patients with breast cancer.
Patient Study Duration:	The patients will be followed up until death, withdrawal of consent or study closure.
Study Duration	2 years
Aim of study	This study seeks to collect tissue (FFPE blocks of primary breast cancer, non-CNS metastasis - if available and/or brain metastasis – if available) and CSF samples (which are routinely collected as part of clinical care) to support analysis of the following objectives:

	i) To investigate molecular/genetic concordance between primary and metastatic cancer sites ii) Investigate potential molecular or genetic markers for diagnosis and/or therapy within CSF and other collected tissue.
Objectives:	• To investigate the concordance between the primary and brain metastasis for ER, PgR and HER2 • The prevalence and concordance of the expression of the androgen receptor within brain metastasis as compared to primary disease. • To investigate the genomic landscape of CNS disease secondary to breast cancer. Please note that the current funding provided allows for all relevant tissue collection, processing and storage at the GCP Laboratories facility. Further funding will be sought (before tissue collection is complete) to conduct analysis and meet the stated objectives in this protocol. Should funding not be secured, the collected tissue samples will be transferred to the LCTC (previously LCTU) Post-Trial Tissue Bank (REC reference: 18NW0621).

3. Schematic of 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 Unit.

Chief Investigator

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

Principal Investigators

In each participating centre a principal investigator and co-investigators 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.

Funder

This study is funded by: Daiichi Sankyo Europe GmBH. Please note that the current funding provided allows for all relevant tissue collection, processing and storage at the GCP Laboratories facility. Further funding will be sought (before tissue collection is complete) to conduct analysis and meet the stated objectives in this protocol. Should funding not be secured, the collected tissue samples will be transferred to the LCTC (previously LCTU) Post-Trial Tissue Bank (REC reference: 18NW0621).

4.1 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 Clinical 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 with brain metastases, independent statistician, patient representative and study coordinator. The role of the TSC is to provide overall supervision for 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).

4.2 Protocol Contributors

Name	Affiliations	Contribution to protocol
Professor Carlo Palmieri	Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine University of Liverpool Level 6 Duncan Building Daulby Street 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 L69 3GL	Reviewed statistics section
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.

5. Introduction

5.1 Background

Breast cancer is the second most common (after lung cancer) primary tumour to metastasise to the brain (also known as Breast Cancer Brain Metastases or CNS involvement secondary to Breast Cancer) accounting for 17% of brain metastases [1,2]. Improvements in the systemic treatment of extracranial metastatic disease have resulted in patients surviving longer with their metastatic disease, which appears to be contributing to the increase in the incidence of cerebral metastases[3].

Local treatment in the form of open surgical resection, stereotactic radiosurgery (SRS) and whole brain radiotherapy (WBRT) have all been applied in isolation or combination in the treatment of cerebral metastases from breast cancer with the aim of improving local control, extending life and preventing further neurological manifestations such as leptomeningeal metastases and Paraneoplastic Disorders. A number of randomised controlled trials have been performed, but treatment guidelines in North America, Europe and the UK reflect deep uncertainty about which strategies serve patient needs best [4]. There is also questionable relevance of many studies to BCBM patients as they are largely underrepresented in older randomised trials [5]. These trials are usually underpowered for stratification by receptor type.

This study aims to improve the current knowledge/literature base following on from recent publications by implementing a nationwide data collection of routine clinical care in Breast Cancer Brain Metastases (BCBM). The study will focus primarily on understanding more on cerebral metastases. However, secondary aims will also place emphasis on understanding the clinical presentation and manifestation of Leptomeningeal disorders and Paraneoplastic Disorders should they be diagnosed among BCBM patients.

Cerebral Metastases

Cerebral metastases is a growing problem among patients living with metastatic breast cancer, particularly for patients with triple negative and human epidermal growth factor receptor 2 (HER2)-positive disease [2], and is associated with significant morbidity and mortality. The median overall survival ranges from 3 months to 26 months [3]. The most recent breast cancer-specific prognostic model, the Modified Breast GPA, confirmed that tumour

receptor status, along with age, performance status and number of brain metastases, predicted overall survival [3].

