

A prospective non-randomised, multi-centre feasibility study to assess if patients with residual cancer following dual-targeted neoadjuvant chemotherapy treatment for HER2-positive, ER-negative early breast cancer can be identified by multiple ultrasound-guided tumour bed core biopsies

Sponsor: University of Birmingham

Protocol Version 4.0, 14th October 2024

ISRCTN Number:	**17170034**
Sponsor Number:	**RG_16-108**
CRCTU Number:	**BR3045**
IRAS Project ID Number:	**211232**



CANCER RESEARCH UK | Birmingham Cancer Research UK Clinical Trials Unit | UNIVERSITY OF BIRMINGHAM

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SIGNATURE PAGE

NOSTRA-Feasibility Study protocol Version 4.0, 14th October 2024

This protocol has been approved by:

Name:	Professor Daniel Rea	**Trial Role:**	Chief Investigator
Signature:		**Date:**	16/OCT/2024.

This protocol describes the NOSTRA-Feasibility Study and provides information about procedures for participants taking part in the NOSTRA-Feasibility Study. The protocol should not be used as a guide for treatment of participants **not** taking part in the NOSTRA-Feasibility Study

Sponsor Statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the Integrated Research Application System (IRAS) Form by the sponsor will serve as confirmation of approval of this protocol.

AMENDMENTS

Amendment Number	Date of Amendment	Protocol Version Number	Type of Amendment	Summary of Amendment
Substantial 1 (AM09)	20-Dec-2019	2.0	Substantial	Changes to allow further Trial Management Group approved chemotherapy regimens Change to allow recruitment until the end of cycle 5 of neoadjuvant chemotherapy Clarification and justification of allowed adjuvant treatments Clarification of SAE reporting procedures Change to allow MRI option instead of mammography post neoadjuvant treatment Clarification of when sites are provided with pertuzumab Clarification of when VAB may be used Changes to ctDNA Sub-Study blood tubes Add two new Case Report Forms Clarification to sample size calculation Minor corrections to typographical errors and staffing changes
Substantial 2 (AM15)	29-Jan-2021	3.0	Substantial	Inclusion of optional consent for retrospective MR image collection and recommended MRI guidelines Exclusion of multi-focal disease Clarification of exclusion: patients with previous ipsilateral breast cancer or DCIS Clinical tumour measurements no longer mandated Further clarification to study sample size calculation Further clarification of SAE reporting procedures, including additional requirement for reporting of COVID-19 cases Additional clarification on core tumour bed biopsy procedure Inclusion of option of adjuvant trastuzumab emtansine (Kadcyla) in patients with residual disease

				Inclusion of optional remote consent procedure Minor clarifications, corrections to typographical errors and staffing changes
Substantial 3 (AM23)	14-Oct-2024	4.0	Substantial	Update to background information Trial staff changes Removal of follow up appointment at 5 years and allowing remote follow-up at 12 months Removal of requirement to report positive COVID-19 cases as SAE Further details of MRI upload procedures Clarification on axillary biopsy procedure Collection of ethnicity data Change of location of tissue sample storage for future research- from University of Edinburgh to University of Birmingham Clarification of new exploratory secondary endpoints- translational Clarification of follow up after treatment discontinuation and relapse Update to wording to match newer version of protocol template Remote re-consent procedure

STUDY SUMMARY

Title:	**NOSTRA-Feasibility Study:** A prospective non-randomised multi-centre feasibility study to assess if patients with residual cancer following dual-targeted neoadjuvant chemotherapy treatment for HER2-positive, ER-negative early breast cancer can be identified by multiple ultrasound (US)-guided tumour bed core biopsies
Chief Investigator:	Professor Daniel Rea
Sponsor:	University of Birmingham
ISRCTN No.	17170034
Study Design:	A prospective non-randomised, single-arm, multicentre, feasibility study for a proposed future randomised phase III clinical trial
Primary Objectives:	To determine whether patients with residual cancer can be identified by histological examination of multiple US-guided tumour bed core biopsies following dual-targeted neoadjuvant treatment for HER2-positive, ER-negative early primary breast cancer
Secondary Objectives:	To examine concordance between local pathology reporting and central review of (i) the multiple tumour bed cores and (ii) Residual Cancer Burden (RCB) score
Translational Research Objective:	Tissue will be retained from consenting patients for future ethically approved research purposes.
ctDNA Sub-Study Objectives (optional):	• Sequential analysis of circulating levels of DNA (ctDNA) to determine predictive marker of treatment response during neoadjuvant treatment and determine if cancer remains and whether surgery is required • Analysis of matched ctDNA and tumour biopsy at diagnosis to determine predictive markers of response to neoadjuvant treatment
Primary Outcome Measures:	The observed number of patients with false negative biopsies (i.e. no tumour in the biopsy but tumour in the surgical specimen) as a proportion of all those assessed by Local Histopathology Review

Secondary Outcome Measures:	1. Concordance between Local and Central Histopathology Review of core biopsies 2. Compliance with treatment 3. Time to local recurrence 4. Time to distant recurrence 5. Overall survival 6. Re-evaluation of the primary outcome using the central pathological review determination of RCB to define false negative biopsies as RCB-0 or 1 (i.e. no tumour or minimal residual disease) in the core biopsies but RCB-2 or 3 in the surgical specimen 7. Ability of the axillary lymph node assessments post-neoadjuvant treatment to identify definitive axillary lymph node involvement determined by surgery histopathology
Translational Research Outcome Measure:	Tissue will be retained from consenting patients for future ethically approved research purposes.
ctDNA Sub-Study Outcome Measures:	1. Ability of sequential ctDNA analysis to provide early warning of the persistence of invasive cancer post-neoadjuvant chemotherapy 2. Determine whether matched ctDNA and tumour biopsy analysis at diagnosis provide predictive markers of response to neoadjuvant treatment
MRI Research Outcome Measures:	1. To assess if parameters derived from baseline and interim Magnetic Resonance Imaging (MRI) scans can differentiate between ‘‘responders’’ and “non-responders” 2. To evaluate if MRI-derived parameters from post treatment scans correlate with the multiple ultrasound-guided tumour bed core biopsies and final pathology
Patient Population:	Female patients with early invasive breast cancer that is HER2-positive and ER-negative and who are able to undergo neoadjuvant chemotherapy with dual-targeted anti-HER2 treatment and surgery
Sample Size:	150 patients
Eligibility Criteria:	**Key Inclusion Criteria:** • Patient with histological diagnosis of operable HER2-positive, ER-negative, early stage invasive breast cancer • Tumour size ≥ 1cm and visible on US* (T1c to T4d) • Patient fit and willing to receive, or is already receiving and has received no more than five cycles of a NOSTRA-Feasibility Study

approved treatment regimen†, in the opinion of the responsible clinician

- Eastern Co-operative Group (ECOG) performance status of 0 or 1
- Women of child-bearing potential, prepared to adopt highly effective contraceptive measures if sexually active in accordance to the respective drug contraception recommendation Female, aged ≥ 18 years
- Able to provide written informed consent for the study
- Availability of embedded paraffin tumour blocks from pre-chemotherapy biopsy
- The radiology team are willing to perform the tumour bed biopsies

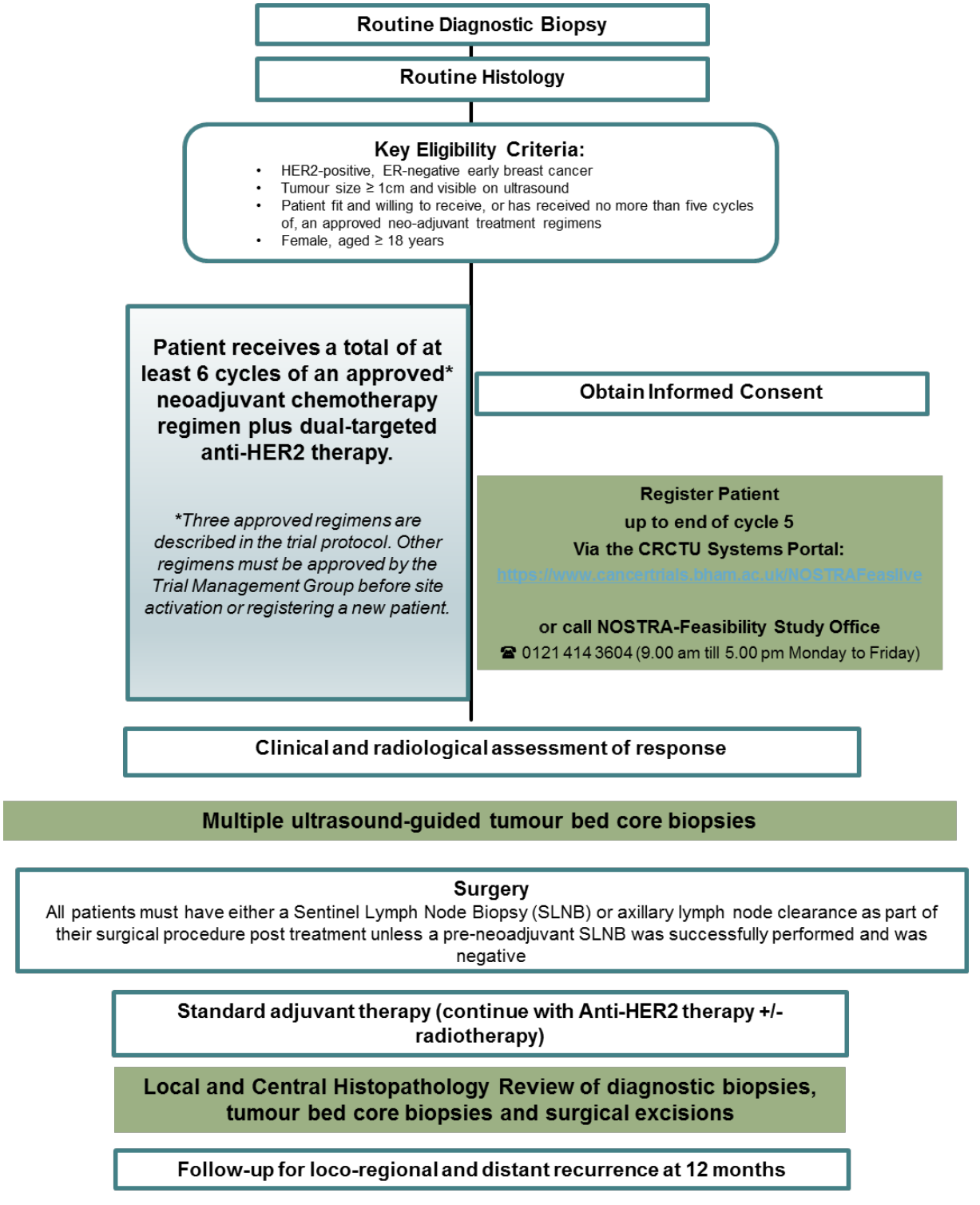
Key Exclusion Criteria:

- Previous ipsilateral breast cancer or DCIS
- Unequivocal evidence of distant metastatic disease at registration
- Multi-focal disease at diagnosis
- Active malignancy
- Previous chemotherapy
- Prior extensive radiotherapy (as judged by the Investigator) to bone marrow
- Risk factors precluding the safe administration of the intended cytotoxic chemotherapy regimen
- Patient unsuitable for the planned dual-targeted anti-HER2 treatment in opinion of the Investigator
- Prior diagnosis of cardiac failure
- Uncontrolled hypertension, coronary heart disease or other significant cardiac abnormality
- Bleeding diathesis
- Any evidence of other disease which in the opinion of the Investigator places the patient at high risk of treatment related complications
- Pregnant (patients must have a urine or blood Human Chorionic Gonadotropin test performed to rule out pregnancy prior to study entry)
- Patient lactating
- Patients who have received live vaccine within 4 weeks of the date of study entry
- Any concomitant medical or psychiatric problems which in the opinion of the Investigator would prevent completion of treatment or follow-up
- Patient unfit and/or unwilling to undergo surgery
- Patient unwilling or unable to comply with scheduled visits, treatment plan and study procedures
- Patient has started non-protocol compliant neoadjuvant chemotherapy
- Patient has started approved neoadjuvant chemotherapy but insufficient data is available to complete relevant Case Report Forms (CRFs)
- Patient has received more than five cycles of approved neoadjuvant chemotherapy

Study Treatment:	Sites may use their standard neoadjuvant regimen(s) in this study. Regimens must include at least six cycles of neoadjuvant chemotherapy, with at least three cycles of dual-targeted anti-HER2 therapy. Regimens must be anthracycline/taxane or taxane/platinum-based. The three regimens described below are examples of those approved for use in the study. If a site wishes to use a different regimen, it must be approved by the Trial Management Group (TMG) before site activation. If a site subsequently wishes to change their chosen regimen(s), then this must be approved before patient registration. **Regimen 1:** 5-Fluorouracil 500mg/m^2*, Epirubicin 100mg/m2, Cyclophosphamide 500-600mg/m^2, cycle 1-3 with Pertuzumab 840mg cycle 1 and 420mg cycles 2 and 3 and Trastuzumab 8mg/kg cycle 1 and 6mg/kg cycle 2 and 3, three weekly (3 cycles) Followed by Docetaxel with Pertuzumab and Trastuzumab: Docetaxel 75-100mg/m^2 cycles 4-6 with Pertuzumab 420mg cycle 4-6 and Trastuzumab 6mg/kg cycles 4-6, three weekly (3 cycles) **Regimen 2:** 5-Fluorouracil 500mg/m^2*, Epirubicin 100mg/m^2, Cyclophosphamide 500-600mg/m^2, three weekly (3 cycles). Followed by Docetaxel with Pertuzumab and Trastuzumab: Docetaxel 75-100mg/m^2 cycles 4-6 with Pertuzumab 840mg cycle 4 and 420mg cycles 5 and 6 and Trastuzumab 8mg/kg cycle 4 and 6mg/kg cycles 5 and 6, three weekly (3 cycles) **Regimen 3:** Docetaxel 75mg/m^2 and Carboplatin AUC 6 with Pertuzumab 840mg cycle 1 and 420mg cycles 2-6 and Trastuzumab 8mg/kg cycle 1 and 6mg/kg cycles 2-6,three weekly (6 cycles) *Omission of 5-Fluorouracil at the discretion of the treating Investigator **Adjuvant Treatment:** Anti-HER2 therapy must be continued following completion of neoadjuvant chemotherapy regimen as per local standard of care, +/- radiotherapy
Tumour Bed Core Biopsies:	Following neoadjuvant treatment, all patients will undergo multiple US-guided tumour bed core biopsies prior to surgery. Eight tumour bed core biopsies will be taken in total. A fan of paired cores will be taken working from the medial tumour margin across the tumour to the lateral edge of the original tumour bed as described below: • Two cores from the medial peripheral margin • Two cores from the central tumour bed medially • Two cores from the central tumour bed laterally • Two cores from the lateral peripheral margin

Translational Research:	Formalin fixed paraffin embedded tumour blocks will be collected for translational research. These include: • Primary diagnostic core biopsies • Post-neoadjuvant treatment tumour bed core biopsies • Representative tumour blocks from the surgical resection specimens (including axilla biopsy specimen and Sentinel Lymph Node Biopsy (SLNB) specimens)
ctDNA Sub-Study (optional):	If patients are recruited before starting neoadjuvant chemotherapy and consent to take part, blood samples will be collected for ctDNA Sub-Study at the following time points: • Prior to commencing neoadjuvant treatment (pre-cycle 1 of chemotherapy and dual-targeted anti-HER2 treatment) • Post-cycle 1 of neoadjuvant treatment • After surgery (first visit to clinic post-surgery)
MRI Research:	On completion of recruitment, any breast MRI scans from consenting patients taken during routine practice will be harvested, de-identified, sent securely to the Image Data Bank at The Cheltenham Imaging Centre, Cobalt Health Medical Charity, Cheltenham and stored to create an image library for research purposes.
Study Duration:	**Treatment period:** anticipated to be 12 months **Follow-up duration:** 1 year (follow-up at 12 months post-surgery)
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STUDY SCHEMA



