TITLE PAGE

Trial Title: Predicting REsponses to chemotherapy from NUTRition In triple negatIVE breast cancer: The PRE-NUTRITIVE Study.

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1. KEY TRIAL CONTACTS

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2. LIST OF ABBREVIATIONS

ACT	Adjuvant Chemotherapy
AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
EPR	Electronic Patient Record
GCP	Good Clinical Practice
ICF	Informed Consent Form
LTHT	Leeds Teaching Hospitals NHS Trust
NACT	Neoadjuvant Chemotherapy
NHS	National Health Service
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
TMG	Trial Management Group
TNBC	Triple Negative Breast Cancer

3. BACKGROUND AND RATIONALE

Adherence to the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) cancer prevention guidelines is linked to reduced cancer incidence and mortality in many cancer types [1-5]. However, the interaction between the molecular changes driven by adherence to these recommendations and response to chemotherapy in patients has not been explored. Detoxification and nutrient metabolism in healthy tissues prevent toxic accumulation of metabolic intermediates and end-products [6]. These pathways also enhance processing of anti-cancer drugs such as chemotherapy agents. Resistance to chemotherapy is a major barrier to successful cancer treatment. Clinical tools which can predict the most effective chemotherapy regimen for an individual patient remain underdeveloped. Multiple studies have shown dietary patterns can influence cancer treatment efficacy at molecular level [7]. There are multiple examples as follows; citrus is contraindicated for ~30% of all prescription drugs owing to irreversible inhibition of detoxification enzyme CYP3A4[8]; dietary fatty acids induce CYP2C8 (a phase 2 detoxification factor) resulting in the inactivation of numerous chemotherapeutics [9]; vitamin A derivatives change expression of phase 1 detoxification efflux pumps linked to chemoresistance in several cancer types [10-12]. Other compounds such as Vitamin D [13-15] and curcumin [16] increase drug efficacy. Our research group recently reported that the cholesterol homeostasis transcription factor LXRα mediates chemotherapy resistance via P-glycoprotein (Pgp) in triple negative breast cancer (TNBC) [17].

TNBC make up ~15% of breast cancers. TNBC are a specific type of breast cancer that lack expression of oestrogen (ER), progesterone (PR), and HER2 receptors. Despite chemotherapy, their prognosis remains the poorest of all breast cancer subtypes (5-year survival ~65%). TNBC chemotherapeutics are also used to treat many other cancers. Therefore, the ability to predict whether these drugs will be efficacious in individual patients could have wider applicability beyond TNBC patients. Traditionally, patients have surgery to remove tumours and then receive adjuvant chemotherapy (after surgery; [ACT]) in order to eliminate potential residual microscopic disease. However, there is no way to assess the efficacy of ACT until years later when the patient's tumour relapse status becomes known. For some cancers the alternative treatment approach is to administer chemotherapy before surgery (neoadjuvant chemotherapy; NACT). One of the aims of NACT administration is to achieve sufficient cancer shrinkage and facilitate less radical surgery. In breast cancer patients this may mean a lumpectomy instead of mastectomy. Therefore, NACT is commonly utilised in patients with TNBC.

During NACT, the response to chemotherapy is monitored with interval MRI scans, and where sub-optimal tumour response (shrinkage) is observed, the chemotherapy regimen can be altered. However, despite this, sub-optimal response to NACT is often observed due to chemoresistance. Furthermore, pathology assessment of remaining cancer after NACT (Residual Cancer Burden) strongly predicts long-term survival[18]. Therefore, it is vital to elucidate the underlying molecular mechanisms responsible for chemoresistance. The proportion of TNBC patients achieving the best possible outcome of pathological complete response (no residual cancer on pathology assessment [pCR]) after NACT is 32% [19].

Our pilot data (submitted as part of the funding application supported by WCRF) identified numerous intersections between nutrient dependent transcription factors (nutrient-TFs) and chemoresistance biomarkers (CR-biomarkers) in TNBC. To identify TNBC specific CR-biomarkers we performed a systematic literature review (PROSPERO #CRD42021243047) selecting 66 CR-biomarkers with expression levels that are prognostic in TNBC patient cohorts, and where a molecular mechanism of chemoresistance has been elucidated. Analysis of these the CR-biomarkers and 36 nutrient-TFs in TNBC patients allowed us to discover several networks initiating from cholesterol metabolism, fatty acid, and retinoids. Our *in silico* results used to support the WCRF and WCRF international application were validated by RNA-Seq analysis and with *in vitro* and *in vivo* data (the accepted grant proposal is available on request).