Current NCCN guidelines recommend brain MRI screening for recurrent or stage IV breast cancer patient only if symptoms are present. Symptoms can present as headaches, vomiting, seizures or forms of neurological deficits [3]. Systemic treatment is the treatment of choice following central nervous system progression in patients who have had prior local therapy. Relative to other cancers, little is known about the tumour microenvironment within the brain, a setting unique to all other metastatic sites. The nature of the systemic therapy will depend on the tumour subtype, preceding systemic treatment and the patient's performance status [6]. These therapies may include chemotherapy, HER2-directed therapy and endocrine therapy. However, therapeutic options for intracranial disease remain limited; therefore, novel therapeutic approaches need to be developed.

Leptomeningeal Metastases (LM)

Whilst most CNS metastases develop in the parenchyma, a minority may arise in the leptomeninges. The exact incidence of LM is difficult to estimate, however they are thought to comprise between 10 to 20% of CNS metastases[7]. Treatment of LM is highly variable between patients, depending on the clinical context and the presence of any co-morbidities. There is also considerable inter-institutional variation with regard to treatment, reflecting controversies that exist in the relative merits of different treatment modalities. Neurosurgery, in the context of LM, has a role in relieving non-communicating hydrocephalus and the insertion of a ventricular "Ommaya" reservoir to provide access for administering intrathecal chemotherapy [8]. CNS-targeted radiotherapy is often given to treat sites of LM, often in combination with systemic anti-cancer drug therapy. Intrathecal chemotherapy usually takes the form of methotrexate, cytarabine or thiotepa [9,10]. Clearance of malignant cells from the CSF following chemotherapy has been shown to be a good prognostic factor. However, survival remains poor, reaching a median of 15 weeks [11].

Thus, breast cancer leptomeningeal disease is an area of significant unmet need. Its relatively uncommon occurrence hinders the development of a standard approach to its management and the lack of a well annotated collection of tissue and cerebrospinal fluid hampers the development of relevant and meaningful research contributing to the need for this study in collecting such data.

Paraneoplastic neurological disorders (PND)

Paraneoplastic neurological disorders are defined as remote effects of malignancy, not due to the presence of metastatic spread to the nervous system. PND are rare, occurring in less than 1% of women with breast cancer [12,13]. However, they are important because they frequently precede the diagnosis of the breast cancer primary and because they cause severe neurological disability. Current thinking is that they are caused by an autoimmune response to 'onconeural' antigens, aberrantly expressed antigens on tumours that are also present on CNS cells, although the precise immunopathogenic mechanisms are unknown [14]. It is likely that there is an important cellular immune response, evidenced by the presence of lymphocytic infiltration and activated cytotoxic T lymphocytes in the CSF of affected patients.

PND may affect any part of the nervous system either focally (e.g. cerebellar degeneration) or diffusely (e.g. encephalomyelitis). Both the central and peripheral nervous system may be affected, with antigenic targets being either intracellular (both nuclear and cytoplasmic) or extracellular (receptors and ion channels). As a general rule, PND associated with antibodies against intracellular targets cause predominantly CNS disorders, while those associated with antibodies against extracellular antigens cause predominantly neuromuscular disorders. PND affecting the CNS are commonly associated with specific anti-neuronal antibodies, which are present in both serum and CSF. The most common PND associated with breast cancer are cerebellar degeneration, encephalomyelitis with rigidity and opsoclonus-myoclonus syndrome. The most common anti-neuronal antibody in breast cancer is anti-Yo [12]. Most CNS syndromes respond poorly to immunomodulatory treatment although occasional improvement is seen when the underlying tumour is treated. In contrast, disorders affecting the neuromuscular junction e.g. Lambert-Eaton myasthenic syndrome (LEMS) do improve with treatments that remove the relevant antibodies directed against voltage-gated calcium channels [15,16]. The prognosis for the majority of PND is poor, even if the tumour is detected and treated, and patients are forced to remain in a severely disabled state.