SCHEDULE OF EVENTS

Assessment	Screening/ Baseline[1]	Neoadjuvant Chemotherapy & Anti-HER2 Treatment				Surgery	Adjuvant treatment	Follow-up at 12 months post surgery[22 & 23]
		Prior to Day 1 of each cycle[4]	After Cycle 3[4]	End of Cycle 5	On completion of neoadjuvant treatment			
Diagnostic biopsy	X						**Continue to complete anti-HER2 therapy as per local practice, +/- radiotherapy**	
US-visible clip insertion[2]	X							
Axillary US scan +/- FNA/biopsy	X				X[5]			
Molecular markers (ER, PgR, HER2)	X							
Informed consent & Registration[3]		X						
Medical history and ECOG performance status	X							
Left Ventricular Ejection Fraction Measurement[6]	X		X		X			
Radiological tumour assessment [7&8] Ultrasound Mammography MRI[10]	X X X		 X		X X[9] X			
Clinical laboratory assessments Pregnancy test[11] Full blood count Urea, Electrolytes, Creatinine, Liver Function Tests[12]	X X X	X X X						
Physical examination	X							
Clinical tumour measurement (caliper)[13]	X		X		X			
Vital signs[14]		X						
Sentinel lymph node biopsy[15]	X					X		
Pre-surgery protocol defined tumour bed core biopsies[16]						X		
Tumour sample collection	X[17]				X[18]	X[19]		
Blood sample collection for ctDNA Sub-Study[20]	X	X				X		
AE review		X			X[21]	X		X
Survival & relapse								X

Notes:

1. These procedures must all have occurred prior to study registration to determine eligibility, regardless of when the patient is recruited, and results/evidence must be documented in the patient's source notes for monitoring purposes
2. Patients must have an ultrasound-visible clip inserted within their first two cycles of neoadjuvant treatment
3. Patients have consented and been registered before the end of their fifth cycle of neoadjuvant treatment
4. Unless patient is recruited after cycle 3 (then complete form retrospectively from notes to document any procedures performed as per standard care)
5. Biopsy/FNA specimen required if involved nodes at diagnosis
6. Left Ventricular Ejection Fraction (LVEF) must be measured for all patients by echocardiogram (ECHO) or Multi Gated Acquisition (MUGA) scan. Modality used must be consistent throughout the study.
7. Radiological tumour measurement of the breast and assessment of ipsilateral axilla
8. Uninformative baseline radiological assessments do not have to be repeated at later time points
9. Breast mammogram may be omitted if a breast MRI has been taken at this time point.
10. MRI breast is recommended (not mandated). Post neoadjuvant treatment MRI, if used, should be undertaken before study biopsies, to avoid artefacts.
11. Pregnancy test (Human Chorionic Gonadotropin urine or blood) must be performed at baseline, for women of child-bearing potential and at risk of pregnancy must have a urine or blood Human Chorionic Gonadotropin test performed to rule out pregnancy prior to study entry)
12. Liver function tests: Alanine Aminotransferase (ALT) or Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) and Bilirubin
13. Clinical Tumour Measurement is optional as per local practice
14. Vital signs as per local practice
15. All patients must have either a SLNB or an axillary lymph node clearance as part of their surgical procedure post-neoadjuvant treatment unless a pre-neoadjuvant SLNB was successfully performed and was negative
16. Protocol defined tumour bed core biopsies should be performed prior to surgery
17. All baseline diagnostic core biopsies
18. Axilla biopsy specimen if involved nodes at diagnosis
19. Surgical excision specimens
20. Sample to be collected prior to cycle one (baseline) of neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment, post-cycle one and at first clinic appointment post-surgery. Patients are eligible if registered prior to start of neoadjuvant treatment only.
21. Adverse events to be reviewed pre-surgery (i.e. following collection of tumour bed core biopsies) and post-surgery
22. Follow-up to be performed at standard routine clinical follow-up visit or via telephone
23. Follow-up at 12 months should be from the date of surgery

ABBREVIATIONS

ABPI	Association of the British Pharmaceutical Industry
AC	Doxorubicin Cyclophosphamide
ADC	Apparent Diffusion Co-efficient
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
AST	Aspartate Transaminase
AUC	Area Under the Plasma Concentration-time Curve
C	Cyclophosphamide
Cb	Carboplatin
CI	Confidence Interval
CHI	Community Health Index
CMF	Cyclophosphamide Methotrexate 5-Fluorouracil
CRCTU	Cancer Research UK Clinical Trials Unit
CRF	Case Report Form
CT	Computerised Tomography
CTAAC	Clinical Trials Advisory & Awards Committee
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating Tumour Deoxyribonucleic Acid
DCIS	Ductal carcinoma in situ
DFS	Disease Free Survival
DW	Diffusion Weighted
DPD	Dihydropyrimidine Dehydrogenase
DCE	Dynamic Contrast Enhanced
E	Epirubicin
ECHO	Echocardiogram
ECOG	Eastern Co-operative Oncology Group
eCRF	electronic Case Report Form
EMA	European Medicines Agency
ER	Oestrogen Receptor
eRDC	electronic Remote Data Capture
F	5-Fluorouracil
FEC	5-Fluorouracil Epirubicin Cyclophosphamide
FN	False Negative
FNA	Fine Needle Aspirate
FNR	False Negative Rate
FSE	Fast Spin Echo
g/l	Gram per litre
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GP	General Practitioner
H	Trastuzumab
Hb	Haemoglobin
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hormone Receptor
IBR	Institute of Biomedical Research
IEP	Image Exchange Portal
ISF	Investigator Site File
iv	Intravenous
LVEF	Left Ventricular Ejection Fraction
LVSD	Left Ventricular Systolic Dysfunction
MDT	Multidisciplinary Team
mg	Milligram
mg/m2	Milligram per square metre
mg/kg	Milligram per kilogram
ml/min	Millilitres per minute
mm/l	Micromoles per litre
MIB	Minimally Invasive Biopsy
MRI	Magnetic Resonance Imaging
MUGA	Multi Gated Acquisition
NEQAS	National External Quality Assessment Scheme
NHS	National Health Service

NICE	National Institute for Clinical Excellence
NIHR	National Institute of Health Research
NPV	Negative Predictive Value
OS	Overall Survival
pCR	pathological Complete Response
PgR	Progesterone Receptor
Pz	Pertuzumab
RCB	Residual Cancer Burden
R&D	Research and Development
REC	Research Ethics Committee
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SABCS	San Antonio Breast Cancer Symposiums
SC	Subcutaneous
SSDL	Site Signature Delegation Log
SLNB	Sentinel Lymph Node Biopsy
SNR	Signal to Noise Ratio
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Docetaxel
TMG	Trial Management Group
TNO	Trial Number
TSC	Trial Steering Committee
TSE	Turbo Spin Echo
ULN	Upper Limit of Normal
UK	United Kingdom
US	Ultrasound
USA	United States of America
VAB	Vacuum Assisted Biopsy
VAB-MIB	Vacuum Assisted-Minimally Invasive Biopsy
vs	Versus
WBC	White Blood Cell Count
WMA	World Medical Association

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1 BACKGROUND AND RATIONALE

1.1 Background

1.1.1 Neoadjuvant chemotherapy for early breast cancer

Neoadjuvant chemotherapy for patients with operable early breast cancer is now well established. It is as effective in terms of improving survival as adjuvant chemotherapy in women with operable cancer (1, 2) with additional benefits of down-staging disease and acting as an *in vivo* assay of sensitivity to treatment. Advantages are that more patients can be managed with breast-conserving surgery and in addition patients not responding can avoid multiple further cycles of treatment with the associated toxicities. A recent consensus statement (3) has recommended that neoadjuvant chemotherapy be considered a standard approach in the management of operable breast cancer. This recommendation is based largely on the information from a meta-analysis confirming that neoadjuvant chemotherapy provides the same Disease Free Survival (DFS) and Overall Survival (OS) benefit as adjuvant chemotherapy (4). The Cochrane review in 2009 also concluded that whilst there was a slightly higher loco-regional recurrence rate there was no significant difference in DFS or OS between women with early stage breast cancer who receive neoadjuvant or adjuvant systemic treatment (5). In Human Epidermal Growth Factor Receptor 2 (HER2)-positive invasive breast cancer, the addition of trastuzumab to chemotherapy and more recently pertuzumab, have resulted in major stepwise improvements in efficacy as evidenced by the high rates of pathological Complete Response (pCR) achieved. One of the important questions to ask now in neoadjuvant treatment is whether surgery could be avoided in patients who have achieved a pCR.

1.1.2 Pathological Complete Response (pCR)

The tumour response, in particular pCR, to neoadjuvant chemotherapy has been shown to provide prognostic information regarding DFS and OS (6, 7, 8, 9, 10). Those patients with pCR following neoadjuvant chemotherapy have a superior DFS and OS (10, 11) compared to those with residual disease. pCR is used as the primary endpoint in neoadjuvant treatment trials because it is strongly correlated with good outcome. However, there are variable definitions of pCR. Some scoring systems permit residual in situ carcinoma or lymph node involvement within the definition of pCR (12). The most recent meta-analysis has shown that prognosis is not affected by the presence of in situ disease and the inclusion of both ypT0, ypN0 and ypT0/is ypN0 pathological classifications (yp indicating post-neoadjuvant pathological assessment) provides a clear prognostic advantage compared to all other post- neoadjuvant pathological outcomes (10). Graded scoring systems, such as the Residual Cancer Burden (RCB), allow a further prognostic refinement by providing a semi-quantitative estimate of the degree of response to neoadjuvant chemotherapy in predicting survival after neoadjuvant treatment (13). The quality of pathological examination and reporting is also of considerable importance (14). Central Histopathology Review is useful in research studies to standardise the quality of pathology reporting. The reporting of RCB score in routine practice is variable. Simplified versions of pCR are being evaluated as alternatives that may be more practical in the routine setting but remain prognostically useful. It is proposed that we explore the potential for simplified reporting as a possible alternative to formal RCB scoring. The rate of pCR has been shown to vary dramatically depending on tumour subtype. pCR performs poorly in oestrogen receptor (ER)-positive HER2-negative tumours as a long term surrogate and alternative forms of neoadjuvant prognostic classification are more useful (15). pCR remains the most discriminating factor for predicting outcome in triple negative and HER2-positive cancers (16, 17).

1.1.3 Increasing pCR with cytotoxic chemotherapy agents and scheduling

The first generation neoadjuvant studies used short anthracycline based regimens such as doxorubicin and cyclophosphamide (AC) with reported pCR rates of 10-15%. The addition of taxanes in a sequential fashion approximately doubles the overall pCR rates (18). Further modest increases in pCR have been achieved with changes in scheduling (19). Longer duration of chemotherapy is also associated with an increase in pCR rates with more than 4 cycles of chemotherapy consistently performing better than four or less cycles. The current standard approach to neoadjuvant chemotherapy is to use sequential taxane/anthracycline-based chemotherapy to deliver at least 6 cycles of chemotherapy.

1.1.4 Neoadjuvant HER2 targeted treatment and its impact on pCR rates

Approximately 15-20% of primary invasive breast cancers over-express HER2, usually a result of amplification of the HER2 receptor oncogene (20). The addition of neoadjuvant trastuzumab to

chemotherapy has been shown to dramatically improve pCR rates in patients with HER2-positive operable invasive breast cancer, without any significant increase in toxicity (8, 21, 22, 23). pCR is most likely in those HER2-positive patients with clinically negative lymph nodes, high tumour grade, and negative ER status (23). The TECHNO and NOAH trials have provided evidence that pCR in HER2-positive breast cancers treated with neoadjuvant anthracyclines, taxane and trastuzumab predicted improved DFS and OS (8). The NOAH trial (24) recruited 334 patients randomised to receive neoadjuvant chemotherapy either with or without trastuzumab, with a comparator group of HER2-negative patients receiving neoadjuvant chemotherapy. A recent overview has reported overall pCR rates in HER2-positive disease treated with chemotherapy and trastuzumab at 30% for ER-positive and 50% for ER-negative cases (8). In patients with HER2-positive and hormone receptor negative disease there is a high rate of pCR. At 5 years DFS is around 90% with pCR, and around 45% with no pCR (Hazard ratio (HR) 0.15 p<0.001) (10).

1.1.5 Evidence for dual HER2-receptor blockade

Trastuzumab has been followed by development of other drugs targeting the HER2-receptor. The monoclonal antibody pertuzumab acts by blocking ligand dependent HER2-3 dimerisation. Lapatinib is a small molecule tyrosine kinase inhibitor that is highly specific to HER1 and HER2 and has activity after the development of trastuzumab resistance in advanced breast cancer. In the neoadjuvant setting, dual-targeted anti-HER2 blockade with chemotherapy has produced pCR rates for both combinations; lapatinib with trastuzumab (25, 26) and pertuzumab with trastuzumab (23).

1.1.6 Pertuzumab

The addition of pertuzumab to trastuzumab and neoadjuvant chemotherapy was investigated in the NeoSphere trial (27). This randomised 417 women with early HER2-positive breast cancer to neoadjuvant treatment with docetaxel and concurrent trastuzumab, pertuzumab or trastuzumab/pertuzumab, and a combined pertuzumab/trastuzumab arm with no neoadjuvant chemotherapy. Patients treated with pertuzumab/trastuzumab/docetaxel for 12 weeks had a pCR rate of 45.8%, significantly higher than in those receiving the other treatments and without significant differences in tolerability. Subgroup analysis revealed a pCR rate of 63.2% in women with ER-negative tumours who received dual-targeted anti-HER2 treatment combined with docetaxel. These excellent results were demonstrated in a treatment group in which only 65% of women were classified as operable prior to neoadjuvant treatment.

The TRYPHAENA trial (28) randomised patients with stage II or III HER2-positive breast cancer to receive concurrent or sequential trastuzumab and pertuzumab and anthracycline-based chemotherapy or concurrent with anthracycline-free chemotherapy. In the study, 225 women with early HER2-positive breast cancer were randomised to one of three regimens:

(i) 3 cycles of FEC (5-fluorouracil, epirubicin and cyclophosphamide) followed by 3 cycles of docetaxel, both concurrent with 6 cycles of trastuzumab plus pertuzumab
(ii) 3 cycles of FEC, followed by 3 cycles of docetaxel concurrent with 3 cycles of trastuzumab plus pertuzumab
(iii) 6 cycles of concurrent docetaxel/carboplatin, pertuzumab plus trastuzumab.

In the ER-negative patients who received a taxane-containing chemotherapy regimen, pCR rates were 79.4%, 65% and 83.8% respectively across the three treatment arms (28) (see Table 1). In a single centre ‘real world’ series 60 evaluable patients with HER-2 positive treated with neoadjuvant dose dense doxorubicin and cyclophospahamide followed by weekly paclitaxel and concurrent trastuzumab and pertuzumab, overall pCR rate was 68% (HR positive 32/49, 65% HR negative 9/11, 82%) (29).

Table 1: Pathological complete responses reported in combination anti-HER2 treatment trials in HER2-positive disease from 5 representative trials.

TRIAL	Neo-ALTTO			CHER-LOB			Holmes			NeoSphere				TRYPHAENA		
Numbers Treated	149	154	152	36	39	46	14	29	23	107	96	107	107	75	72	76
Chemotherapy Regimen	Pac	Pac	Pac	Pac FEC	Pac FEC	Pac FEC	FEC Pac	FEC Pac	FEC Pac	Doc	Doc	Doc	Nil	FEC Doc	FEC Doc	Carbo Doc
Anti-HER2 Agent	T	L	T+L	T	L	T+L	T	L	T+L	T	P	T+P	T+P	T+P	#T+P	T+P
pCR %	29	25	51	42	37	47	54	45	74	29	24	46	16.	62	57	66
pCR % ER+	16	23	42	25	23	36	NA	NA	71	20	6	6.5	5.6	46	49	65
pCR% ER-	34	33	**61**	26	36	**56**	NA	NA	**78**	37	30	**63**	27	**79**	50	**84**

Pac= Paclitaxel; FEC= 5-fluorouracil/epirubicin/cyclophosphamide; Doc= docetaxel; Carbo= carboplatin; L= lapatinib; T= trastuzumab; P= pertuzumab
#Indicates only given during Docetaxel component of FEC-Doc sequence

1.1.7 Cardiac safety with anti-HER2 directed treatment in the neoadjuvant context

Anthracyclines are recognised as cardiotoxic agents but continue to be used to treat early breast cancer because they are effective. Trastuzumab is also recognised as a cardiotoxic agent; the mechanism of cardiac insult differs from anthracyclines and is both treatable and reversible (30). In the adjuvant setting, trastuzumab is associated with an average 5 fold increased risk of congestive heart failure, from 0.5% to 2.56%, and significant increase in the incidence of reduced left ventricular ejection fraction (LVEF), from 0.5% to 0.9% (31). There are now numerous neoadjuvant trials where anthracycline and trastuzumab have been combined with reassuringly low rates of cardiac complications. In summary, these reports document almost one thousand patients (965) exposed to anthracycline/trastuzumab combination treatments in the neoadjuvant setting. The overall reported incidence of symptomatic cardiac failure across these trials is less than 1%. Asymptomatic declines in LVEF are modest and not obviously worse than reported for trastuzumab and non-anthracycline based chemotherapy (27, 32) or in adjuvant studies of trastuzumab after chemotherapy (30) and appear to recover. Limited follow-up data shows no evidence of delayed cardiotoxicity. Trastuzumab now has marketing authorisation as neoadjuvant treatment in combination with doxorubicin or epirubicin.