There is a clinical need to accurately predict which TNBC patients are less likely to respond optimally to NACT. Development of such a predictive tool will provide substantial potential benefits to patients including optimisation of chemotherapy regimen, with avoidance of ineffective regimen and associated side effects, and maximisation of tumour response and patient survival. This feasibility study will aim to inform the design of such prediction model. To do this, we will gather data on metabolite, nutritional, and chemoresistance mechanisms in TNBC, and gather nutritional data from patients based on molecular analysis and survey questionnaires. This feasibility study will test the hypothesis that nutrient-metabolism parameters in TNBC and adherence to the WCRF cancer prevention recommendations can influence the expression of functional chemoresistance biomarkers. Our overarching aim is to establish if data regarding the activity levels of these molecular pathways, and measures of nutritional intake, can be used to predict chemotherapy response in TNBC patients. This trial will aim to elucidate the molecular mechanisms responsible for the tumour's nutrient sensitive transcription factors to evade the cytotoxic effects of chemotherapy.

4. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

This is a feasibility study that will collect data on the nutritional status of TNBC patients, the activity of nutritional and metabolic related genes in their tumours, and efficacy of their chemotherapy regimen. The study will primarily assess the feasibility of collecting this information in the clinical setting. Preliminary data that is gathered will be used to identify specific nutrient/metabolic parameters that predict chemoresponse in TNBC patients. The data gathered from this study will be used to inform the design of future multi-centre clinical trial that is sufficiently powered.

	Objectives	Outcome measures/Endpoints
Primary	To assess the feasibility of collecting nutritional metrics from TNBC patients during standard clinical care pathways and compare this to chemotherapy response.	To determine the patient's nutritional status via survey, and by nutritional profiling of blood and tumour biopsy. These data will be compared to the patients' tumour response on imaging and pathology.
Secondary	1. Establish patient recruitment rate 2. Establish adherence to WCRF cancer prevention recommendations 3. Assess intra-tumour variability of candidate metabolic pathways 4. Test power of candidate metabolic pathways to predict chemoresponse 5. Establish feasibility of designing a sufficiently powered multicentre clinical trial to develop a chemoresponse prediction tool	1. Patient uptake rate of blood and tumour tissue donation 2. Completion rate of patient survey 3. Determine potential variation in nutritional status of the study participants (based on molecular assays and survey questionnaire) 4. Accuracy and compliance of WCRF guideline adherence using FFQ and myfood24 based methods of data collection. 5. RNA yield from tumour biopsies and selection of best candidate metabolic pathways that predict chemoresponse

5. STUDY DESIGN

This is a prospective, non-randomised, feasibility, cohort study. Patient recruitment period will be 18 months. A minimum of 15 and maximum of 20 TNBC patients undergoing NACT at Leeds Teaching Hospitals NHS Trust (LTHT) will be recruited. Histological confirmation of TNBC will be determined by local pathology team at LTHT based on the assessment of ER/PR/HER2 status.

Once patients have agreed to participate in the study, the clinical team will collect pseudonymised information relevant to the study from the hospital electronic patient record (EPR): body mass index and waist-hip ratio, type/frequency/duration of chemotherapy, tumour response to NACT using MRI scans, type of breast and axillary surgery performed, pathology assessment of diagnostic biopsy and surgical resection. Study participants will be asked to complete a questionnaire at the start of NACT to evaluate baseline adherence to the WCRF recommendations, and at the end of NACT to evaluate any potential dietary changes. Study participants will also be asked to provide a blood sample at the start and end of NACT to ascertain micro- and macro-nutrient levels in plasma/serum.

Prior to starting NACT, patients undergo an additional procedure to have a marker clip placed into the tumour. This marks the original tumour location and is especially important when planning surgical resection for patients who achieve complete radiological response during NACT. The marker clip is placed by the radiology team under ultrasound guidance and provides a widow of opportunity in the patient's standard clinical pathway to obtain research biopsies. Patients will therefore be asked to donate two tumour core biopsies (14 gauge) during clip placement. These biopsies will be performed by the same radiology team performing the clip placement. RNA will be extracted from the fresh biopsy tissue and analysed by RNA-sequencing or Taqman™ Low Density Array (depending on yield). For the small number of patients where the marker clip was already placed at the time of initial diagnostic biopsy, patients will be offered the option of undergoing an additional research biopsy, but this will not be mandatory for participating in the remaining components of the trial. At LTHT, 71% of TNBC patients undergoing NACT in 2020 did not have a marker clip placed at the diagnostic biopsy. These patients therefore required a second procedure to have clip placed as part of standard clinical pathway. This trial will assess the feasibility of obtaining tumour biopsy tissue in patients who require this second procedure, as well as determine the acceptability of patients who agree to undergo an additional research biopsy.