Currently, there is a lack of active research into PND in general as well as in breast cancer specifically, as well as no centralised resource of relevant biological material for research.

Logistically, The PRIMROSE Tissue Study anticipates using the trainee collaborative model [17] to establish an observational database collecting presentation, diagnosis, management and outcome data from patients with newly diagnosed CNS involvement secondary to breast cancer in the UK.

5.2 Rationale

There is an urgent need to better understand the outcomes and disease patterns after local therapy in the BCBM patient group in the context of current modern oncological treatment. Furthermore, an understanding of how local therapy influences quality of life and neurocognitive functioning in breast cancer patients is needed, particularly as patients may undergo a number of different local therapies over their disease course. Finally, it is recognised that there is inequitable access to specific types of local therapy nationwide. Mapping out the geographic variation in availability of these local therapies will help guide future policy and local clinical commissioning to improve breast cancer patient outcomes.

Basic and translational research to understand the pathophysiology of breast cancer involving the CNS remains limited by a lack of access to annotated clinical material. Such research is needed if preventative and novel treatment strategies are to be developed. Moreover, there is currently a lack of basic information regarding the incidence and management of cerebral metastasis in the UK, how it may vary and its impact on patient outcomes. Furthermore, clinical studies have been hampered by a lack of a central resource to aid feasibility work; as well as, when open, to identify possible eligible patients. Finally, any future advances in systemic therapy may ultimately be limited, in certain breast cancer subtypes, by the development and progression of intracranial disease.

The PRIMROSE Tissue will facilitate the collection of relevant clinical data including the collection of a well annotated diagnostic and fresh tissue in order to foster the development of relevant and meaningful research. It will enable research in support of the overarching objectives. We seek to establish this tissue collection in order to improve our ability to achieve our aims, to the betterment of research both locally and across the UK.

5.3 Risks and Benefits

5.3.1 Potential Risks

The study collects tissue and CSF samples acquired during the patients' routine care. No additional procedures above those undertaken as part of routine care will be undertaken and therefore there is no additional risk for the patients by participation. For patients undergoing diagnostic or therapeutic lumbar puncture consent to allow a 10ml blood sample and an extra 10ml CSF sample to be taken in addition to the routine sample CSF taken as part of the

procedure. There are anticipated to be no additional risk to patients by the taking of this additional sample.

5.3.2 Potential Benefits

It is likely that the patients registered will not derive any direct benefit from this study. The establishment of this study will enable focused translational research into CNS disease secondary to breast cancer. It is hoped that this will lead to a better understanding of CNS disease including the identification of patients at particular risk so enabling the development of possible preventive strategies, as well as novel treatment of those who develop CNS disease.

5.4 Objectives

This study seeks to collect tissue (FFPE blocks of primary breast cancer, non-CNS metastasis - if available and/or brain metastasis – if available) and CSF samples (which are routinely collected as part of clinical care) to support analysis of the following objectives[1]:

- To investigate the concordance between the primary and brain metastasis for ER, PgR and HER2
- The prevalence and concordance of the expression of the androgen receptor within brain metastasis as compared to primary disease.
- To investigate the genomic landscape of CNS disease secondary to breast cancer.

[1]Please note that the current funding provided allows for all relevant tissue collection, processing and storage at the GCP Laboratories facility. Further funding will be sought (before tissue collection is complete) to conduct analysis and meet the stated objectives in this protocol. Should funding not be secured, the collected tissue samples will be transferred to the LCTC (previously LCTU) Post-Trial Tissue Bank (REC reference: 18NW0621)

6. Study Design

This is a prospective non-interventional multicentre research study. Patients with histological confirmed locally advanced or metastatic breast cancer who meet the entry criteria will be provided with details of the study and consented. The study enables the collection of biological material collected during the course of routine care.