1.1.8 Cardiac safety with combined dual-targeted anti-HER2 treatment

The primary endpoint of the TRYPHAENA trial was cardiac safety and demonstrated a low incidence of symptomatic and asymptomatic left ventricular systolic dysfunction (LVSD) across all arms. The study also reported a low incidence of symptomatic cardiotoxicity. Small mean declines in LVEF were observed across all arms but these recovered during the adjuvant phase of the trial and are similar to the declines seen in adjuvant treatment with single agent trastuzumab (28). The NeoSphere study investigated taxane chemotherapy in combination with trastuzumab and pertuzumab and also reported a good cardiac safety profile (27). Similar reassuring cardiac safety has been reported in the trials combining chemotherapy with trastuzumab and lapatinib; the Neo-ALTTO study contained multiple arms including an arm with trastuzumab, lapatinib and paclitaxel. Cardiac tolerability was very good in this study with no differences noted between any of the arms (33). The CHER-LOB study showed similar results with a reassuring cardiac safety profile with no episodes of symptomatic heart failure and no significant decline in LVEF (34).

1.1.9 Identification of patients who do not achieve a pCR and therefore may require surgery

There is no imaging modality that can robustly identify pCR and there is also conflicting data about which imaging modalities best identify residual disease post-neoadjuvant treatment. Magnetic Resonance Imaging (MRI) data is particularly conflicting (35, 36). In a study of the accuracy of MRI in the prediction

of pCR (37), overall pCR was correctly predicted in 78% of patients. In the presence of pCR, MRI was concordant in 52% of cases and MRI suggested complete response in 8.7% of cases when there was residual disease. There was a statistically significant improvement in the ability of MRI to predict the presence or absence of residual tumour in HER2-positive and triple negative tumours compared with luminal subtypes. However, this conflicts with other data (38) suggesting no relationship between tumour subtype and ability of MRI scanning to detect residual disease. The clinical relevance of the ability to detect small volume residual disease is unknown in this scenario (following breast conserving surgery clinically undetectable disease is sufficiently sterilised by radiotherapy to make the outcomes equivalent to mastectomy) but MRI specificity is not sufficient to identify pCR.

Data was published in 2015 from the first study to attempt post-treatment tumour bed core biopsies (39). The study included a heterogeneous group of patients and there was no defined tumour bed core biopsy protocol. In all, 164 patients were included in this multi-centered pooled analysis of prospectively assembled data. Core-Cut Minimally Invasive Biopsy (CC-MIB) or Vacuum-Assisted Biopsy-Minimally Invasive Biopsy (VAB-MIB) was performed after neoadjuvant chemotherapy and before surgery. Negative Predictive Values (NPV) and False-Negative Rates (FNR) to predict a pCR in surgical specimen (diagnose pCR through Minimally Invasive Biopsy (MIB)) were the main outcome measures. pCR in the surgical specimen was seen in 93 (56.7%) cases of the whole cohort. The NPV of the MIB diagnosis of pCR was 71.3% (95% CI: (63.3%; 79.3%)). The FNR was 49.3% (95% CI: (40.4%; 58.2%)). The presence of a clip marker tended to improve the NPV (odds ratio 1.98; 95% CI: (0.81; 4.85)). None of the mammographically guided VABs (n= 16) were falsely negative (FN 0%, NPV 100%).

In 2018, Kuerer et al (40) aimed to determine the accuracy of fine-needle aspiration (FNA) and vacuum-assisted core biopsy (VACB) in assessing the presence of residual cancer after neoadjuvant treatment. Their single USA centre study included 40 patients with T1-3 N0-3 triple negative or HER2 positive disease. Patients underwent image-guided FNA and VACB before surgery. Combined FNA/VACB demonstrated an accuracy of 98% (95% CI, 87%–100%), false-negative rate of 5% (95% CI, 0%–24%), and negative predictive value of 95% (95% CI, 75%–100%) in predicting residual breast cancer. In 2022 Kuerer et al (41) published their subsequent randomised multicentre trial. They evaluated radiotherapy alone, without breast surgery, in patients with early-stage triple-negative breast cancer and HER2-positive breast cancer treated with neoadjuvant chemotherapy who had pathological complete response determined by image-guided VACB. The primary outcome was biopsy-confirmed ipsilateral breast tumour recurrence rate. 50 patients underwent VACB. The VACB identified a complete pathological response in 31 patients (62% [95% CI 47.2-75.4]). They saw no serious adverse effects related to the biopsy and no ipsilateral recurrences have been seen at a median follow up of 26.4 months.

Tasoulis et al (42) in 2020 pooled data from 166 women having undergone image guided VAB (143 [86.1%]) and core-cut biopsy in London, Seoul and Houston. The false-negative rate across the whole cohort was 18.7% (95% CI, 10.6%-29.3%). Subgroup analysis of patients with a complete/partial clinical response and residual imaging abnormality of 2 cm or smaller with at least 6 VABs taken (76 [45.8%]) demonstrated a false-negative rate of 3.2% (95% CI, 0.1%-16.7%), a negative predictive value of 97.4% (95% CI, 86.5%-99.9%), and an overall accuracy of 89.5% (95% CI, 80.3%-95.3%).

The RESPONDER study (43) in 2021, evaluated the accuracy of VAB to reliably exclude residual disease after neoadjuvant treatment. Twenty-one sites in Germany took part in the study, recruiting 398 female patients with all sub-types of breast cancer. Uni- and multivariable logistic regressions were performed to identify patient and VAB characteristics associated with false-negative cases. In the multivariable analysis, a false-negative VAB result was significantly associated with accompanying ductal carcinoma in situ (DCIS) in the initial diagnostic biopsy [odds ratio (OR), 3.94; p=0.001], multicentric disease on imaging before NST (OR, 2.74; p = 0.066), and age (OR, 1.03; p = 0.034). Exclusion of women with DCIS or multicentric disease (n = 114) and classification of VABs that did not remove the clip marker as uncertain representative VABs decreased the FNR to 2.9% (3/104).

For this initial feasibility study only patients with tumours that are visible on ultrasound (US) are eligible, to ensure accurate diagnostic biopsy, placement of tumour bed clips, assessment of response and multiple tumour bed core biopsies following neoadjuvant treatment all under US guidance. This feasibility study will inform the accuracy of multiple US directed tumour bed core biopsies at detecting residual cancer as well as a comparison with the amount of residual disease, as assessed by RCB. The main phase III trial will answer whether the ability to detect a small residual cancer burden is of any

clinical relevance.

1.1.10 Outcomes after neoadjuvant chemotherapy where surgery was omitted

Although there is no data available from patients who have not had surgery following targeted treatments known to produce a high pCR rate, there is data from patients who had chemotherapy in a previous era which produced much lower pCR rates, in the region of 15%. In the largest series of 453 patients identified for neoadjuvant chemotherapy between 1986 and 1999, 136 (30%) had a complete clinical response, 67 underwent surgery as their primary loco-regional treatment modality, and 69 had no surgery but had radiotherapy alone. There were no significant differences in DFS between patients who had surgery and those who had radiotherapy alone (64% vs 61% at 5 years p= 0.7). Similarly, there were no differences in distant DFS between the two groups (70% vs 75% at 5 years; p= 0.6). Overall survival at 5 years was 74% in those undergoing surgery and 76% in those without surgery. These results were maintained at 10 year follow-up (60% vs 70%; p= 0.9). In patients who had surgery after chemotherapy, the extent of surgery (mastectomy vs conservative surgery) did not affect DFS or OS (44). No significant differences were seen in rates of loco-regional recurrences, but there was a trend toward higher recurrence rates for the no surgery group than for the surgery group (loco-regional only recurrence rate, 21% vs 10% at 5 years; p= 0.09). Similar findings have been reported in a French study (45). This limited data from previous studies is reassuring in showing no survival detriment to avoiding immediate surgery, suggesting that despite the increased incidence of local relapse, salvage surgery is effective in controlling disease.

1.1.11 Adjuvant targeted treatment

Several studies have demonstrated the utility of 18 cycles (approximately one year) of adjuvant treatment with trastuzumab (30, 46, 47) as gold standard care. The value of additional anti-HER2 agents has produced mixed results. The ALTTO study comparing adjuvant lapatinib or trastuzumab with the combination of both trastuzumab and lapatinib showed no statistically significant additional benefit at 4.5 years median follow-up for DFS from lapatinib added to trastuzumab and chemotherapy (48). The TEACH study has provided some evidence that lapatinib monotherapy has adjuvant activity in early HER2-positive disease in the absence of trastuzumab, the impact was marginal and of uncertain clinical relevance in the adjuvant Trastuzumab era (49). The ExteNET study has recently reported positive preliminary results from the addition of adjuvant neratinib after completion of adjuvant trastuzumab however longer follow-up is recommended to confirm long lasting benefit (50). The APHINITY study completed recruitment in August 2013 of 4,800 patients to a comparison of adjuvant trastuzumab with combined trastuzumab and pertuzumab in combination with adjuvant chemotherapy. Results from this study are now published and demonstrate a further improvement in DFS with the addition of adjuvant pertuzumab to trastuzumab reducing invasive recurrence by approximately 20% (51). The absolute benefits in the trial were modest but in node positive cases the reduction in invasive recurrence at 3 years is 2% and as a result adjuvant pertuzumab has been recommended by NICE (52) for high risk HER-2 positive breast cancer. It is anticipated that many patients recruited to the NOSTRA-Feasibility study will meet the criteria for adjuvant pertuzumab and where this is funded and meets local treatment guidelines treatment with adjuvant pertuzumab is recommended. Results from trials of reduced duration of trastuzumab are controversial. Non inferiority of a six month course of trastuzumab compared to 12 months is reported in the PERSEPHONE trial (53), but paradoxically the PHARE trial (54) with a similar design was unable to demonstrate non inferiority using a more stringent definition of non-inferiority. A patient level meta-analysis is planned and may help identify where de-escalation of trastuzumab monotherapy is appropriate. The use of pCR to determine the indication for attenuated adjuvant anti HER-2 therapy has not been directly evaluated in clinical trials.

Trastuzumab emtansine (Kadcyla) has recently been approved for use as adjuvant therapy where residual disease is present at surgery despite neoadjuvant trastuzumab, pertuzumab and taxane-containing chemotherapy. This is now available in many hospitals since the publication of the UK Technology appraisal guidance [TA632] (55) in June 2020.

The use of trastuzumab emtansine is recommended in NOSTRA-Feasibility patients as follow on adjuvant therapy as an alternative to continued trastuzumab and pertuzumab, where access to this agent is available, where residual disease is present at surgery, or is present in the end of treatment core biopsy, as per NICE guidance (55).

1.1.12 Conclusion

The exceptionally high pCR rates recently reported in HER2-positive, ER-negative early invasive breast cancer with the neoadjuvant combination of chemotherapy, trastuzumab and pertuzumab makes this an ideal setting to launch a feasibility study addressing the question of whether surgery is necessary in patients who have achieved a pCR to neoadjuvant treatment. The current evidence suggests a feasibility study is now timely to address the need for surgery in this specific group of patients who are treated with dual-targeted neoadjuvant treatment. Calls for this type of approach have been made by leading, senior researchers at several recent international cancer conferences (Gabriel Hortobagyi, San Antonio Breast Cancer Symposium 2012; Alastair Thompson, European Society for Medical Oncology 2012;).

The NOSTRA-Feasibility Study is the only study currently evaluating using a protocol-driven fan of core biopsies across the entire tumour bed amongst patients with early invasive breast cancer. It is the only study that specifically targets HER-2 positive ER negative disease treated with maximally effective neoadjuvant therapy.

1.2 Study Rationale

1.2.1 Justification of patient population

Approximately 15% of early invasive breast cancer patients in the UK have HER2-positive tumours (National ICC NEQAS audit) (http://www.ukneqasiccish.org). Within the neoadjuvant population HER2-positive cases split roughly 50:50 between ER-positive and ER-negative disease; thus an estimated 6-7.5% of all operable breast cancer patients have tumours with the HER2-positive, ER-negative phenotype. In the UK, this is approximately 3500 patients per year. At least half of these would be candidates for a dual-targeted (a combination of two anti-HER2 therapies plus chemotherapy) neoadjuvant approach, which results in consistently high pCR rates, as reported in multiple studies and in the region of 80%, which are linked to good outcome. These patients are thus an appropriate group in which to initially explore omitting surgery.

This feasibility study requires recruitment of 150 patients to ascertain that in this patient group the ability to detect residual disease by post-neoadjuvant treatment US-guided tumour bed core biopsies is sufficiently accurate to inform a randomised phase III trial design. If the results of this feasibility study are successful, a proposed phase III trial will test the hypothesis that surgery following an effective targeted treatment and chemotherapy combination is without benefit to the patient and is an unnecessary 'adjuvant treatment' for which there is no indication and can safely be omitted.

1.2.2 Justification for design

The NOSTRA-Feasibility Study is a single-arm study designed as a prerequisite to the conduct of a randomised phase III trial which will determine if it is safe to omit surgery after the planned neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment. It has been established from extensive consultation, that before this phase III trial can commence the clinical research community and patients require a demonstration that in the proposed trial population (ER-negative HER2-positive patients suitable for neoadjuvant chemotherapy) examination of tumour bed core biopsies will identify the majority of women who harbour residual invasive cancer following completion of neoadjuvant treatment such that the probability of women in this trial harbouring residual disease and being randomised to no surgery is 5% or less. This feasibility study is restricted to patients matching the entry criteria for the proposed phase III trial and will determine if a protocol defined tumour bed core biopsy procedure can identify residual cancer with sufficient accuracy that the above conditions can be achieved and the phase III trial may be conducted with sufficient confidence that patient safety will not be compromised.

The NOSTRA-Feasibility Study also has an important translational research component, which involves the collection of diagnostic core biopsies, surgical excision specimens (including axilla biopsy specimens and Sentinel Lymph Node Biopsy (SLNB) specimens) and protocol defined tumour bed core biopsies. The translational research component is regarded as an essential part of the study with the primary aim to conduct exploratory analysis of tumour samples to identify novel biomarkers thus refining the ability to predict pCR. The collection of blood samples will assess the ability of sequential circulating tumour DNA (ctDNA) analysis to provide early warning of the persistence of invasive cancer post-neoadjuvant treatment and determine whether matched ctDNA and tumour biopsy analysis at diagnosis provide predictive markers of response to neoadjuvant treatment.

1.2.3 Approved treatment regimens

Sites may use their standard neoadjuvant regimen(s) in this study. Regimens must include at least six cycles of neoadjuvant chemotherapy, with at least three cycles of dual-targeted anti-HER2 therapy. Regimens must be anthracycline/taxane or taxane/platinum-based.

The three example regimens described in this protocol are approved for use in the study. If a site wishes to use a different regimen, it must be approved by the Trial Management Group (TMG) before site activation. If a site subsequently wishes to change their chosen regimen(s), then this must be approved before patient registration.

The three approved regimens provide Investigators with three distinct treatment options. A regimen with exposure to concurrent pertuzumab and trastuzumab alongside both anthracycline and taxane based chemotherapy. An anthracycline free chemotherapy backbone of docetaxel and carboplatin with concurrent trastuzumab and pertuzumab and an option to use an anthracycline but without concurrent anti-HER2 treatment, reserving the dual-targeted anti-HER2 treatment for the taxane component of a block sequential regimen. There is no clear consensus among Investigators regarding the optimal schedule. Each of these regimens have potential advantages and disadvantages with respect to toxicity profile (Table 2) and potential efficacy. The availability of all three schedules permits wider acceptability of the study to Investigators and may provide further clarification on differences in activity and toxicity.