Patients will be offered the option of donating tumour tissue from surgery, but this will not be mandatory for participating in the remaining components of the trial.

6. STUDY SETTING

This trial will be run jointly from LTHT and School of Food Science and Nutrition (FS&N) at University of Leeds. Patient recruitment, along with all the previously cited activities on study participants, will be performed at LTHT. FS&N will perform analytical methods including RNA extraction from tumour tissue, as well as analysis of micro- and macro-nutrients in blood samples using commercial kits. RNA sequencing will be performed by external research organisations (NOVOGENE). Liquid chromatography mass spectrometry (LC-MS)/mass

spectrometry (MS) will be performed in-house or with collaborators for micro- and macro-nutrient analysis. All data analysis will be performed at FS&N. All data will be held at FS&N on secured password protected servers.

7. STUDY PARTICIPANTS

7.1 Inclusion criteria

- Female or male patients aged 18 years or older
- Uni- or bilateral TNBC in the breast and/or axilla (including primary, second primary, locoregional recurrence)
- Clinical recommendation for NACT
- Ability to provide informed consent
- Uni- or multi-focal invasive breast cancer of any histological subtype (if multifocal, dominant lesion that is triple negative to be biopsied) and any tumour (T) and clinical node (N) staging.

7.2 Exclusion criteria

- Patients diagnosed with pure non-invasive breast cancer (e.g. DCIS) or with non-TNBC invasive subtypes
- Patients diagnosed with distant metastatic disease at the time of primary breast cancer diagnosis
- Inability to provide informed written consent

TNM staging system has been stated as per Cancer Research UK definitions (https://www.cancerresearchuk.org/about-cancer/breast-cancer/stages-types-grades/tnm-staging).

8. STUDY PROCEDURES

8.1 Participant Identification

Study participants will be identified at the breast multidisciplinary (MDT) meeting, in breast surgery and breast oncology clinics by the clinical team directly involved in the patient's care at LTHT.

8.2 Recruitment and informed consent

The direct clinical team (consultant breast surgeons, oncologists, or radiologists) and an appropriately trained member of the hospital team (including research nurses, as per the delegation log; Annex 1) will discuss the option of study participation with the patient at the clinic and provide a patient information sheet (Annex 2). This is in accordance with Good Clinical Practice (GCP) guidelines and will have been documented and approved by the Principal Investigator on the delegation log. Patients will be given a minimum of 24 hours to consider the study and enable sufficient time to consider the information and ask any questions they may have about participation. For patients who agree to participate in the study, they will sign and date the latest approved version of the informed consent form (ICF) (Annex 3) enabling the study specific procedures and data collection to proceed. Written consent can be taken by the direct clinical team and/or appropriately trained member of the team (including research nurses or trials assistants, as per the delegation log). All personnel who obtain consent will be Good Clinical Practice (GCP) trained, and suitably qualified and experienced.. The completion of consent form will occur during the patient's scheduled hospital visits so not to incur additional hospital visits as a result of agreeing to participate in the study. In addition, patients will have the additional option of undergoing postal consent (e.g. due to changes in COVID-19 restrictions). Participants undergoing face-to-face or postal consent will be given a fully signed copy of the ICF before study participation.

The original signed ICF will be retained in the trial site file and a copy will be placed in the patient's notes. Patients will be reminded that participation is voluntary and that they can withdraw at any time point without this affecting their care and without providing a reason. Participants will be informed that data and samples acquired and analysed prior to withdrawal will be retained. The reason for withdrawal will be recorded if provided by the patient. Where a participant is required to re-consent or new information is required to be provided to a participant, it is the responsibility of the CI to ensure this is done in a timely manner.

Potential participants must be able to give consent and a person is assumed to have mental capacity to decide unless shown to be absent. Participants who are unable to consent for themselves will not be approached. If a participant who has consented but later becomes incapacitated, the participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

8.3 Additional consent and long-term storage provision of study participant data and specimens in related future studies.