Blinding is not required for this study. There are no interventions and randomisation is not required. Patients will continue to receive standard care and no patient-reported outcomes or additional assessments are required to be collected.

6.1Study Setting

6.1.1 Selection of Participating Sites

All oncological and neurosurgical centres in the UK treating breast cancer or CNS involvement will be eligible to participate in the study.

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 ward setting. Relevant trainees from across the UK will be invited to participate in the study through the National Research Collaborative network, recently established Breast Cancer Trainee Collaborative model[17] and other relevant professional bodies. We plan to engage with the Society of British Neurological Surgeons (SBNS), the joint Liverpool Manchester North West Surgical Trials Centre (Director Professor Nigel Bundred), one of five national Surgical Clinical Trials Units, trainee networks and, we will also approach individual neurological centres.

The study intends to collaborate with the British Neurological Surveillance Unit (BNSU) to use their well-established network to identify relevant cases presenting to neurologist, in particular suspected cases of PND. It is accepted that diagnosing PND can be problematic. Where a diagnosis of PND is probable or possible, these patients may still be recruited but will be recorded as probable or possible cases of PND within the database.

Details of reviewing the sites and the formal greenlighting processes will be kept with TMG.

6.1.2 Selection of Principal Investigators

All principal investigators will be required to demonstrate relevant experience and commitment. 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.

The study intends to engage with the British Neurosurgical Trainees Research Collaborative (BNTRC) to appoint a trainee PI within each neurosurgery centre and the model for this has already been successfully established for trauma (RESCUE-ASDH) and hydrocephalus (BASICS) randomised controlled trials.

Hence the principal investigator at each site can constitute:

- Consultant level clinicians
- Trainee level clinicians

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

7. Eligibility Criteria

The PRIMROSE Tissue Study aims to recruit 300 patients. All patients must provide written, informed consent before any study procedures occur and must meet all eligibility criteria as described below.

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. Histologically and/or cytologically confirmed breast cancer with CNS involvement, as defined as having one or more of the following:

    a) Metastases to the brain parenchyma
    b) Metastases to the leptomeninges
    c) Paraneoplastic Neurological Disorders

4. FFPE of biopsied or resected primary breast cancer, non-CNS metastasis and/ or brain metastasis is available or will be available.
5. Informed consent

7.2 Exclusion Criteria

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

1. Unable to comply with study procedures or give informed consent

7.3 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 Outcomes

Outcome	Outcome measures
• To investigate the concordance between the primary and brain metastasis for ER, PgR and HER2	Comparison of the following receptors in different tissue: Expression of ER, PgR and HER2
• The prevalence and concordance of the expression of the androgen receptor within brain metastasis as compared to primary disease.	Comparison of the following receptors in different tissue: Expression of androgen receptor
• To investigate the genomic landscape of CNS disease secondary to breast cancer within CSF	Measure the amount of cell free DNA within CSF

9. Participant Timelines and Assessments

8.1 Participant Identification

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

8.3 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 provided with the relevant Patient information Sheet and Consent form (PISC) detailing the study. Patients will be given the opportunity to read through and have an initial discussion of the study. Patients will be approached during their first or second clinic visit/appointment. Patients will be given a verbal introduction to the study by a member of the clinical research team with knowledge of the study. The patient will be provided with the relevant Patient information Sheet and Consent Form detailing the study. The patient will be given the opportunity to read through and have an initial discussion of the study. Patient 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 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)

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 Screening and Registration Process

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 study, 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.**

Patients who meet the eligibility criteria outlined in section 7 and successfully fulfil the screening visit requirement will be enrolled into the study. The patient's GP and referring consultant will be informed of patient's decision to participate in the study according to consent provided by the study participants.

After a patient has been screened and their details MUST be entered onto 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 Tissue Study team.

PRIMROSE Tissue Study 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 documents.