Table 2: Adverse events (grade 3 & 4) in the TRYPHAENA trial.

	Treatment regimen		
	FEC+H+P x3→T+H+P x3	FEC x3 →T+H+P x3	TCH+P x6
Adverse event, n (%)	n = 72	n = 75	n = 76
Neutropenia	34 (47.2)	32 (42.7)	35 (46.1)
Febrile neutropenia	13 (18.1)	7 (9.3)	13 (17.1)
Leukopenia	14 (19.4)	9 (12.0)	9 (11.8)
Diarrhoea	3 (4.2)	4 (5.3)	9 (11.8)
Anaemia	1 (1.4)	2 (2.7)	13 (17.1)
Thrombocytopenia	0 (0.0)	0 (0.0)	9 (11.8)
Vomiting	0 (0.0)	2 (2.7)	4 (5.3)
Fatigue	0 (0.0)	0 (0.0)	3 (3.9)
Alanine aminotransferase	0 (0.0)	0 (0.0)	3 (3.9)
Drug hypersensitivity	2 (2.8)	0 (0.0)	2 (2.6)
FEC= Fluorouracil/Epirubicin/Cyclophosphamide; H = Trastuzumab; P= Pertuzumab; T = Docetaxel; TCH = Docetaxel/ Carboplatin/Trastuzumab			

1.2.4 Tumour bed core biopsies

Currently clinical and radiological response is insufficiently sensitive to determine pCR after neoadjuvant treatment with both false-negative and false-positive prediction. Pilot work from several groups have explored the technique of post-treatment tumour bed core biopsies. This is a technique that can be performed under US scan guidance.

Preliminary studies have demonstrated that these techniques are capable of identifying residual cancer in majority of cases. The accuracy of the test applied to a cohort where pCR is already very high has not yet been systematically studied. This study will provide definitive information regarding the accuracy of this approach and will generate data to determine if a randomised study of no surgery after a negative tumour bed core biopsy to radiotherapy alone vs surgery and radiotherapy can be safely conducted.

We have conducted pilot work in a single centre to inform the development of the sampling technique described in this protocol(56). A randomised study of tumour bed core biopsies in combination with radiological exceptional response is opening in the USA. This approach will however not include cases where pCR has been achieved but clinical and radiological abnormalities have not fully resolved this situation is frequently encountered. This study will explore the potential advantages and disadvantages of incorporating radiological features in addition to the tumour bed core biopsy information as an entry criteria for a randomised study.

2 AIMS, OBJECTIVES AND OUTCOME MEASURES

2.1 Aims and Objectives

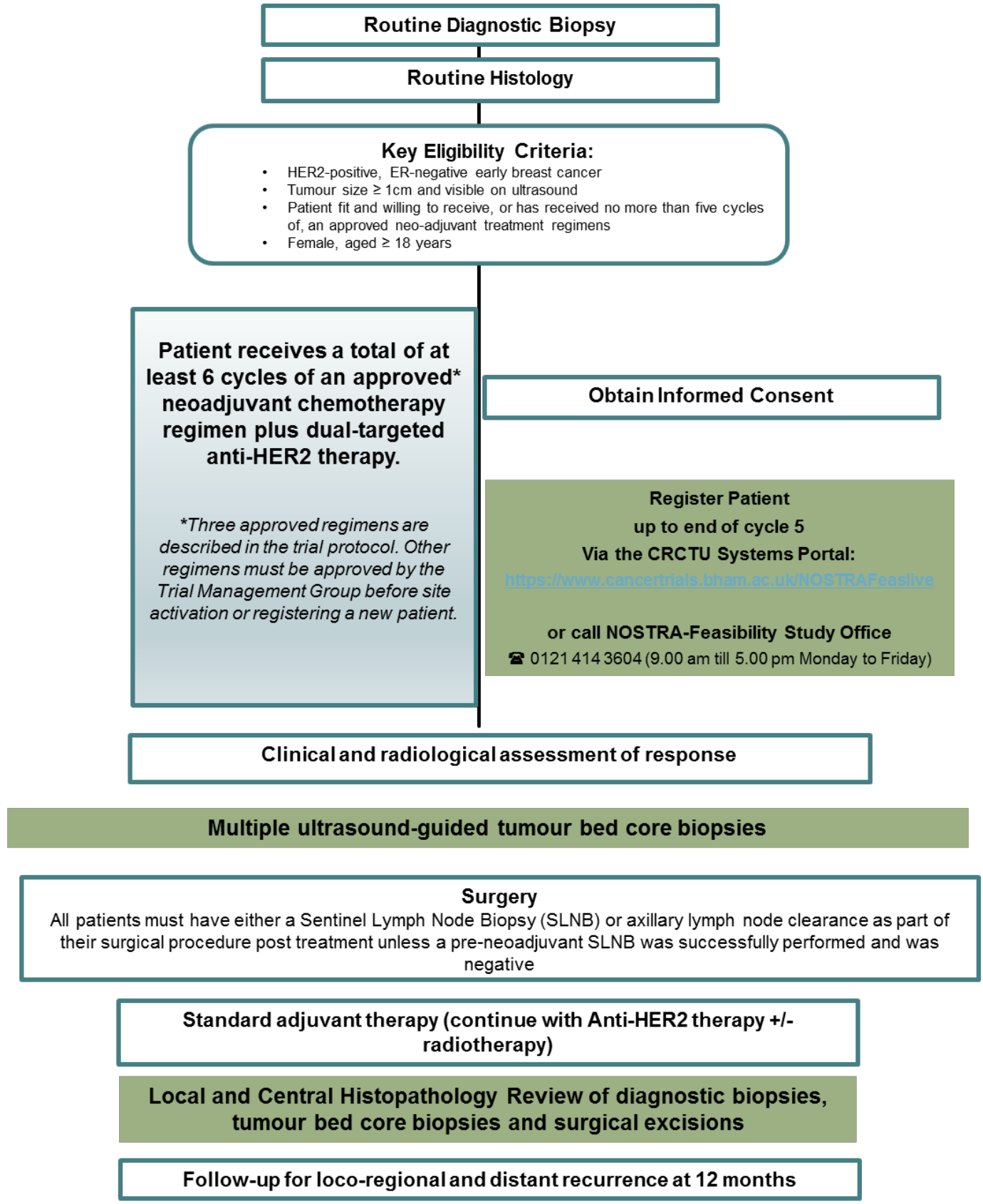
Primary Objectives:	To determine whether patients with residual cancer can be identified by histological examination of multiple US guided tumour bed core biopsies following dual-targeted neoadjuvant treatment for HER2-positive, ER-negative early primary breast cancer.
Secondary Objectives:	To examine the concordance between local pathology reporting and Central Histopathology Review of (i) the multiple pre-surgical cores and (ii) RCB score. During this feasibility stage, the study histopathologists will centrally review all tumour bed core biopsies (blinded to the pathology at resection) to assess the presence and amount of residual disease. Concordance between the Central Histopathology Review (as RCB score) and the locally reported histopathology of the excisional specimen will also be assessed and the outcome of this will inform both the protocol and the need for the Central Histopathology Review in a proposed future randomised trial where patients with negative tumour bed core biopsies may undergo radiotherapy alone.
Translational Research Objective:	Tissue will be retained from consenting patients for future ethically approved research purposes.
ctDNA Sub-Study Objectives (optional):	• Sequential analysis of ctDNA to determine predictive marker of treatment response during neoadjuvant treatment, determine if cancer remains and whether surgery is required. • Analysis of matched ctDNA and tumour biopsy at diagnosis to determine predictive markers of response to neoadjuvant treatment.
MRI Research Objectives (optional):	• To assess if parameters derived from baseline and interim Magnetic Resonance Imaging (MRI) scans can differentiate between ‘‘responders’’ and “non-responders”. • To evaluate if MRI-derived parameters from post treatment scans correlate with the multiple ultrasound-guided tumour bed core biopsies and final pathology.

2.2 Outcome Measures

Primary Outcome Measure:	The observed number of patients with false negative biopsies (no tumour in the core biopsies but tumour in the surgical specimen) as a proportion of all those assessed.
Secondary Outcome Measures:	1. Concordance between local and central histopathology reporting of pCR 2. Compliance with neoadjuvant and adjuvant treatment 3. Time to local recurrence 4. Time to distant recurrence 5. Overall survival 6. Re-evaluation of the primary outcome using the Central Histopathological Review determination of RCB to define false negative biopsies as RCB-0 or 1 (no tumour or minimal residual disease) in the core biopsies but RCB 2 or 3 in the surgical specimen 7. Ability of the axillary lymph node assessments post-neoadjuvant treatment to identify definitive axillary lymph node involvement determined by surgery histopathology 8.
Translational and exploratory Research Outcome Measure:	1. Evaluation of primary outcome using alternative simplified methods of assessment of tumour response that approximate to RCB score.
ctDNA Sub-Study Outcome Measures:	1. Ability of sequential ctDNA analysis to identify existence of residual invasive cancer post-neoadjuvant treatment. 2. Determine whether matched ctDNA and tumour biopsy analysis at diagnosis provide predictive markers of response to neoadjuvant treatment.

3 STUDY DESIGN

A prospective multi-centre, non-randomised, single-arm feasibility study to inform a future phase III randomised trial. Female patients with early invasive breast cancer that is HER2-positive and ER-negative will be offered an approved neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment regimen followed by surgery. Patients will be recruited before commencing, or up to the end of their fifth cycle of, neoadjuvant treatment. The treatment period is anticipated to be 12 months for those registering with the study prior to starting neoadjuvant treatment, with follow-up at 12 months after surgery.


Routine Diagnostic Biopsy
Routine Histology
Key Eligibility Criteria:
HER2-positive, ER-negative early breast cancer
Tumour size ≥ 1cm and visible on ultrasound
Patient fit and willing to receive, or has received no more than five cycles of, an approved neo-adjuvant treatment regimens
Female, aged ≥ 18 years
Patient receives a total of at least 6 cycles of an approved* neoadjuvant chemotherapy regimen plus dual-targeted anti-HER2 therapy.
*Three approved regimens are described in the trial protocol. Other regimens must be approved by the Trial Management Group before site activation or registering a new patient.
Obtain Informed Consent
Register Patient
up to end of cycle 5
Via the CRCTU Systems Portal:
https://www.cancertrials.bham.ac.uk/NOSTRAFeaslive
or call NOSTRA-Feasibility Study Office
☎ 0121 414 3604 (9.00 am till 5.00 pm Monday to Friday)
Clinical and radiological assessment of response
Multiple ultrasound-guided tumour bed core biopsies
Surgery
All patients must have either a Sentinel Lymph Node Biopsy (SLNB) or axillary lymph node clearance as part of their surgical procedure post treatment unless a pre-neoadjuvant SLNB was successfully performed and was negative
Standard adjuvant therapy (continue with Anti-HER2 therapy +/- radiotherapy)
Local and Central Histopathology Review of diagnostic biopsies, tumour bed core biopsies and surgical excisions
Follow-up for loco-regional and distant recurrence at 12 months

4 ELIGIBILITY

Patients meeting the criteria below are eligible to participate in the study. Eligibility must be confirmed by an Investigator (defined as the individual who has the responsibility for the medical care of the patient within the NHS i.e., typically a consultant level doctor). Confirmation of eligibility at multi-disciplinary meetings is satisfactory.

4.1 Inclusion Criteria

1) Patient with histological diagnosis of operable, HER2-positive*, ER-negative**, early stage invasive breast cancer
2) Tumour size ≥1cm and visible on US (T1c to T4d)***
3) Patient fit and willing to receive a NOSTRA-Feasibility Study approved treatment regimen in the opinion of the responsible clinician:
- Patients must not have clinically significant cardiac abnormalities. Cardiac function should be assessed by physical examination and baseline measurement MUST be made of LVEF by Multi Gated Acquisition (MUGA) scan or echocardiogram (ECHO). LVEF must be 55% or greater and above the lower limit of normal range as defined locally by the treating centre (usually at least 50%)
- Patients must have adequate bone marrow, hepatic and renal function****

Or
Patient already started approved chemotherapy, has received five or fewer cycles, and data is available to complete all relevant Case Report Forms (CRFs) *****.

4) Eastern Co-operative Oncology Group (ECOG) performance status of 0 or 1 (see Appendix1)
5) Women of child-bearing potential, prepared to adopt highly effective contraceptive measures if sexually active in accordance with the respective drug contraception recommendation
6) 18 years or older
7) Female
8) Able to provide written informed consent for the study
9) Availability of embedded paraffin tumour blocks from pre-chemotherapy biopsy
10) The radiology team are able and willing to perform the tumour bed core biopsies

*HER2-positive tumours are defined as those with 3+ reactivity on immunohistochemistry, or 2+ scoring with HER2 gene amplification by *in situ* hybridisation techniques, as per UK national guidelines (57)
** ER-negative tumours are those in which less than 1% of tumour cells show any nuclear reactivity. All laboratories undertaking ER and HER2 assessment must participate successfully in appropriate external quality assurance scheme, such as United Kingdom (UK) National External Quality Assessment Scheme (NEQAS)
*** TNM Staging System for Breast Cancer (see Appendix 2)
**** **Defined as: Considered adequate for safe administration of selected treatment by treating Clinician**
For guidance the following pararmeters normally indicate adequate organ function and will be considered as evidence of adequate organ function. Patients with parameters outside the following ranges but still considered fit for treatment should be discussed with the Chief Investigator and the outcome of the discussion should be documented.

Hepatic function:
Alanine Aminotransferase (ALT) or Aspartate Aminotransferase (AST) ≤1.5 x Upper Limit Normal (ULN)
Alkaline phosphatase (ALP) ≤2 x ULN
Bilirubin within normal range. If AST/ALT and ALP are within normal limits then isolated elevation of bilirubin to ≤3 ULN and a presumptive diagnosis of Gilbert's syndrome is permitted

Renal function:
1. Creatinine ≤1.5 x ULN
2. Glomerular filtration rate (GFR) must be >50 ml/min estimated using Cockroft-Gault formula

Bone marrow function:
1. Haemoglobin (Hb) >100g/L
2. White Blood Cell (WBC) >3 × 10^9/L
3. Platelets >100 x 10^9/L

***** Missing data items on the Baseline Form may be acceptable, but sites must discuss with the NOSTRA-Feasibility Study team prior to registering a patient.

4.2 Exclusion Criteria

1) Previous ipsilateral invasive breast cancer or Ductal Carcinoma in Situ (DCIS)
2) Unequivocal evidence of distant metastatic disease at registration
3) Multi-focal disease at diagnosis
4) Active malignancy
5) Previous chemotherapy
6) Prior extensive radiotherapy (as judged by the Investigator) to bone marrow
7) Risk factors precluding the safe administration of the intended cytotoxic chemotherapy regimen:
LVEF below institutional lower limit of normal and or echocardiographic evidence of haemodynamically significant valvular heart disease or impaired ventricular contractility
8) Patient unsuitable for the planned dual-targeted anti-HER2 treatment in opinion of the Investigator
9) Prior diagnosis of cardiac failure
10) Uncontrolled hypertension, coronary heart disease or other significant cardiac abnormality
11) Bleeding diathesis
12) Any evidence of other disease which in the opinion of the Investigator places the patient at high risk of treatment related complications
13) Pregnant
14) Patient lactating
15) Patients who have received live vaccine within 4 weeks of the date of study entry
16) Any concomitant medical or psychiatric problems which in the opinion of the Investigator would prevent completion of treatment or follow-up
17) Patient unfit and/or unwilling to undergo surgery
18) Patient unwilling or unable to comply with scheduled visits, treatment plan and study procedures
19) Patient has started protocol non-compliant neoadjuvant chemotherapy
20) Patient has started approved neoadjuvant chemotherapy but insufficient data is available to complete relevant CRFs
21) Patient has already received more than five cycles of approved neoadjuvant chemotherapy

4.3 Additional Inclusion Criteria for ctDNA Sub-Study

1. Patient has <u>not</u> yet started neoadjuvant treatment.
2. Patient is willing and able to give blood samples as per the ctDNA Sub-Study Guidelines.