We will retain any unused serum/tissues/cellular samples for potential future analysis at the end of the study in a pseudonymised form with prior specific consent. This includes the storage and use of data and samples for future research related to this trial and submission to a data repository. For the ancillary/future studies, only data and samples of study participants who provide this additional optional consent to reuse their data/sample will be used. Study participants can opt out of this additional consent and still participate in the main trial. If study participants decide to withdraw from a future study, data and specimens will be eliminated for that specific study. Study participants will be asked to provide optional consent to store personal information to be recalled for future studies. Consent will be obtained specifically for the use of RNA and DNA. At the end of the study, HTA relevant and non-relevant materials will be stored by the research team pending ethical approval for use in another project at HTA licenced premises at the UoL where required. Other samples will be disposed in accordance with the HTA's Code of Practice.

8.4 Blinding

Blinding is not applicable to this study.

8.5 Baseline assessment (T0)

Study observation period starts from when the study participants sign the informed consent and continues until the time when the patient finishes NACT and completes surgical treatment and pathology assessment. The total duration is estimated to be around nine months. The following baseline measures will be collected as part of the trial or as part of routine clinical pathway if already available:

Blood samples for micro- and macro-nutrient level determination (50ml): blood collection will be performed in fasting state (>8 hours fast) through venepuncture on the opposite arm to where the breast cancer is (as per hospital protocol). Serum/plasma sample will be obtained by using a yellow top blood sample vacutainer/EDTA pink top blood sample vacutainers. The samples will be labelled and immediately transported to the FS&N laboratories to be processed.

Biopsies for RNA analysis: The patient will be biopsied under US guidance with local anaesthesia where 2 x 14G core biopsies will be obtained from the dominant mass by a member of the radiology team trained to perform biopsies. Similarly, 2 x 14G core biopsies

will be obtained from the dominant mass by the trained surgical team once the cancer has been resected from the patient. The samples will be labelled, placed in RNAlater on ice, and immediately transported to the FS&N laboratories to be processed. Coded samples of extracted RNA will be sent to external company for RNA sequencing or processed internally by Taqman™ Low Density Array (depending on yield).

Validated FFQ (EPIC-Norfolk) for UK population (Annex 4) and myfood24.org: surveys will be conducted to determine adherence to six 2018 WCRF/AICR recommendations about healthy diet (Eat wholegrains, vegetables, fruit and beans; limit 'fast foods'; limit red and processed meat; limit sugar sweetened drinks; limit alcohol consumption; do not use supplements for cancer prevention). Adherence to the WCRF/AICR recommendations will be scored according to previous studies Winkels et al. [20], Romaguera et al. [21], and Malcomson et al. [3].

Physical activity EPAQ2 (Annex 5): survey [22, 23] will be conducted to determine adherence to 2018 WCRF/AICR recommendations about physical activity.

Breastfeeding and smoking information: information about past or current breastfeeding and past or current use of tobacco will be asked to determine adherence to 2018 WCRF/AICR recommendations.

MRI scan: MRI scan will be performed at baseline prior to commencing NACT as standard of care (SOC). Briefly, MRI is performed at 1.5 T with the patient lying prone in a bilateral breast coil. T1 and T2-weighted volume imaging of both breasts pre-contrast is performed. Twelve sets of fat suppressed high spatial resolution T1-weighted volumes (acquisition time 34 s) are acquired before during and after administration of a bolus of 0.1 mmol/kg Gd-DOTA; imaging continuing for approximately 7 mins. Total examination time ~ 30 minutes.

Anthropomorphic measures: body weight will be measured to the nearest 0.1 kg, using a technical balance and height with a stadiometer to the nearest 0.1 cm. Body weight and height will be used to calculate the body mass index (BMI) (BMI=weight (kg)/height $(m)^2$) of each subject to assess the adherence to the WCRF/AICR recommendations regarding body fatness. Waist and hip circumferences will be measured with a tape measure to the nearest 0.5 cm.

8.6 Visit 1 (T1)

The T1 timepoint will coincide with the completion of two cycles of the first treatment regimen. At this timepoint a second planned routine breast MRI scan will have been performed to assess the response to treatment. The result of this MRI scan will be collected.

8.7 Visit 2 (T2)

The T2 timepoint will coincide with the completion of two cycles of the second treatment regimen. As per the treatment protocol, this may coincide with the 4th or 6th cycle of treatment overall, depending on response seen on the MRI at the T1 timepoint. At this timepoint a third planned routine breast MRI scan will have been performed to assess the response to treatment. The result of this MRI scan will be collected at T3.

8.8 Visit 3 (T3)

At T3 participants will undergo surgical resection of tumour, pathological assessment and evaluation of residual cancer burden as per standard clinical care. Blood collection for micro- and macro-nutrient analysis and food and physical activity surveys (as per section 8.5) will be repeated at T3.