9.3 Schedule for Assessments and Follow-up

Once consent has been obtained and documented and eligibility reviewed, the patient will be scheduled in for CSF collection as per their standard care pathway. This will be taken either by a lumbar puncture (with atraumatic needles) or aspiration of CSF from Ommaya Reservoir procedure or at the time of neurosurgery.

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

Procedures	At Registration (1st or 2nd Visit)	CSF Extraction Visit	Imaging/ Scan Visit	Disease Progression Visit
Data Collection				
Demographic Information	X			
Disease history and characteristics	X			
Prior cancer therapy (top line)	X			
Current anti-cancer treatment and concomitant medications	X			X
Progression Details				X
Scan Data only Collection (no images to be collected)				
Scan Data Collection (as per standard care – CT, MRI etc.)			X	
Tissue and CSF Sample Collection				
Routinely collected/	X			X

Diagnostic FFPE block i) Collection of Primary Breast Biopsy and/or ii) Collection of non-CNS recurrent/metastatic tissue and/or ii) Collection of CNS Biopsy				
At time of standard care CSF sample extraction - *Excess CSF sample collection – 10ml* - *EDTA Blood tube – 10ml*		X		

10. Procedures for all patients

10.1 Screening and Enrolment

Screening and enrolment will be completed as detailed in Section 9.4.

10.2 Registration Visit

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

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

The available diagnostic and routine FFPE blocks 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 CSF Extraction Visit

The following procedures will occur at this visit:

- Blood sample collection (1 x 10ml EDTA blood 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 or at standard care neurosurgery as per standard procedure (atraumatic needles should be used unless discussed with PI & CI). The CSF samples for the PRIMROSE Tissue study will be excess samples taken at the time of the standard care CSF extraction procedure.

10.4 Scan/Imaging Visit

If the patient is booked in for a routine scan as part of their standard care pathway (e.g. MRI/CT scan), the Scan/Imaging visit eCRF should be completed. (No images to be collected only related data as per eCRF).

10.5 Disease Progression Visit

If patient comes in for routine visit and there are no changes in 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)

10.6 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 withdraws consent.
- Contraindications to collection procedures.
- Death
- Clinician-led:
    - Any change in the patient's condition which results in a contraindication to collection procedures or causes the patient to fall under the exclusion criteria.
    - Any change in the patient's condition that justifies discontinuation of involvement in the clinician's opinion.

Data to be collected at the time of discontinuation includes 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. 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 via eCRF 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 this should be recorded onto eCRF.

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.

10.7 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.

10.8 End of Study

The end of the study is defined to be the date on which data for all participants is frozen and data entry privileges are withdrawn from the study database. The study may be closed prematurely by the Trial Steering Committee.

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 Tissue samples should be collected, processed, stored and shipped according to the PRIMROSE Tissue 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 following FFPE blocks will be collected for patients enrolled on the PRIMROSE Tissue Study where these samples were taken as part of the patient's routine care (or are planned to be taken as part of the patient's routine care) – e.g. diagnostic biopsies:

- Primary breast tumour tissue
- Non-CNS metastasis tumour tissue (where available)
- Brain metastasis tumour tissue (where available)

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

11.2 Blood (EDTA) Samples

A blood sample (*1 x 10 ml EDTA*) will be taken at the CSF Extraction visit (immediately prior to the CSF Extraction Procedure).

This blood sample should be processed at each trial site, stored at -80^{o}C and transferred in batches to the GCP Laboratory Facility.

11.3 Fresh CSF Sample

Cerebrospinal Fluid samples will be collected at the time of a patient's routine CSF extraction procedure. This will either be carried out via Lumbar Puncture or Ommaya Reservoir aspiration or at standard care neurosurgery as per standard procedure. The CSF samples for the PRIMROSE Tissue study will be an excess sample taken at the time of the standard care CSF extraction procedure.

The following samples will be collected for the PRIMROSE Tissue Study:

- 1 x 10ml CSF sample

Samples will be processed, stored and transferred to the GCP Laboratory Facility as per the PRIMROSE Tissue Study Laboratory Manual.