5 SCREENING AND CONSENT

Potential patients will be identified at Multidisciplinary Team (MDT) meetings in participating hospitals. Potential patients will be approached about their willingness to participate in the study by the Investigator and/or Research Nurse and will be given information about the study.

5.1 Patient Pathway

Please see Flowchart 1 for the recommended patient pathway to registration. Sites should review the applicability of this pathway to local circumstances and may adapt as appropriate to fit to locality practices.

5.2 Screening

Investigators are expected to maintain a Screening/Enrolment Log of all HER2+ and ER-ve potential patients considered for the study. This Log will include limited information about the potential participant (e.g., year of birth) and the date and outcome of the screening process (e.g., enrolled into trial, reason for ineligibility or refused to participate). The reason for screening failures should be captured on the Patient Screening/Enrolment Log which will be reviewed by the NOSTRA-Feasibility Study Office in order to monitor recruitment and identify issues with eligibility. An Eligibility Checklist must be completed for each patient. Baseline pathology and screening data will be captured on a Baseline Form while radiological data will be collected on a Radiology Assessment Form.

For patients who appear to meet all of the criteria for participation in the study, the Investigator will provide the potential participant with the current approved Participant Information Sheet to allow them to make an informed decision regarding their participation. If informed consent is given (see section 5.3), the Investigator will conduct a full screening evaluation to ensure that the patient satisfies all inclusion and exclusion criteria (note, participants must meet the all the eligibility criteria at the time they are entered into the study, see section 6). This evaluation should be documented in the medical records. A patient who gives written informed consent and who satisfies all the inclusion and exclusion criteria may be entered into the study (see section 6). Note that assessments conducted as standard of care do not require trial specific informed consent and may be provided as screening data if conducted within the stipulated time period prior to trial entry. Assessments required at screening are listed in the flowchart of assessments and detailed below.

Screening procedures detailed below must be undertaken to confirm eligibility before the patient is registered into the study, regardless of when in the patient's course of treatment they are recruited.

If the patient has already commenced neoadjuvant treatment at registration, then the procedures are likely to have already taken place. All results and/or evidence must be available in the source documentation for verification of eligibility, completion of the baseline Case Report Form and for monitoring purposes. In this situation there is no need to repeat these procedures prior to study registration. If any screening procedures did not take place prior to neoadjuvant treatment, for example pregnancy test (if applicable/ appropriate), then these must take place prior to registration. Please discuss with the NOSTRA team prior to registering the patient if in any doubt.

- Diagnostic biopsy and US-guided visible marker clip insertion
- Medical history
- Full physical examination including height and weight
- ER, Progesterone Receptor (PgR) and HER2 status
- Radiological measurement of primary breast tumour(s) by standard practice MRI (optional), US scan and mammogram
    - US scan should include assessment of axillary lymph node involvement and abnormal nodes should be biopsied as detailed below. It is strongly advised to repeat any baseline radiological measurements that have been performed more than 6 weeks prior to study entry as close to (but before) cycle 1 as possible
- Fine Needle Aspirate (FNA) or core biopsy of clinically or radiologically enlarged/abnormal axillary nodes is required to define involvement
- Clinical tumour measurements (usually by caliper) are optional according to local practice

- TNM staging to exclude metastatic disease in accordance with standard early breast cancer practice according to local site policy. However we recommend cross sectional imaging e.g. computerised tomography (CT) scan of the chest and abdomen/pelvis for patients with a significant risk of metastatic disease. Where staging is indicated this should be performed before study entry if possible
- LVEF measured by ECHO or MUGA within 12 weeks (provided there has been no documented cardiac event in which case the patient should be re-evaluated) prior to study entry
- ECOG performance status
- SLNB at clinician's discretion and only if no abnormal lymph nodes detected on US scan
- Biochemical screen to include:
    - ALT or AST
    - ALP
    - Bilirubin
    - Urea
    - Creatinine
    - Sodium
    - Potassium
    - Estimated GFR
- Pregnancy test for
- Routine full blood count to include:
    - Haemoglobin level
    - WBC count including differential count
    - Platelet count

Patients with abnormal blood test results are normally ineligible. The Chief Investigator should be contacted to discuss specific cases where identifiable reasons for abnormal test results can be demonstrated which will not influence the safe administration of study treatment.

For patients with an abnormal blood test result, or clinically involved axillary nodes, full staging is recommended and this should be performed prior to registration.

5.3 Informed Consent

It is the responsibility of the Investigator to obtain written informed consent for each patient prior to performing any study related procedures. Therefore, any patients recruited during neoadjuvant chemotherapy must receive all treatment up to that point as per standard care. The Investigator may delegate the task of obtaining informed consent to an appropriately qualified Research Nurse or Specialist Registrar trained in obtaining informed consent and in Good Clinical Practice (GCP). However, assessing patient suitability and the final confirmation of the patient eligibility is the responsibility of the Investigator. This delegation of responsibilities must be clearly documented on the Site Signature Delegation Log (SSDL). To facilitate the informed consent process, a Patient Information Sheet is provided to the patient. Investigators must ensure that they adequately explain the aim, study treatment, anticipated benefits and potential hazards of taking part in the study to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the study at any time, without compromising their care. The patient should be given ample time (e.g. 24 hours) to read the Patient Information Sheet and to discuss their participation with others outside of the site research team. The patient must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the study without giving a reason must be respected.

If the patient expresses an interest in participating in the study they should be asked to sign and date the latest version of the Informed Consent Form. Patients may take part in the main study but opt out of additional blood samples for the ctDNA Sub-Study (if applicable), for their tissue samples to be retained for future research and/or their MRI scans to be used for research purposes. The Investigator must then sign and date the Informed Consent Form on the same day. A copy of the Informed Consent Form should be given to the patient, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into the study, the patient's unique Trial Number (TNO) should be entered on the Informed Consent Form maintained in the ISF. In addition, if the patient has given explicit consent a copy of the signed Informed Consent Form must be sent in the post to the NOSTRA-Feasibility Study Office for review.

The informed consent process is expected to involve an interview between the investigator team and the patient which should facilitate two-way communication. In exceptional circumstances, it is acceptable for patients to provide consent to participate remotely. For example, the PIS and ICF could be posted to the patient and the initial discussion could be held by telephone/video call, with patient consent documented on the ICF at the time. In this instance, confirmation of the participant's identity must first be sought. The Informed Consent Form should be wet ink signed by the patient and the patient should be instructed to bring the signed ICF to their next appointment to allow the form to be signed by the Investigator, but this must be prior to their entry onto the trial and clearly documented in the patient's notes.

Details of the informed consent discussions should be recorded in the patient's medical notes. This should include date of, and information regarding, the initial discussion, the date consent was given, whether this was given remotely, with the name of the study and the version number of the Patient Information Sheet and Informed Consent Form.

Throughout the study the patient should have the opportunity to ask questions about the study and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient, in which case the process above should be followed and the patient's right to withdraw from the study respected. Participants are permitted to re-consent at the same visit that new information is provided if they wish to do so. Details of these discussions (as specified above) should also be recorded in the participant's medical records. Alternatively, it may be more appropriate for patients to provide re-consent remotely. In that instance, the following procedure should be followed:

- An appropriate member of the clinical research team remotely (e.g. by telephone or videoconference) explains the new information and need for re-consent to the patient
- The new PIS and ICF or Addendum to the previous PIS is posted to the patient with a covering letter using the ethically approved template
- A reminder letter using the ethically approved template may also be posted if required
- The patient signs the form at home and returns by post to the research team
- A member of the research team delegated consent cross-checks the patient's signature with their original consent form and counter signs
- A fully signed copy is returned by post to the patient and Study Office
- The process is clearly documented in the patient's notes

Electronic copies of the Patient Information Sheet and Informed Consent Form are available from the NOSTRA-Feasibility Study Office and should be printed or photocopied onto the headed paper of the local institution.

Details of all patients approached about the study should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent their General Practitioner (GP) should also be informed that they are taking part in the study. A GP Letter is provided electronically for this purpose. The Patient Identification Log should be completed as a record of which patients are taking part in the trial.

Flowchart 1: Recommended Patient Pathway



Keys:

Trial Pathway

Mandated

Local MDT option

For patients registered before starting neoadjuvant treatment and who consented to ctDNA Sub-Study

*MRI as per local protocol is recommended but not mandated

6 STUDY ENTRY

6.1 Time Frame for Study Entry

Ideally patients should, where possible, be entered into the study before starting neoadjuvant chemotherapy. However, patients may be approached within their first five cycles of an approved neoadjuvant chemotherapy regimen provided that they have already undergone the required baseline assessments and this data is available.

6.2 Registration

Patients should be registered into the study once eligibility is confirmed through screening assessments. Written Informed Consent must be obtained prior to registration. In order to register a patient, both the Eligibility Checklist and the Registration Form must be completed.

Patient registration should be performed by sites using the NOSTRA-Feasibility electronic Remote Data Capture (eRDC) system which has been developed by CRCTU, University of Birmingham or by telephone or email. The registration telephone line will be open during office hours (9:00am to 5:00pm, Monday to Friday). Investigators must be registered with the NOSTRA-Feasibility Study Office before they are permitted to register patients into the study (see section 15.1 Site Set-up and Initiation).

The following information must be provided:

- Name of site and responsible Investigator
- Patient's initials
- Date of birth
- Patient's hospital number
- Date of informed consent
- Intended treatment regimen option at the discretion of the treating clinician

eRDC System: https://www.cancertrials.bham.ac.uk/NOSTRAFeasLive

Enquires: ☎ 0121 414 3604 or email NOSTRA@trials.bham.ac.uk
(9.00 am till 5.00 pm, Monday to Friday)

At the end of the registration process patients will be assigned a unique TNO. The TNO will be used to identify the patient and should be recorded on the CRFs and on any further correspondence with the NOSTRA-Feasibility Study Office. The TNO should also be documented on the original signed Informed Consent Form filed in the ISF.

On completion of the registration process, an email to key site personnel (including the Investigator, the person performing the registration and responsible pharmacist) will then be sent confirming the details of registration, and containing a link to the NOSTRA-Feasibility eRDC system, from which site users with the correct permissions and a username may log in and download a study entry confirmation report.

Once a patient has been registered into the study their name should be added to the Patient Identification Log. With the patient's prior consent their GP should be informed that they are taking part in the study. A GP Letter is provided electronically for this purpose.

The completed and signed Eligibility Checklist, Registration Form and signed Informed Consent Form (if consented for copy to be sent to NOSTRA-Feasibility Study Office) should then be sent to the NOSTRA-Feasibility Study Office.

Once patients have been registered into the study, if they have consented to do so and have not yet started chemotherapy, a baseline blood sample should be taken for translational research prior to commencing neoadjuvant treatment (see section 11.4).

7 TREATMENT DETAILS

All patients will receive neoadjuvant chemotherapy plus dual-targeted anti-HER2 treatment with trastuzumab and pertuzumab using NOSTRA-Feasibility Study approved combination regimens with established efficacy and tolerability. Three example regimens are described in the protocol. If a site wishes to use a different regimen, it must be approved by the Trial Management Group (TMG) before site activation.

7.1 Study Required Treatment

For the purposes of this study 5-fluorouracil (F), epirubicin (E), cyclophosphamide (C), pertuzumab (P) and trastuzumab (H) are to be used within their licenced indications. Docetaxel (T) and carboplatin (Cb) are, however, used outside the licenced indication but used in combination with pertuzumab, trastuzumab in compliance with National Institute of Clinical Excellence (NICE) guidelines for neoadjuvant treatment for HER2-positive early breast cancer.

7.1.1.1 5-Fluorouracil

5-Fluorouracil is an antineoplastic agent licensed by the European Medicines Agency (EMA) for use in the management of common malignancies, particularly cancer of the colon and breast, either as a single agent or in combination with other cytotoxic agents. For the purposes of the NOSTRA-Feasibility Study it is being used within its licensed indication.

5-Fluorouracil is off patent and is available from a number of manufacturers.

7.1.1.2 Epirubicin

Epirubicin is an antineoplastic agent licensed by the EMA and is used in the treatment of a range of neoplastic conditions including breast cancer. Epirubicin can be used in combination with other cytotoxic agents. For the purposes of the NOSTRA-Feasibility Study it is being used within its licensed indication.

Epirubicin is off patent and is available from a number of manufacturers.

7.1.1.3 Cyclophosphamide

Cyclophosphamide is an antineoplastic agent licensed by the EMA for the treatment of malignant disease in adults and children. It is frequently used in combination with other cytotoxic drugs. For the purposes of the NOSTRA-Feasibility Study it is being used within its licensed indication.

Cyclophosphamide is off patent and is available from a number of manufacturers.

7.1.1.4 Docetaxel

Docetaxel is an antineoplastic agent licensed by the EMA for use as adjuvant treatment for operable breast cancer in combination with doxorubicin and cyclophosphamide. It is therefore being used outside of its licensed indication for the purposes of the NOSTRA-Feasibility Study. However the use of this drug in combination with cyclophosphamide as adjuvant treatment of breast cancer is now wide spread in the UK, and the use of docetaxel after prior use of FEC is also widely practised in the neoadjuvant setting. The reverse sequence of docetaxel followed by FEC is often preferred in the neoadjuvant context and forms the control arm of the ARTemis trial, a recent national multicentre trial (58).

Docetaxel solution for infusion was originally manufactured by Sanofi Aventis but is now off patent and is available from a number of manufacturers.

7.1.1.5 Carboplatin

Carboplatin is an antineoplastic agent licensed by the EMA for the treatment of ovarian cancer and small cell lung cancer. It is frequently used in combination with other cytotoxic drugs and outside the licenced indication in the treatment of a wide range of solid tumours including breast cancer in both early stage and advanced stage disease both as a single agent and in combination with other anticancer agents. For the purposes of the NOSTRA-Feasibility Study it is being used outside its licensed indication.

Carboplatin was first manufactured by Bristol Myers Squibb but is now off patent and is available from a number of manufacturers.

7.1.1.6 Pertuzumab

Pertuzumab is indicated for use in adult patients with HER2-positive breast cancer . In the NOSTRA-Feasibility Study it is being used within the licensed indication.

Pertuzumab (Perjeta®) is manufactured by Roche. Perjeta is indicated for use in early breast cancer in combination with trastuzumab and chemotherapy for the neoadjuvant treatment of adult patients with HER2-positive, locally advanced, inflammatory, or early stage breast cancer at high risk of recurrence and the adjuvant treatment of adult patients with HER2-positive early breast cancer at high risk of recurrence.

7.1.1.7 Trastuzumab

Trastuzumab is a monoclonal antibody licensed by the EMA for use in HER2-positive early and metastatic breast cancer. It is licensed for use in combination with neoadjuvant chemotherapy followed by adjuvant trastuzumab, for locally advanced (including inflammatory) disease or tumours >20 mm in diameter. It is used in combination with FEC or with docetaxel based regimens. For the purposes of the NOSTRA-Feasibility Study it is being used within the licensed indication.

Trastuzumab (Herceptin®) was first manufactured by Roche, but is now off patent and is available from a number of manufacturers.

7.1.1.8 Supply of Treatment

All chemotherapy agents used should be supplied from hospital pharmacy stocks.

Pertuzumab for neoadjuvant use only will be supplied to UK sites, if required, for cases where it cannot be funded as standard of care. Hospitals in the UK may use standard commercially sourced pertuzumab to initiate treatment. Commercial stock will be replaced on a like for like basis. Ongoing supply of study supplies or replacement of commercially sourced pertuzumab used for patients in this study will be through a dedicated supply or resupply ordering process as detailed in the Drug Supply File. The Drug Supply File contains full details of supply, ordering, and accountability of pertuzumab. The Drug Supply File will be provided to the responsible Pharmacist on Site Initiation.

7.2 Tumour Positional Marking Prior to Treatment

Tumours must be visible on US scan and a minimum of one US-visible clip should be inserted before the start of treatment, as per standard UK practice. A standard local protocol for tumour clip insertion should be followed. A minimum of one US-visible clip should be placed as centrally as possible in the tumour bed, or alternatively multiple clips may be placed at the edges of the lesion. A post-clip insertion mammogram should be taken as standard practice.

All patients will have axillary US scan plus or minus core needle biopsy or fine needle aspiration (FNA) of abnormal lymph nodes according to local protocol and NICE guidelines. Centres that routinely clip biopsied nodes should continue to do so.