8.9 Withdrawal criteria

Participants are free to withdraw at any time of the study without giving a reason. Patients who decide to withdraw will be not able to continue any study specific procedures and/or sample collection for translational analysis. During the trial, information about the time and reasons withdrawal will be recorded.

Subjects will be withdrawn from the study for any of the following reasons:

- Subject withdraws consent
- Patients lost to follow-up
- Occurrence of adverse event/serious adverse event

8.10 End of Study

The recruitment period will end 18 months after the study opens for patient recruitment at LTHT or if the trial is stopped for another reason by the funding body or regulatory body. The trial will close when the last patient has completed their surgical follow up, *i.e.* about nine months after the last participant has been recruited to the study. The REC who provided the

favourable opinion will be notified of the trial conclusion in writing. The CI will notify the REC of the end of a clinical trial within 90 days of its completion.

9. STORAGE OF DATA AND SAMPLES

The clinical information gathered as part of standard clinical care will be pseudonymised by the research team at LTHT. The linkage between the pseudonymised clinical information and the data generated from the laboratory analysis will be password protected and stored securely. Samples collected by the LTHT research team will be immediately transferred to FS&N with the codes already applied. FS&N will not have access to the personal data and data and samples will be non-identifiable to FS&N researchers. The samples and the pseudonymised research data will be under the custodianship of James L. Thorne, Chief Investigator.

Serum/plasma samples will be immediately processed and/or stored in cryotubes at -80°C (+/-10) freezer in the FS&N centre research for a maximum of 10 years. After this time samples will be discarded. All the required materials will be provided by the Sponsor. Serum and/or plasma samples will then be analysed by using validated scientific methods to quantify micro-/macronutrient content. University of Leeds will use/retain pseudonymised samples for the duration of the study. After the end of the trial, the link between donor and sample will be broken and samples destroyed, unless the participant consented for the re-use of samples in future study. In this case, samples will be maintained pseudonymized for 10 years and then destroyed. Serum sample destruction will be performed according to the University of Leeds procedures and recorded on laboratory documents (FS&N). Unspent biopsy samples and tumour tissue collected at surgery will be stored liquid nitrogen at the University of Leeds until the end of the trial or for up to 10 years as according to HTA and University ethical guidelines in case participants consented for re-use of their samples in future studies. Biological material will be retained at the University of Leeds to support future potential research projects that will undergo a separate ethics application.

It will be the responsibility of the trial site LTHT to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the 2018 Data Protection Act. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act. If transport of material to third parties will be

necessary for research purposes, a tissue transfer agreement (TTA) will be signed by the Sponsor and the relative third party.

10. DATA MANAGEMENT

Data collection will occur in accordance with GCP, Caldicott principles and the General Data Protection Regulation (GDPR) 2018, and will work in line with NHS confidentiality guidelines and codes of conduct. Data for each patient will be pseudonymised using a unique alphanumeric study identification number. No patient identifiable data will be recorded into the Case Report Form (CRF) (Annex 6) for the purpose of the study.

10.1 Case report forms

The study CRF is the primary data collection instrument for this study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. If the answer to a question is unknown, write “NK” (Not Known). Where a choice is requested, cross (X) the appropriate response. All entries should be printed legibly in block capital letters using black ink. If any entry error has been made, to correct the error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialled and dated. DO NOT ERASE ERRORS by any method. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. Never use correction fluid. The Investigator must review all entries for completeness and correctness. The PI at LTHT is responsible for the quality of the data recorded in the case report form. The data recorded should be a complete and an accurate account of the participant’s record collected during the study. Only the data required by the protocol are captured in the CRF.

10.2 CRFs as Source Documents

Data can be entered directly onto the CRF by site research team, and if this is done then this would then be considered a source document. The PI at LTHT will keep records of all participants (sufficient information to link records e.g. CRFs and samples), all original signed informed consent forms and copies of the CRF pages.

10.3 Source Data

All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and

evaluation of the trial are considered source data. Source data are contained in source documents (original records or certified copies). Source data and records will respect the following criteria: Accurate; Legible; Contemporaneous; Original; Attributable; Complete; Consistent; Enduring; Available when needed.

10.4 Source Documents

Original documents, data and records (*e.g.*, past and present clinical records, including copies of original records and signed copies of consent form, laboratory notes, participant files, and records kept at the laboratories, and at medico-technical departments involved in the clinical trial, study CRF will be considered source documents.