12. Safety Reporting

Adverse events/safety events experienced by patients **will not** be recorded or reported for this study as all patients are receiving standard treatment decided by their clinical team and their care will not be influenced by the patient taking part in the PRIMROSE Tissue Study.

13. Data Management and Study Monitoring

For the PRIMROSE Tissue 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 these 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.

13.1 Source documents

For this study, source documents will include hospital records, laboratory notes and samples stored at laboratory departments involved in the study. Each participating site should maintain appropriate medical and research records for this study.

Date(s) of informed consent processes including date of provision of patient information, study ID number and the fact that the patient is participating in a clinical trial should be added to the patient's medical record chronologically.

13.2 Data collection method

Data are to be entered into the trial eCRF database by members of the research team at site. Relevant manuals on data entry will be provided.

13.3 Monitoring

Monitoring is conducted to ensure protection of patients participating in the trial and all aspects of the trial (procedures, laboratory, 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 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, accuracy and timeliness of data collection etc.

Trial Oversight Committees related to the monitoring of the trial are detailed in Section 4.

13.4 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.

13.5 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 Tissue Study is categorised as a Type A study.

13.6 Confidentiality

This study 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 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 trial Sponsor and The University of Liverpool's Data Protection Officer and appropriate processes followed.

13.6 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.

- Data quality checks and monitoring procedures will be undertaken in line with the Trial Data Management Plan.

13.7 Records Retention

The retention period for the PRIMROSE Tissue Study data and information is 10 years from the official End of Trial date.

The PI at each investigational site must make arrangements to store the essential study documents (as defined by ICH 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 electronic CRFs 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.

14. Statistical Considerations

14.1 Introduction

Details of provided on the statistical considerations given to the design of the study and analysis of study data. Full details of the statistical analysis of all trial data will be included in a separate statistical analysis plan.

14.2 Sample Size

As this is an exploratory study, there are no formal hypothesis being tested. Any analysis performed will be predominantly descriptive meeting the exploratory objectives stated in this protocol (subject to further funding for analysis being secured) in order to develop some specific hypothesis that can be tested in future research. There are no formal power calculations to determine a sample size. The number of samples obtained for this study is not therefore capped and the intention is to obtain as much data as resources will allow.

14.3 Statistical Methodology

14.3.1 Groups for Analysis

Primary analysis will be conducted on the full analysis set following the intention to treat principle, retaining all samples/patients in the analysis irrespective of any protocol deviations. Sensitivity or sub-group analysis based on per-protocol or any other defined patient group will also be performed as required.

14.3.1 Missing Data

Missing data are anticipated to be small and all analyses are planned on a complete case basis.

14.3.2 Levels of significance

All comparative analysis will be compared against the nominal p=0.05 level to determine statistical significance with results presented alongside 95% confidence intervals.

14.3.3 Analysis Methods

Analyses will be descriptive in nature. Continuous data will be presented as Median (IQR) [Range] and categorical data will be presented as frequencies of counts with associated percentages. Any comparisons across groups will be performed using Chi-square/Fisher test for categorical data and Wilcox Test/T-test for continuous data.

15. Regulatory And Ethical Considerations

15.1 Statement of Compliance

This study is designed to comply with Good Clinical Practice (GCP) and will be managed and conducted in compliance with the protocol and LCTC Standard Operating Procedures. In addition, sample collection 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 Breaches

Protocol deviations will be managed according to local site and LCTC procedures as appropriate. They will be reported to study oversight committees.

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 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 ten 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 Daiichi Sankyo Europe GmBH in accordance with the stipulated guidance in the grant letter. The PRIMROSE Tissue Study 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.

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 (01/10/2020)

Original Approved version

19 Documents Supplementary To The Protocol

Documents referenced within the protocol are separately maintained and version controlled. Any of the supplementary documents subject to CA and / or Ethical review are submitted as separate version controlled documents.

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