7.3 Timing of Neoadjuvant Treatment

Neoadjuvant chemotherapy should start as soon as possible after the date the diagnostic core biopsy was taken (and within a maximum of 9 weeks of that date unless delayed by patient choice; for instance to allow for participation in fertility protection process) and ideally within 2 weeks of registration.

7.4 Treatment Summary

7.4.1 Neoadjuvant Treatment

Patients with HER2-positive, ER-negative early invasive breast cancer will receive neoadjuvant chemotherapy plus dual-targeted anti-HER2 treatment with pertuzumab and trastuzumab using approved combination regimens with established efficacy and tolerability (Table 3).

Patients registered into the study will receive an approved treatment regimen specified below at the discretion of the treating clinician (see Table 3 and Figure 1). The efficacy of the neoadjuvant treatment will be monitored and is restricted to one of three approved treatment regimens.

7.4.2 Adjuvant Treatment

All patients will continue with adjuvant anti-HER2 therapy as per local practice, +/- radiotherapy.

Sites may use their standard neoadjuvant regimen(s) in this study. Regimens must include at least six cycles of neoadjuvant chemotherapy, with at least three cycles of dual-targeted anti-HER2 therapy. Regimens must be anthracycline/taxane or taxane/platinum-based.

The three example regimens described below are approved for use in the study. If a site wishes to use a different regimen, it must be approved by the TMG before site activation. If a site subsequently wishes to change their chosen regimen(s), then this must be approved before patient registration.

Table 3: Approved Treatment Regimens

Regimen 1: FEC with Pertuzumab and Trastuzumab followed by Docetaxel with Pertuzumab and Trastuzumab
FEC with Pertuzumab and Trastuzumab: 5-Fluorouracil 500mg/m^2*, Epirubicin 100mg/m^2, Cyclophosphamide 500-600mg/m^2, cycle 1-3 with Pertuzumab 840mg cycle 1 and 420mg cycles 2 and 3 and Trastuzumab 8mg/kg cycle 1 and 6mg/kg, cycle 2 and 3, three weekly (3 cycles). **Followed by Docetaxel with Pertuzumab and Trastuzumab:** Docetaxel 75-100mg/m^2 cycles 4- 6 with Pertuzumab 420mg cycle 4-6 and Trastuzumab 6mg/kg cycles 4-6, three weekly (3 cycles).
Regimen 2: FEC followed by Docetaxel with Pertuzumab and Trastuzumab
FEC: 5-Fluorouracil 500mg/m^2*, Epirubicin 100mg/m^2, Cyclophosphamide 500-600mg/m^2 three weekly (3 cycles). **Followed by Docetaxel with Pertuzumab and Trastuzumab:** Pertuzumab 840mg cycle 4 and 420mg cycles 5 and 6 plus Trastuzumab 8mg/kg cycle 4 and 6mg/kg cycles 5 and 6 with Docetaxel 75-100mg/m^2 cycles 4-6, 6, three weekly (3 cycles).
Regimen 3: Docetaxel and Carboplatin with Pertuzumab and Trastuzumab
Docetaxel 75mg/m^2 and Carboplatin AUC 6 plus Pertuzumab 840mg cycle 1 and 420mg cycles 2-6 plus Trastuzumab 8mg/kg cycle 1 and 6mg/kg cycles 2-6, three weekly (6 cycles).
Standard adjuvant treatment (continue with anti-HER2 therapy as per local practice +/-, radiotherapy)

*Omission of 5-fluorouracil at the discretion of the treating Investigator

Patients will have an ECHO or MUGA scan to assess LVEF at baseline (prior to the first cycle of chemotherapy) and this will be repeated at the end of cycle 3 of neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment and on completion of all neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment. Cardiac monitoring will continue during the administration of adjuvant trastuzumab.

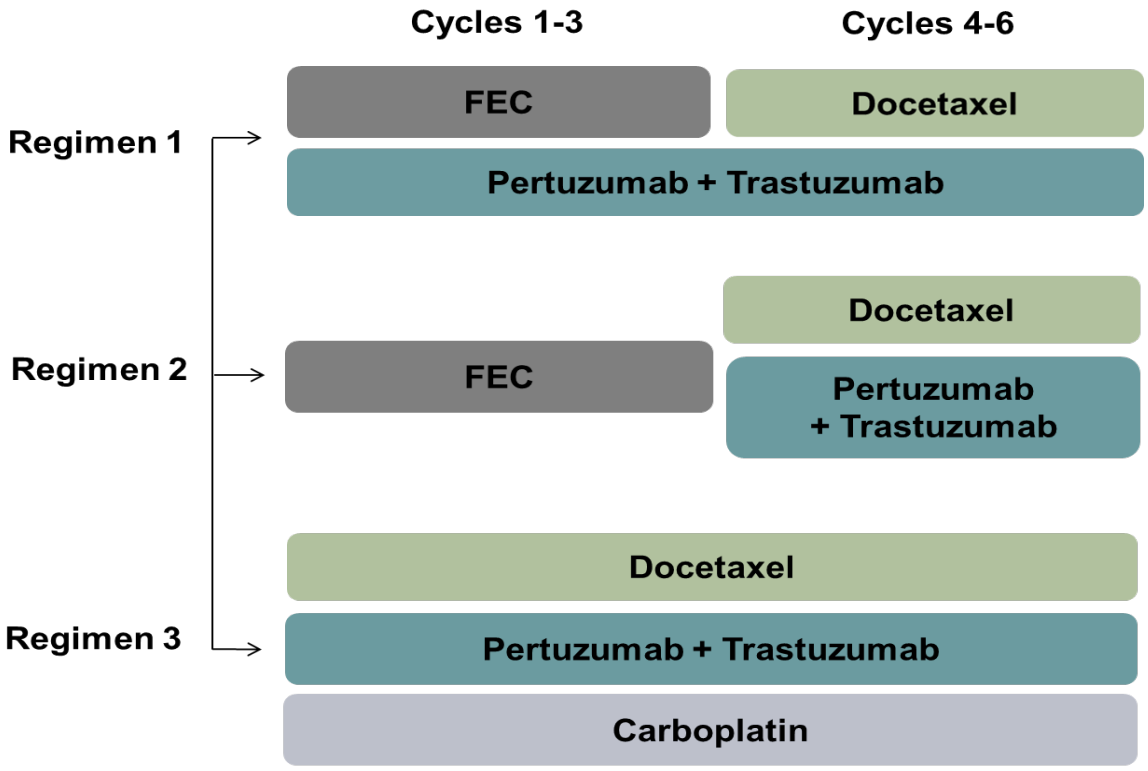


Figure 1: Illustration of approved treatment regimens. Omission of 5-fluorouracil is at the discretion of the treating Investigator

7.5 Neoadjuvant Chemotherapy plus Dual-targeted Anti-HER2 Treatment

7.5.1 Neoadjuvant Chemotherapy

7.5.1.1 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC)
Patients prescribed regimen 1 or 2 will receive 3 cycles of iv 5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2, and cyclophosphamide 500-600mg/m^2 (FEC100) at three weekly intervals. Drugs should be prepared and administered according to institutional standard practice (including any dose banding protocols provided these deliver doses of within 6% of the calculated dose). Supportive medication, dose reductions and delays are at the discretion of the Investigator (see section 7.8, 7.9 and 7.17.6). Omission of 5-fluorouracil is at the discretion of the treating Investigator.

7.5.1.2 Docetaxel (T)
Patients prescribed regimen 1 or 2 will receive 3 cycles of iv docetaxel at three weekly intervals. Docetaxel 75-100mg/m^2 is prescribed as per local practice. Patients prescribed regimen 3 will receive 6 cycles of iv docetaxel at 3 weekly intervals at 75mg/m^2. Drugs should be prepared and administered according to institutional standard practice (including any dose banding protocols provided these deliver doses within 6% of the calculated dose). Supportive medication, dose reductions and delays are at the discretion of the Investigator (see section 7.8, 7.9 and 7.17.6).

7.5.1.3 Carboplatin (Cb)
Patients prescribed regimen 3 will receive 6 cycles of iv carboplatin at 3 weekly intervals. Carboplatin will be administered in a dose of AUC 6 (area under the plasma concentration-time curve). Drugs should be prepared and administered according to institutional standard practice (including any dose banding protocols provided these deliver doses within 6% of the calculated dose). Supportive medication, dose reductions and delays are at the discretion of the Investigator (see section 7.8, 7.9 and 7.17.6).

7.5.2 Dual-targeted Anti-HER2 Treatment

7.5.2.1 Pertuzumab (P)

Pertuzumab should be administered iv using a loading dose of 840mg with cycle 1. 420mg should be administered iv for all subsequent doses. Regimen 1 and 3 require a total of 6 doses, regimen 2 requires a total of 3 doses.

Pertuzumab should be prepared and administered according to institutional standard practice (including any dose banding protocols provided these deliver doses within 10% of the calculated dose). Supportive medication, dose reductions and delays are at the discretion of the Investigator (see section 7.8, 7.9 and 7.17.6).

Patients at high risk of recurrence (for example with node positive disease) may also receive adjuvant Pertuzumab as per standard local practice.

7.5.2.2 Trastuzumab (H)

Trastuzumab should be administered iv at 8mg/kg for the first loading dose of chemotherapy and at 6mg/kg for all subsequent cycles. Trastuzumab should be continued following completion of study chemotherapy and anti-HER2 treatment should be given as per local standard of care.

If the interval between delivery of last dose of pre-operative chemotherapy and surgery is greater than 3 weeks, then a further dose of neoadjuvant single agent trastuzumab as iv or sc should be given to avoid interruption of the 3 weekly trastuzumab schedule. Three weekly dosing should continue uninterrupted through the pre-operative period into the adjuvant phase of treatment as per local practice.

Trastuzumab should be prepared and administered according to institutional standard practice (including any dose banding protocols provided these deliver doses within 10% of the calculated dose).

7.5.2.3 Pertuzumab and Trastuzumab Cardiac Toxicity

Cardiac monitoring of LVEF should be maintained throughout anti-HER2 treatment according to local policy. If trastuzumab and pertuzumab are discontinued during chemotherapy, then chemotherapy should continue uninterrupted if this is considered safe by the Investigator. Trastuzumab and pertuzumab can be reintroduced when deemed safe to do so, when LVEF returns to normal range and is within 10% of baseline value. Reintroduction of trastuzumab should be scheduled to coincide with the next chemotherapy administration. If trastuzumab has been delayed by more than 4 weeks from the previous cycle (delay greater than 1 week in administration of a 3 weekly cycle) during the neoadjuvant treatment a reloading schedule of 8mg/kg should be used with the first reintroduction followed by the usual 6mg/kg maintenance dose at 3 weekly intervals thereafter. Similarly, a reloading dose of pertuzumab at 840mg should be used when reintroducing if pertuzumab has been delayed by more than 4 weeks from the previous cycle (delay greater than 1 week in administration of a 3 weekly cycle).

7.6 Dose Calculation

Body surface area calculation should be performed using institutional methodology. Dose recalculation should be performed where body weight has changed by more than 10%.

Carboplatin dose must be calculated as an AUC dose according to renal function. Renal function (ml/min) must be estimated using the Cockroft Gault formula (140 - age (years) x weight (kg)/serum creatinine (µmol/L). Dose calculation for AUC 6 x (25 + GFR (ml/min)).

7.7 Dose Modifications and Delays

The chemotherapy schedules in NOSTRA-Feasibility Study are in widespread use in the UK and sites will be familiar with managing the toxicity associated with these drugs. The following guidance is provided to encourage a consistent approach, but Investigators may use local toxicity management guidelines.

Trastuzumab and pertuzumab are also in widespread use in the UK and sites will be familiar with the toxicity associated with these HER-2 directed treatments. Toxicity primarily comprises of acute reactions to administration and cardiac toxicity manifesting either as symptomatic left ventricular failure or more commonly as asymptomatic decline in LVEF. Sites should follow institutional practice in the management of trastuzumab or pertuzumab induced cardiac dysfunction. Pertuzumab and trastuzumab administration should be discontinued for 3 weeks if LVEF is noted to fall by more than 10% or falls below the institutional normal range (normally LVEF of 50%). Cardiology advice should be sought. Introduction of angiotensin converting enzyme inhibitors and beta blockade should be considered. LVEF should be monitored. On recovery to within 10% of baseline value anti-HER2 treatment may be re-introduced.

The decision whether to continue with study treatment should be based on the individual circumstance and the responsible clinician's judgment that continuation is in the patient's best interest. Investigators are encouraged to discuss unusual or complex toxicity management with the Chief Investigator or the Clinical Coordinator but overall responsibility for patient care rests with the Investigator.

7.8 Dose Modifications and Delays in Response to Toxicity

7.8.1 Non Permissive Haematological Parameters

Non-permissive haematological parameters are ultimately defined locally, but the following guidance may be used:

- Delay for minimum period required for recovery to permissive parameters and practicalities of rescheduling
- Introduce secondary Granulocyte Colony Stimulating Factors (G-CSFs) prophylaxis without dose reduction for delay due to uncomplicated neutropenia
- Dose reduce all cytotoxics by 20% for non-permissive thrombocytopenia on day 21
- Use red cell support to maintain haemoglobin above 100g/L
- A further 20% dose reduction is recommended where initial dose reduction (and G-CSF support) has not resulted in recovery of haematological parameters by day 21
- Where prolonged myelosuppression (dose delay >4 weeks) despite G-CSF prophylaxis and dose reduction by two increments has been experienced chemotherapy should be discontinued

7.8.2 Febrile Neutropenia

- Manage acute febrile neutropenia and neutropenic sepsis using institutional guidelines
- Treatment delay is not required where recovery has been rapid and uncomplicated
- Use secondary G-CSF prophylaxis with all subsequent cycles of chemotherapy
- 20% dose reduction in all cytotoxic drugs should be introduced for life threatening neutropenic sepsis (High Dependency Unit or Intensive Care Unit involvement in management) even where primary prophylaxis was not used
- 20% dose reduction (with maintained G-CSF prophylaxis) of all cytotoxic agents should be introduced after a second episode of febrile neutropenia or neutropenic sepsis

7.8.3 Emesis

- Emesis should be minimised by escalation of antiemetic co-medication
- Dose reduction should be reserved for refractory vomiting
- Where available aprepitant should be introduced for persistent or severe chemotherapy related emesis control and is recommended as standard treatment for all patients receiving FEC100

7.8.4 Diarrhoea

- Diarrhoea is a common side effect of chemotherapy but is increased by the addition of pertuzumab
- In the TRYPHAENA trial all grade diarrhoea was reported in 44-55% of patients, with grade 3 or 4 diarrhoea in 11.8% of patients treated with carboplatin, docetaxel, trastuzumab and pertuzumab and 4.2 to 5.3 % of patients treated with FEC, trastuzumab, pertuzumab x3 docetaxel trastuzumab pertuzumab x3 and FEC x3 docetaxel, trastuzumab, pertuzumab x3 respectively
- Diarrhoea should be actively managed using loperamide and treatment escalated after clinical

assessment if diarrhoea is not adequately controlled

7.8.5 Mucositis

- Antiseptic mouth wash prophylaxis is recommended for all patients
- Secondary infection should be treated promptly and antifungal prophylaxis with nystatin or fluconazole is recommended in patients experiencing grade 2 or above mucositis
- 20% dose reduction is recommended for grade 3 or 4 mucositis

7.8.6 Hypersensitivity

Local protocols for the management of hypersensitivity reactions should be followed. Substitution of docetaxel with nab-paclitaxel (if available) should be considered for docetaxel hypersensitivity. We encourage discussion with the Chief Investigator who can provide advice on dose and schedule for individual patients.

7.8.7 General Guidance

Where an initial 20% dose reduction has not reduced the toxicity to acceptable levels a second dose reduction by a further 20% is recommended. Discontinuation of chemotherapy is recommended where two dose reductions have not resolved recurrent toxicity problems. Individual drugs suspected of causing severe toxicity may be omitted completely (e.g. 5-fluorouracil after severe diarrhoea associated with suspected dihydropyrimidine dehydrogenase (DPD) deficiency). Re-escalation after dose reduction is not recommended.

7.9 Supportive Therapy and Concomitant Medication

Patients may continue with previous concomitant treatments (i.e. prescription, non-prescription or alternative therapies) at the same doses and schedule as prior to the start of study treatment, at the discretion of the local Investigator, provided the medication is not prohibited within the exclusion criteria for study participation.