Source documents are where data is first recorded, and from which participants' data are obtained. These will include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. Source data verification will be monitored to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki 1964 as amended October 1996. Monitoring by the Chief Investigator or authorised authorities will ensure:

- Sufficient data is recorded to enable accurate linkage between hospital records, CRF, and the data generated from the laboratory analysis
- Source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit
- Staff working on the trial will meet requirements of the EU Directive

10.5 Data handling and record keeping

The CI and the research team will ensure that the participants' identity is protected at every stage of their participation within the study. Personal data will only be available to the research team at the LTHT. Codes will be created for each participant personal information and data pseudonymised. No deletion of entered data will be performed. All information collected during the study will be kept strictly confidential. Information will be held securely on paper and electronically at LTHT and University of Leeds and both will comply with all aspects of the Data Protection Act 2018 and operationally this will include:

- Consent from patients to record personal details including name, date of birth, address and telephone number, email address, NHS ID and hospital ID

- Appropriate storage, restricted access and disposal arrangements for patient personal and clinical details
- Consent from patients for access to their medical records by responsible individuals from the research staff, the Sponsor, and from regulatory authorities, where it is relevant to trial participation
- Consent from patients for the data collected for the trial to be used to evaluate safety and develop new research.

Data completeness is an integral part of any study. A CONSORT style will be used to monitor data completeness from eligibility screening, approach, study acceptance through to the final follow-up visit. This information will be made available to the study steering group as regular reports. The team will also report the number of:

- Patients screened per month
- Patients approached per month (and reasons why not approached)
- Patients recruited per month
- The number of patients who complete each follow up visit or are lost to follow-up
- The number of patients who complete the trial.

No one except for the CI and statistician are authorized to make data changes. If data are transformed during processing, it should always be possible to compare the original data and observations with the processed data. At the end of the study, research data will be fully anonymised and files will be retained with the purpose to share them with other researchers when not bound by copyright restrictions, confidentiality requirements, or contractual clauses. The results of the analyses conducted on individual participants will not be made available to the participant or their doctor. However, participants can request and obtain a copy of study results.

10.6 Access to Data

Direct access of coded data will be granted to the research team at the University of Leeds, authorised representatives from the Sponsor and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent.

10.7 Archiving

Fully anonymized research data will be deposited with the University of Leeds data repository. Raw sequencing data will be stored by LeedsOmics facility for 5 years and then deposited and hosted with a digital object identifier (DOI). The Sponsor will be responsible for the archiving of all trial documents. The trial database and essential documents will be retained in file or in locked spaces (for paper documents) at the LTHT site. Personal data documents will be

archived for 25 years at LTHT after completion of trial. Pseudonymised data will be held by the Sponsor for 25 years for patients who consented to be contacted for future research The Destruction of essential documents will require authorisation from the Sponsor.

11. SAFETY REPORTING

11.1 Definitions

An *Adverse Event* (AE) is any untoward medical occurrence in a subject to whom an intervention has been administered, including occurrences which are not necessarily caused by or related to that intervention. An AE can therefore be any unfavourable and unintended sign, symptom or disease temporarily associated with study activities.

A response to a toxic or involuntary medical product is considered as an *Adverse Reaction* (AR). It is a reasonable possibility that an AR could be linked to any AE in this context. ARs can be caused by medicinal products within or outside (i.e. off-label use, overdose, misapplication, abuse and medical errors) the purpose of their marketing authorisation or due to occupational contact.

A *serious adverse event/reaction* (SAE/SAR) is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect
- Any other significant clinical event, not falling into any of the criteria above, but which in the opinion of the Investigator requires reporting.

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

11.2 Recording and reporting of events not considered AEs for this study

Any medical condition present at baseline where the severity and/or frequency do not get worse during the study is not considered as AE. Current health conditions/diseases must be

reported in the CRF. NACT-related toxicity will be not considered as AE as this is not the result of any study-related procedure or intervention.

11.3 Recording and reporting of AEs

Any AE related to the administration of NACT is not a reportable AE for this study. In all the other cases AEs that are critical to the safety evaluation of the participant will be reported to the Sponsor and NHS site. Information elicited and all clearly related signs and symptoms will be recorded in the AE and SAE section in the CRF.

11.4 Recording and reporting of SAEs

Any SAE related to the administration of NACT is not a reportable SAE for this study. Only SAE related and unexpected occurring to a research participant will be recorded in the CRF and reported to the main REC and to the Sponsor by the CI/PI within one working day to the Sponsor and 15 days to the main REC after becoming aware of the event through the SAE report form (Annex 7). All SAEs, related and unexpected, occurring from the time of written informed consent until 30 days post cessation of intervention must be recorded and emailed to the Sponsor within one working day of the research staff becoming aware of the event. Once all resulting queries have been resolved, the original SAE report form will be kept in the trial Master file and a copy will be sent to the Sponsor.

For each SAEs the following information will be collected:

- event duration (start and end dates, if applicable);
- action taken;
- outcome;
- causality (*i.e.* relatedness to trial investigation);
- whether the event would be considered anticipated.

12. **RESPONSIBILITIES**

12.1 Notification of deaths

All deaths will be reported to the Sponsor.

12.2 Medication Errors, Overdose, Abuse, and Misuse

Not applicable

12.3 Reporting urgent safety measures

The CI/PI may take urgent safety measures to ensure the safety and protection of trial participants from any immediate hazard to their health and safety. The measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI/PI to inform the Sponsor, NHS site and REC of this event within three days. Notice in writing setting out the reasons for the urgent safety measures and the plan for further action will be sent.

13. STATISTICS AND DATA ANALYSIS

This feasibility study is not powered to predict a clinical endpoint and sample size is calculated solely on practical considerations regarding time and financial constraints. The data collected in this feasibility study will inform the design of subsequent trial that is sufficiently powered. We will create network diagrams of patients who respond or not respond. SPSS will be use to assess correlation between candidate markers and clinical data (MRI, pCR, WCRF adherence scoring).

14. MONITORING, AUDITING AND INSPECTION

The purpose of monitoring is to determine and verify the protection and well-being of participants, the accuracy of the data, and the compliance of the conduct and quality of approved protocol, GCP and regulatory requirement. The members of the Trial Management Group (TMG) are represented by CI, PIs, and Statistician. The TMG is responsible for the day-to-day management of the trial and will oversee all aspects of the conduct of the trial. Regular TMG meetings will be held. The Sponsor will monitor and audit the conduct of research as required, periodically reviewing safety data and issues. Monitoring will be conducted on the sites of enrolment and intervention. The site TMG member will be responsible for data recording, according to data protection laws and requirements, CRF, SAEs, reports, notifications, applications and submissions availability and that these documents are accurate, complete, dated and identify the trial. The site TMG member will also ensure that all personnel part of the study team have adequate training, resources and facilities, including laboratories, equipment and staff, to safely and properly conduct the trial and the these remain adequate throughout the study period. The PIs at NHS site will ensure that any code breaks are properly handled and documented according to the protocol.

15. ETHICAL AND REGULATORY CONSIDERATIONS

15.1 Research Ethics Committee (REC) review & reports

Before the start of the trial, approval will be sought from a REC for the trial protocol, informed consent forms and other relevant documents. Substantial amendments that require review by REC-HRA will not be implemented until the REC-HRA grant a favourable opinion as appropriate. All correspondence with the REC-HRA will be retained in the Investigator Site File. An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. It is the CI's responsibility to produce the annual reports as required. The CI will notify the REC of the end of the trial. If the trial is ended prematurely, the CI will notify the REC, including the reasons for the premature termination. Within one year after the end of the trial, the CI will submit a final report with the results, including any publications/abstracts, to the REC.

15.2 Peer review

This trial was peer reviewed by the scientific committee from the World Cancer Research Fund (WCRF) and awarded funding for the grant application.

15.3 Public and Patient Involvement

We gained valuable input from a patient representative group (Breast Cancer Research Action Group) in the conception and study design, especially in drafting the patient information sheet and the patient consent form, which are concise and easy to understand from the patient and family member's point of view. We have ensured that these documents contain appropriate wording and phrases for patients eligible to join the study. Members of the Breast Cancer Research Action Group completed a patient survey we provided to inform the study design. In response to this feedback the study design was refined with regards to acceptability for research biopsy, blood collections, paper and/or electronic food questionnaires, and study participation. We also received further public and patient involvement from the NIHR CanNut working group for the WCRF grant proposal lay section.

15.4 Regulatory Compliance

The trial will not commence and funds not released by WCRF until authorisation is obtained from HRA and REC. Before the site can enrol participants into the study, the CI/PI or designee will ensure that appropriate approvals from participating organisations are in place. Specific

arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance.

For any amendment to the study, the CI or designee in agreement with the Sponsor, will submit information to the appropriate body in order for them to issue approval for the amendment. The CI or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

15.5 Protocol compliance

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations and must not be used e.g. it is not acceptable to enrol a participant if they do not meet the eligibility criteria or restrictions specified in the trial protocol. Accidental protocol deviations can happen at any time and they must be adequately documented on the relevant forms and reported to the CI and Sponsor immediately. Deviations from the protocol which are found to frequently recur, are not acceptable, will require immediate action and could potentially be classified as a serious breach.