Institutions should follow their local and or national/international administration guidelines for supportive medication for the selected chemotherapy regimen. Detailed guidance on concomitant medication is provided in the Summary of Product Characteristics for each drug and should be referred to for concerns relating to specific drugs.

7.9.1 Anti-emetic Therapy

Anti-emetics should be prescribed at the Investigator's discretion and according to local policy and established national and international guidelines. However, aprepitant is recommended as a standard treatment for all patients receiving FEC100.

7.9.2 Granulocyte–Colony Stimulating Factors

The use of primary neutropenia prophylaxis with G-CSFs but may be prescribed at the Investigator's discretion. When the use of G-CSFs is indicated, it should be administered according to the prescribing information for the particular agent being used.

7.9.3 Prophylactic Antibiotics

At the Investigator's discretion, primary oral prophylaxis with fluoroquinolones may be prescribed as an alternative or in addition to G-CSFs e.g. levofloxacin 500mg daily or ciprofloxacin 500mg given twice daily in accordance with local practice.

7.10 Disease Progression While on Neoadjuvant Treatment

In the event of progression of disease on treatment, the Investigator should treat the patient at their discretion and according to their opinion on optimal treatment.

Details of disease progression should be captured on a Relapse Form and early treatment discontinuation should be recorded on the Neoadjuvant Treatment Discontinuation Form (see section 10.2).

It is assumed in this instance that the patient will not undergo study biopsies. However, study data will continue to be collected on other subsequent forms unless the patient explicitly withdraws their consent. It is appreciated that study time-points are unlikely to be met and there will gaps in data collected post-relapse, as this must be collected without inconvenience to the patient (i.e. from notes).

8 TUMOUR BED BIOPSIES POST-CHEMOTHERAPY

Following neoadjuvant treatment, all patients will undergo multiple US-guided tumour bed core biopsies using one incision, prior to surgery in accordance with the Radiologists Guidelines. Most patients will have 8 cores taken (minimum 14 gauge) but it is recognised that in patients with small tumours or if technical or patient issues dictate, not all 8 cores will be possible.

In some cases, ultrasound-guided Vacuum-Assisted Biopsy (VAB) may be preferable. Sites wishing to use VAB should first discuss this with the NOSTRA study team.

A fan of paired cores (1 mm x 1 mm piece of tissue about 10-15 mm long) In most cases, this should only require a single incision (with angulation of the needle) and will be taken working from the medial tumour margin across the tumour to the lateral edge of the original tumour bed as described below:

- Zone 1: Two cores from the medial peripheral margin
- Zone 2: Two cores from the central tumour bed medially
- Zone 3: Two cores from the central tumour bed laterally
- Zone 4: Two cores from the lateral peripheral margin

The aim is to widely sample the tumour bed, post-neoadjuvant chemotherapy, in a systematic and reproducible way. This is why it is necessary to take a ‘fan of 14G cores’ rather than attempting to remove the ‘centre of the tumour’ using vacuum needle.

The number and the exact location of the tumour bed core biopsies should be captured on the Tumour Bed Core Biopsies Form. If it is not possible to take core biopsies at all or as per the Radiologist Guidelines, this should be reported on a deviation form.

For further details see section 11. Also refer to the separate NOSTRA-Feasibility Tissue Collection and Radiologist Guidelines for additional information.

9 SURGERY

9.1 Primary Surgery

All patients should proceed to surgery in the NOSTRA-Feasibility Study. Surgery should proceed when the Investigator is satisfied that the patient is sufficiently recovered from neoadjuvant chemotherapy and Tumour Bed Core Biopsies, at the earliest 3 weeks after day 1 of the last cycle of chemotherapy and ideally within 6 weeks of day 1 of the last cycle of chemotherapy. Surgery to the breast should be according to local protocol and will be finally determined after the post-neoadjuvant MDT where all imaging and clinical factors will be reviewed. Appropriate surgical options should be discussed and agreed with patients as standard clinical practice.

9.2 Axillary Treatment

Pre-neoadjuvant treatment:

- US-guided biopsy mandated if any abnormal axillary lymph nodes are evident on US scan.
- SLNB is permissible at Investigators discretion only if the US scan of the axilla is normal. If this demonstrates axillary node metastasis a post-neoadjuvant axillary node clearance must be performed.

Post-neoadjuvant treatment:

- Where practical and safe, US-guided biopsy (FNA or core) should be performed if axillary lymph nodes were positive at the pre-neoadjuvant treatment US scan. This is preferably to be done at the time of tumour bed biopsies.
- SLNB is permissible if pre-treatment US scan of the axilla was normal **OR** pre-treatment axillary core biopsy or FNA showed no evidence of axillary metastases **OR** pre-treatment biopsy revealed metastases but a repeat US scan of axilla +/- biopsy of any abnormal nodes post-neoadjuvant treatment are negative.
- Axillary node clearance must be performed in patients with confirmed nodal metastases on pre-treatment SLNB and in those with an axillary biopsy confirming nodal metastases post-neoadjuvant treatment.

All patients must have either a SLNB or an axillary lymph node clearance as part of their surgical procedure post-neoadjuvant treatment, unless a pre-neoadjuvant SLNB was successfully performed and was negative.

10 SCHEDULE OF ASSESSMENTS

10.1 Screening/Baseline

See section 5.2 for screening and baseline tests.

10.1.1 Prior to 1st cycle of neoadjuvant chemotherapy and anti HER2 treatment

If the patient has provided consent to take part in the optional ctDNA Sub-Study, and has yet to start neoadjuvant chemotherapy, a blood sample should be collected prior to commencing the first cycle of neoadjuvant treatment (see section 11.4). This should be taken in conjunction with the routine blood samples.

10.1.2 Prior to each cycle of neoadjuvant chemotherapy and anti-HER2 treatment

Safety assessments should be performed prior to day 1 of each cycle of neoadjuvant chemotherapy (excluding cycle 1) according to each site's standard practice. This data will not be collected for the study but should be documented locally and made available for monitoring purposes. Suggested assessments include:

- Vital signs:
  - Blood pressure
  - Pulse
  - Temperature
- Full blood count within 3 days prior to day 1 of each 3 weekly treatment cycle to include:
  - Haemoglobin
  - WBC count including differential count
  - Platelets
- Biochemical screen within 3 days prior to treatment cycle to include:
  - ALT or AST
  - ALP
  - Bilirubin
  - Urea
  - Creatinine
  - Sodium
  - Potassium
- Adverse Events (AEs) review
- Clinical tumour measurement (calipers)*

* Clinical assessment of breast lesions is not required unless there is concern or at discretion of the Investigator

10.1.3 End of cycle 3 of neoadjuvant chemotherapy and anti-HER2 treatment

- A clinical tumour assessment, normally using calipers, is recommended to confirm absence of early disease progression at the end of cycle 3 (i.e. prior to commencing cycle 4 treatment).
- LVEF measurement by ECHO or MUGA (as per the baseline assessment, as the modality used should be consistent throughout the study).
- Imaging assessments during neoadjuvant chemotherapy should be performed as indicated and as per institutional standard of care. This may include mid-treatment MRI.
- If the patient was recruited post-cycle 3, then the 'End of treatment cycle 3' form must be completed retrospectively to document any of the above procedures which happened as per standard care.

10.1.4 On completion of all neoadjuvant chemotherapy and anti-HER2 treatment

The following assessments should be performed on completion of all neoadjuvant chemotherapy and anti-HER2 treatment:

- AE review
- Clinical and radiological tumour measurement
  - Clinical measurement of breast tumour by calipers (optional, recommended)
  - Radiological measurement of tumour(s) by US scan (can be done at same time as tumour bed core biopsies)

- Radiological measurement of tumour(s) by mammogram and/or MRI is mandated at this point. Choice of imaging is as per the institution's standard care. MRI assessment both before and after neoadjuvant chemotherapy is recommended as the most informative imaging modality in most cases. Serial MRI is now a standard of care in many institutions, but is not mandatory for this study. MRI, if chosen, must be performed before the tumour bed core biopsies, to avoid artefacts.
- A Radiology Assessment Form should be completed to document the radiological response.
- Axillary US scan should be performed and any abnormal nodes should be (re)sampled (by FNA or biopsy) under image control. This can be done at the same time as the tumour bed core biopsies.

- LVEF measurement by ECHO or MUGA (as per the baseline assessment; the modality used should be consistent throughout the study).
- Once acute toxicity has resolved- Study Tumour Bed Core Biopsies (as per section 8).
- FNA or core biopsy of the axilla, if node positive at baseline, as per section 9.2.
- Tumour bed biopsy AE assessment should be performed within 30 days of study biopsies (in person or on telephone).

10.1.5 Surgery

All patients must be scheduled for surgery after completing cycle 6 of neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment.

Surgery should ideally be performed within six weeks of completion of neoadjuvant treatment but allowing time for healing post-study biopsies, in accordance with section 9. Tumour samples should be submitted for Central Histopathology Review as detailed in section 11 and a copy of the patient's anonymised pathology report should be sent to the NOSTRA-Feasibility Study Office. If the patient has provided consent to take part in the optional ctDNA Sub-Study, a blood sample should be taken at the first clinic visit after surgery (see section 11.4).

10.1.6 Adjuvant Anti-HER2 Treatment

Adjuvant anti-HER2 treatment should be continued following completion of neoadjuvant chemotherapy regimen. All patients should continue with anti-HER2 treatment as per local practice. The Adjuvant Treatment Form should be completed to document the adjuvant treatment given. Cardiac monitoring should be continued during the administration of adjuvant anti-HER2 treatment.

10.1.7 Adjuvant Radiotherapy

Radiotherapy will be given after definitive surgery according to local protocols. The Adjuvant Treatment Form should be completed to document the radiotherapy given.

10.1.8 Follow-up

Patient participation will end upon completion of surgery. Centres will be asked to provide follow-up information at 12 months from the date of surgery. The 12 months follow-up should ideally be performed in clinic face to face, but may be performed remotely if no clinic visit is scheduled at that time point. Details must be captured in the Adjuvant Treatment Form. Follow-up assessments can be conducted by an appropriately trained breast practitioner (clinician, clinical nurse specialist, research nurse/practitioner or radiographer). Site staff are advised to contact the patient's GP if the patient does not attend appointments or is not contactable by other means to obtain follow-up information.

10.1.9 Relapse

See Table 4 illustrates definitions of relapse.

Table 4: Definition of Relapse

Type of Relapse	Definition
Loco-regional relapse	A loco-regional relapse is defined as a recurrence of breast cancer in the ipsilateral breast/chest wall, axillary lymph nodes and/or ipsilateral supraclavicular fossa and/or internal mammary nodes
Distant relapse	Distant relapse is defined as a recurrence (metastasis) of breast cancer developing beyond the ipsilateral breast/chest wall, axillary lymph nodes and/or ipsilateral supraclavicular fossa nodes
New primary	Confirmation of the presence of a second unrelated and new cancer. For the purposes of NOSTRA-Feasibility Study this will include contralateral malignant breast disease

As soon as definite confirmation has been obtained that a patient has relapsed or has developed a new second primary cancer, a Relapse Form should be completed and returned to the NOSTRA-Feasibility Study Office.

If a patient has a relapse or new primary diagnosed before they are due to have the studies biopsies, it is assumed they will not take place. However, study data will continue to be collected on other subsequent forms unless the patient explicitly withdraws their consent. It is appreciated that study time-points are unlikely to be met and there will gaps in data collected post-relapse/ second primary, as this must be collected without inconvenience to the patient (i.e. from notes).

10.1.10 Death

As soon as possible following notification that a patient has died, a Death Form should be completed and returned to the NOSTRA-Feasibility Study Office. Every effort should be made to obtain a date and cause of death.

10.2 Neoadjuvant Treatment Discontinuation and Withdrawal of Consent

10.2.1 Neoadjuvant Treatment Discontinuation

It is expected that patients would discontinue study treatment and not proceed to study biopsies in the following circumstances, at the Investigator's discretion:

- As a result of toxicity as specified in section 7.8
- If the patient experiences a dose delay of >4 weeks as a result of toxicity (see section 7.8)
- On confirmation of disease progression
- On diagnosis of second primary
- If the patient becomes pregnant or fails to use adequate birth control (for those patients who are able to conceive)
- At the request of the patient

A Neoadjuvant Treatment Discontinuation Form should be completed to document the reason for neoadjuvant treatment discontinuation. Please note that study treatment discontinuation does not mean that the patient's study participation ends. Such patients should be followed up as closely to the study timelines as possible/appropriate unless the patient withdraws consent.

10.2.2 Withdrawal of Consent

Patients may withdraw consent at any time during the study. They may withdraw from the Main NOSTRA Feasibility study and/or the ctDNA and/or the MRI Sub-Studies. For the purposes of this study, three types of withdrawal are defined:

- The patient would like to withdraw from study tumour bed core biopsies. In these cases, patients should be followed up as closely to the study timelines as possible/appropriate unless the patient withdraws consent.
- The patient has undergone the tumour bed core biopsies but does not wish to attend study visits in accordance with the schedule of assessments but is willing to be followed up at standard clinic visits (i.e. the patient has agreed that data can be collected at standard clinic visits and used in the study analysis)
- The patient has undergone tumour bed core biopsies but would like to withdraw from the study and is not willing to be followed up for the purposes of the study at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the study analysis)

The details of withdrawal (date, reason and type of withdrawal) should be clearly documented in the source data. A Withdrawal of Consent Form should be completed to notify the NOSTRA-Feasibility Study Office of the patient's withdrawal from the study. The Withdrawal of Consent Form must be co-signed by an Investigator.

All information and tissue samples collected up until the point of full withdrawal of consent will be retained and analysed. In addition, safety data (including SAEs) on the participant will continue to be collected as described in Section 13).

10.3 Concurrent Studies

Investigators wishing to enrol patients into another trial should contact the NOSTRA-Feasibility Study Office in the first instance. The NOSTRA-Feasibility TMG will consider the enrolment of NOSTRA-Feasibility patients into other trials which do not interfere with the analysis of the primary outcome or introduce bias. Examples include other trastuzumab therapy trials, trials of imaging, supportive treatment and adjuvant radiotherapy, or after completion of trial specific neoadjuvant chemotherapy or surgery. The NOSTRA-Feasibility Study Office maintain a contemporary record of trials approved by the TMG. Where a trial has not been considered, the NOSTRA-Feasibility Study Chief Investigator will provide advice based on the above principles.