15.6 Data protection and participants confidentiality

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 and the EU General Protection Data Regulation (GDPR) with regards to the collection, storage, processing and disclosure of personal information and will uphold the both legislations core principles. Personal information will be collected, kept secure and maintained at the NHS site. Codes will be created for each participant's personal information and data pseudonymised during the trial and fully anonymized at the end of the trial. Data will be maintained secure and linking code will be placed in separate secure locations. After the end of the trial, personal data will be stored for 25 years under the custody of the LTHT, and anonymised research data will be deposited with the University of Leeds data repository. Personal data will NOT be used by research teams or shared with external research organisations, such as universities and commercial companies in this country and abroad for scientific research purposes.

15.7 Indemnity

The University, when acting as Sponsor, has insurance cover in force, which meets claims against it and where those claims arise from the Universities own negligence in its role and activities relating to the study (and which is subject to the terms, conditions and exceptions of

the relevant policy). Clinical negligence indemnification will rest with the participating NHS Trust under standard NHS arrangements.

15.8 Amendments

Changes made to the research after review body approval are amendments. These may be substantial or non-substantial. The CI and the Sponsor's authorised representative will be responsible for the decision to amend the protocol and for deciding whether an amendment is substantial or non-substantial. The notice of amendment form will be created using the Amendment Tool which can be downloaded from IRAS website. Relevant extracts or new versions of revised documents will be submitted, showing the new version number and date and giving both the previous and new wording which is clearly identifiable. Amendments will be tracked in the protocol appendix and the version of the protocol will be updated.

16. DISSEMINATION POLICY

The CI will be in charge and responsible for any data published from this study. On completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared to be sent to REC-HRA the within 12 months of the end of the study. Data will be published in scientific journals, which will depend on the outcome of data analysis. Funding and supporting bodies will be acknowledged within the publications. LeedsOmics will curate and deposit data in the European Nucleotide Archive with a unique DOI. Overall study outcomes will also be made freely available to trial participants. Full trial report, pseudonymised participant level dataset, and statistical code for generating the results will not be publicly available.

The author of the final report will be the Chief Investigator. Named authors of manuscripts for publications will be in accordance with the International Committee of Medical Journal Editor's defined authorship criteria for manuscripts submitted for publication. No professional medical writers will be hired.

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Appendix 1 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	1.2	17.08.2022	G. Cioccoloni	We have spotted a mistake in reporting the recruitment period time. The actual recruitment period time is 18 months and not 24 months as it was previously reported in IRAS and protocol. Since Leeds Teaching Hospital treats with Neoadjuvant Chemotherapy about 15-20 triple negative breast cancer patients each year a recruitment period of 18 months will give us a margin to reach the patient number needed to complete this study (15-20 triple negative breast cancer patients in total). Furthermore, there was a miscalculation of the end date of the study. The length of the study was reported to be 2 years. The correct length is 2 years and 3 months consisting of 18 months of recruitment plus 9 months of follow

				up since the last patient could be recruited in the last month of recruitment period. Up to 9 months is the time from the diagnosis to receiving neoadjuvant chemotherapy and surgical resection of breast cancer. In the OID "Predicted Site Initiation Visit Date " and "Predicted Start Date for participant recruitment" date were reported incorrectly. Predicted Site Initiation Visit Date is not 11/01/2023 but 01/01/2023, and Predicted Start Date for participant recruitment is not 01/01/2023 but 02/01/2023. There was inconsistency regarding the use of data and samples in case of withdrawal reported in ICF, PIS and protocol. As specified in the ICF, data and samples acquired and analysed prior to withdrawal will be retained. PIS and protocol were corrected accordingly.
2	1.3	08.12.2022	G.Cioccoloni	In order to follow the clinical pathway adopted at the Leeds Teaching Hospital NHS Trust (LTHT), the study design needs to be modified for timing of MRI scans at T2. We previously specified that MRI scan at T2 will be done after the 4th cycle of neo adjuvant chemotherapy (NACT). However, MRI scan at T2 can coincide with the 4th or 6th cycle of NACT, so we

				changed the protocol accordingly. A change in local PI was made. Dr Sebastian Trainor will replace Mr Beak Kim as PI at LTHT. Two PhD students will be involved in this study. Miss Ibithal Barawi and Miss Aleksandra Rzeszutek will join the research team at University of Leeds site.
5	1.4	11.01.2024	G.Cioccoloni	