SCHEDULE OF EVENTS

Assessment	Screening/ Baseline[1]	Neoadjuvant Chemotherapy & Anti-HER2 Treatment				Surgery	Adjuvant treatment	Follow-up at 12 months post surgery[22 & 23]
		Prior to Day 1 of each cycle[4]	After Cycle 3[4]	End of Cycle 5	On completion of neoadjuvant treatment			
Diagnostic biopsy	X						**Continue to complete anti-HER2 therapy as per local practice, +/- radiotherapy**	
US-visible clip insertion[2]	X							
Axillary US scan +/- FNA/biopsy	X				X[5]			
Molecular markers (ER, PgR, HER2)	X							
Informed consent & Registration[3]		X						
Medical history and ECOG performance status	X							
Left Ventricular Ejection Fraction Measurement[6]	X		X		X			
Radiological tumour assessment [7&8] Ultrasound Mammography MRI[10]	X X X		 X		X X[9] X			
Clinical laboratory assessments Pregnancy test[11] Full blood count Urea, Electrolytes, Creatinine, Liver Function Tests[12]	X X X	X X X						
Physical examination	X							
Clinical tumour measurement (caliper)[13]	X		X		X			
Vital signs [14]		X						
Sentinel lymph node biopsy[15]	X					X		
Pre-surgery protocol defined tumour bed core biopsies[16]						X		
Tumour sample collection	X[17]				X[18]	X[19]		
Blood sample collection for ctDNA Sub-Study[20]	X	X				X		
AE review		X			X[21]	X		X
Survival & relapse								X

Notes:

1. These procedures must all have occurred prior to study registration to determine eligibility, regardless of when the patient is recruited, and results/evidence must be documented in the patient's source notes for monitoring purposes
2. Patients must have an ultrasound-visible clip inserted within their first two cycles of neoadjuvant treatment
3. Patients have consented and been registered before the end of their fifth cycle of neoadjuvant treatment
4. Unless patient is recruited after cycle 3 (then complete form retrospectively from notes to document any procedures performed as per standard care)
5. Biopsy/FNA specimen required if involved nodes at diagnosis
6. Left Ventricular Ejection Fraction (LVEF) must be measured for all patients by echocardiogram (ECHO) or Multi Gated Acquisition (MUGA) scan. Modality used must be consistent throughout the study.
7. Radiological tumour measurement of the breast and assessment of ipsilateral axilla
8. Uninformative baseline radiological assessments do not have to be repeated at later time points
9. Breast mammogram may be omitted if a breast MRI has been taken at this time point.
10. MRI breast is recommended (not mandated). Post neoadjuvant treatment MRI, if used, should be undertaken before study biopsies, to avoid artefacts.
11. Pregnancy test (Human Chorionic Gonadotropin urine or blood) must be performed at baseline, for women of child-bearing potential and at risk of pregnancy must have a urine or blood Human Chorionic Gonadotropin test performed to rule out pregnancy prior to study entry)
12. Liver function tests: Alanine Aminotransferase (ALT) or Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) and Bilirubin
13. Clinical Tumour Measurement is optional as per local practice
14. Vital signs as per local practice
15. All patients must have either a SLNB or an axillary lymph node clearance as part of their surgical procedure post-neoadjuvant treatment unless a pre-neoadjuvant SLNB was successfully performed and was negative
16. Protocol defined tumour bed core biopsies should be performed prior to surgery
17. All baseline diagnostic core biopsies
18. Axilla biopsy specimen if involved nodes at diagnosis
19. Surgical excision specimens
20. Sample to be collected prior to cycle one (baseline) of neoadjuvant chemotherapy and dual-targeted anti-HER2 treatment, post-cycle one and at first clinic appointment post-surgery. Patients are eligible if registered prior to start of neoadjuvant treatment only.
21. Adverse events to be reviewed pre-surgery (i.e. following collection of tumour bed core biopsies) and post-surgery
22. Follow-up to be performed at standard routine clinical follow-up visit or via telephone
23. Follow-up at 12 months should be from the date of surgery

11 TISSUE AND ctDNA BLOOD SAMPLE COLLECTION

11.1 Tissue Sample Collection

Tissue samples must be collected in accordance with the NOSTRA-Feasibility Study Pathology and Tissue Sample Collection Guidelines.

Principal Investigators are asked to nominate one individual at their site to act as a Lead Pathologist. The Lead Pathologist will be provided with the NOSTRA-Feasibility Study Pathology and Tissue Sample Collection Guidelines describing the labelling and shipment of samples.

Paraffin embedded material will be submitted to the University of Birmingham for Central Histopathology Review by the Study Pathologists and will then be retained for future research purposes (for consenting patients). This will include tissue from:

- The diagnostic biopsy (if this took place at another site, please organise transfer to the recruiting site)
- Study tumour bed core biopsies (including axilla biopsy/FNA if applicable)
- Surgical excision specimens (including axilla biopsy specimen and SLNB specimen if applicable)

For further details please refer to the NOSTRA-Feasibility Study Pathology and Tissue Sample Collection Guidelines.

All material will be handled and reported in local laboratories as per UK NHS Breast Screening Programme and Royal College of Pathologists joint guidelines. Local pathologists will ensure that accurate documentation of block taking is included in the histology reports from the surgically excised lesions (as per UK guidelines) in order to allow the central review to re-assess and, for example to determine tumour size in more than one dimension.

Please retain the above samples at site until the NOSTRA-Feasibility Study Office arrange courier collection.

All material will be sent by courier to:
Human Biomaterials Resource Centre (HBRC)
University of Birmingham
Hospital Drive
Birmingham, B15 2TT

☎ 0121 414 7668
✉ hbrc-tissuebank@contacts.bham.ac.uk

A copy of the associated pathology report will be requested for each patient. This should be anonymised to contain only the patient's unique TNO and sent to the NOSTRA-Feasibility Study Office.

If diagnostic material is urgently needed, please contact the NOSTRA-Feasibility Study Office who will arrange for immediate retrieval of the material and return to the Pathology Department from whom it was requested. Please note that where possible sections will be cut from the diagnostic tissue block prior to return. In cases where material is sparse, the NOSTRA-Feasibility Pathology Lead will ensure that sufficient material remains for diagnostic purposes.

Please be aware that it will be the responsibility of the local research team to obtain their patient's pathology material if the material is stored at a separate site to the registering hospital.

Pathology Departments will receive a per-patient payment for supplying the requested samples.

11.2 Central Histopathology Review

There will be a Central Histopathology Review of all patients to determine pCR and RCB (see Appendix 3). The Central Histopathology Review Pathologists will review all provided material from the diagnostic biopsy, post-neoadjuvant treatment tumour bed core biopsies and surgical excision specimens (including axilla biopsy specimen and SLNB specimen). The Central Histopathology Review

Pathologists will record the RCB score and group, as this is not routine practice in the UK (11). pCR will be defined on the basis of Central Histopathology Review of the surgical slides (see Appendix 3).

11.3 Collection of Tissue Sample for Future Translational Research

Upon completion of Central Histopathology Review, material from the diagnostic biopsies, post-neoadjuvant treatment tumour bed core biopsies and surgical excision specimens (including axilla biopsy specimen and SLNB specimen) will be kept at the University of Birmingham for consenting patients, for future research purposes.

It is appreciated that in some instances there may be insufficient diagnostic material available for future research purposes. If the local pathology team are concerned that there is insufficient tissue available to be retained, this should be communicated to the NOSTRA-Feasibility Study Office.

11.4 Blood Sample Collection for ctDNA Sub-Study (Optional)

For patients who have provided consent to take part in the optional ctDNA Sub-Study and have not yet commenced neoadjuvant chemotherapy, blood samples should be collected at three time points (see Schedule of Events):

1. Prior to commencing neoadjuvant treatment (pre-cycle 1 of chemotherapy and anti-HER2 treatment)
2. Post-cycle 1 of neoadjuvant treatment (pre-cycle 2 review visit)
3. After surgery is complete (first visit to clinic post-surgery)

These time points should coincide with routine blood tests taken within clinic. One 9ml sample should be collected in an EDTA tube and up to 40ml should be collected in four ctDNA tubes at each time point. Sites are not required to process the blood samples. The blood samples should be packaged and shipped together with the ctDNA Blood Sample Collection Form on the same day to the Institute of Cancer and Genomic Sciences, University of Birmingham.

Samples should be sent immediately to:
Beggs Lab Institute of Biomedical Research (IBR),
2nd Floor, West Extension
Institute of Cancer and Genomic Sciences
College of Medical and Dental Sciences
University of Birmingham
Birmingham
B15 2TT

☎ 0121 414 7945 or 0121 415 8783

For additional information on blood collection, labelling and packaging, refer to the separate NOSTRA-Feasibility Study ctDNA Blood Collection Guidelines.

12 MAGNETIC RESONANCE IMAGING

12.1 Monitoring Response with Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is used for monitoring response to neoadjuvant treatment as part of routine practice within most UK hospitals. It is recommended but not mandated in this study. Breast MRI may take place at any of these three time-points: baseline, after cycle 3 and at the end of neoadjuvant treatment.

12.2 Breast MRI Methods

There is a range of acceptable methods of performing breast MRI and sites should follow their standard departmental practice. The following imaging protocol is suggested, should sites require guidance.

12.2.1 MRI Equipment

MRI 1.5T or 3T utilising a dedicated phased array breast-coil (minimum 8 channels) to provide high signal to noise ratio (SNR) images and simultaneous coverage of both breasts, axial plane recommended.

12.2.2 Image Acquisition

1. Undertaken in the prone position.
2. Scan protocol to include high-resolution (≤ 3 mm slice thinness with no gap) axial T2 weighted turbo spin echo (TSE) or fast spin echo (FSE) sequence without fat suppression in the axial plane.
3. Dynamic contrast enhanced (DCE) imaging to evaluate lesion enhancement undertaken with fat-suppressed, T1 weighted 3D multi-phase gradient echo sequences acquired before and immediately after the administration of 0.1 mmol/ kg gadolinium-containing contrast media with high in-plane spatial resolution (≤ 1mm X 1mm) and thin slices (≤ 2 mm) either 2D or volumetric. Subsequent, dynamic imaging performed in at least five consecutive series at intervals (≤ 60 seconds enabling good temporal resolution).

OPTIONAL

1. High resolution post contrast T1 weighted 3D gradient echo sequence with water excitation and isotropic (≤1mm) voxels.
2. Diffusion weighted (DW) imaging utilising a spin-echo planar sequence in axial orientation with diffusion gradients to measure isotropic apparent diffusion co-efficient (ADC) could assess cellularity and microstructure of tissues. Motion sensitising gradients b50 and b850. This should be undertaken prior to DCE sequences.
3. Spectroscopy – Proton single voxel spectroscopy.

12.3 Retrospective MR Image Collection

On completion of trial recruitment, MR images from consenting patients will be harvested retrospectively, de-identified, sent to the Image Data Bank at The Cheltenham Imaging Centre, Cobalt Health Medical Charity, Cheltenham, and stored to create an image library. This organisation performs more than 50,000 investigations annually for the NHS and has secure IG structures for image transfer and storage.

This will enable evaluation of parameters from scans which may contribute to predicting response and correlate features on post treatments scans with pathological characteristics:

1. To assess if parameters derived from baseline and interim MRI scans can differentiate between ‘‘responders’’ and “non-responders”
2. To evaluate if MRI-derived parameters from post treatment scans correlate with the multiple ultrasound-guided tumour bed core biopsies and final pathology

Images will be collected using the Image Exchange Portal (IEP). This is advantageous as the processes for transferring images via IEP are well known and robust. IEP is in use by almost all NHS sites in the UK and is in routine use to facilitate the transfer of medical images from one healthcare site to another. At most clinical sites, a large number of staff are trained in the use of IEP and indeed most sites have a robust image transfer request service in place (usually run by the PACS team).

In order to prevent the need to store Patient Identifiable Data, sites should de-identify the images before transfer using their usual software/method.

Participating sites which do not use Picture Archiving Computer Stores or an equivalent platform, should submit de-identified images on CD via post to The Cheltenham Imaging Centre, Cobalt Health Medical Charity, Cheltenham, including the patient's TNO and date of MRI only as identifiers.

The image library of de-identified scans will also be available for future image analysis studies.

Further details can be found in the NOSTRA-Feasibility Study Radiology Guidelines.

13 ADVERSE EVENT REPORTING

The collection and reporting of Adverse Events will be in accordance with the UK policy framework for health and social care research 2017 and the requirements of the Health Research Authority (HRA). Definitions of different types of Adverse Event are listed in Appendix 4. The Investigator should assess the seriousness and causality (relatedness) of all Adverse Events experienced by the patient (this should be documented in the participant's medical records) with reference to the protocol.

13.1 Reporting Requirements

13.1.1 Adverse Events

AEs (see Appendix 4 for definition) are commonly encountered in patients receiving treatment for breast cancer. The safety of these treatments is already well characterised, therefore only adverse reactions experienced due to the study tumour bed core biopsies and certain adverse reactions which may be due to pertuzumab and/or trastuzumab will be reported (see section 13.1.5). All AEs experienced by the patient must be recorded in the patient notes.

13.1.2 Serious Adverse Events

Investigators should report AEs that meet the definition of an SAE (see Appendix 4 for definition) following the process outlined in section 13.3. Investigators should only report SAEs which may be attributable to the trial protocol. Expected SAEs are excluded from the expedited reporting process.

13.1.3 Expected Serious Adverse Events

Patients receiving chemotherapy, surgery or radiotherapy may require admission to hospital for appropriate medical intervention following development of some of the more severe known side effects of treatment. The following are regarded as expected SAEs for the purpose of this study and should not be reported on an SAE Form:

- SAEs relating to chemotherapy unless the condition is life threatening or proves fatal
- SAEs relating to radiotherapy unless the condition is life threatening or proves fatal
- SAEs relating to surgery (haematoma, wound infection or seroma, as a result of primary breast surgery) unless the condition is life threatening or proves fatal
- Pre-planned elective procedures unless the condition worsens
- Treatment for the progression of the patient's cancer
- Progression or death as a result of the patient's cancer as this information is captured on the Relapse or Death Form

13.1.4 Monitoring pregnancies for potential Serious Adverse Events

It is important to monitor the outcome of pregnancies of patients in order to provide SAE data on congenital anomalies or birth defects.

In the event that a patient becomes pregnant during the SAE reporting period please complete a Pregnancy Notification Form and return to the NOSTRA-Feasibility Study Office as soon as possible. Provide details of the outcome of the pregnancy on a follow-up Pregnancy Notification Form and if appropriate also complete an SAE Form. All pregnancy notifications will be reported to the Manufacturer (Roche), within 30 calendar days of the CRCTU awareness date. Pregnancies will be followed-up until the outcome of the pregnancy is known and for the first year.

13.1.5 Adverse Events of Special Interest (AESI) for Pertuzumab and Trastuzumab

The following AEs occurring in patients treated with pertuzumab and trastuzumab (even if not considered related to either of those drugs) must be reported on the SAE Form and will be reported to the Manufacturer (Roche), within 15 calendar days of the CRCTU awareness date.

- Heart failure
- Significant asymptomatic decline in left ventricular ejection fraction (i.e. greater than 10% decline and less than 50%) also known as left ventricular systolic dysfunction or decreased ejection fraction.

13.2 Reporting period

AEs assessment will be performed at the following timepoints:

- Prior to each cycle of neoadjuvant chemotherapy and anti-HER2 treatment
- On completion of all neoadjuvant chemotherapy and anti-HER2 treatment
- Prior to surgery and after surgery and at 12 month follow-up

Details of all SAEs will be documented and reported from patient registration until 30 days after the last received dose of anti-HER2 treatment.

13.3 Reporting Procedure

13.3.1 Site

13.3.1.1 Serious Adverse Events

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in Section 5 of the ISF.

AESI and SAEs which may be attributed to the trial protocol, are defined as serious and which require reporting as an SAE (excluding events listed in section 13.1.3) should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4.0.

On becoming aware that a patient has experienced an SAE or an AESI, the Investigator (or delegate) must complete, date and sign an SAE Form. The SAE Form should be faxed together with a SAE Cover Sheet to the NOSTRA-Feasibility Study Office using one of the numbers listed below or alternatively email the SAE Form, replicating the Cover Sheet details, to the email address provided below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, email the SAE Form with an SAE Cover Sheet to :

reg@trials.bham.ac.uk

Include "NOSTRA SAE" in the subject line

On receipt, the NOSTRA-Feasibility Study Office will allocate each SAE or AESI a unique reference number. This number will be transcribed onto the SAE Cover Sheet which will then be e-mailed back to the site or will be recorded in an email sent from the trial mailbox as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the NOSTRA-Feasibility Study Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE or AESI. The SAE Cover Sheet completed by the NOSTRA-Feasibility Study Office must be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the NOSTRA-Feasibility Study Office in the post and a copy kept in the ISF.

Investigators should also report SAEs to their own Trust in accordance with local practice.

13.3.1.2 Provision of follow-up information

Patients should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form (refer to the SAE Form Completion Guidelines for further information).

13.3.1.3 Study Office

On receipt of an SAE Form seriousness and causality will be determined independently by a Clinical Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the study treatment will be regarded as a related SAE. The Clinical Coordinator will also assess all related SAEs for expectedness. If the event is unexpected (i.e. is not defined in the protocol as an expected event) it will be classified as an unexpected and related SAE.

13.3.2 Reporting to the Research Ethics Committee

13.3.2.1 Unexpected and Related Serious Adverse Events

The NOSTRA-Feasibility Study Office will report all events categorised as Unexpected and Related SAEs to the Research Ethics Committee (REC), Sponsor and Manufacturer (Roche) within 15 days.

13.3.2.2 Other safety issues identified during the course of the study

The REC will be notified immediately if a significant safety issue is identified during the course of the study.

13.3.2.3 Investigators

Details of all Unexpected and Related SAEs and any other safety issue which arises during the course of the study will be reported to Principal Investigators. A copy of any such correspondence should be filed in the ISF.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Collection

The Case Report Form (CRF) will comprise the following forms:

Table 5: NOSTRA-Feasibility Study Case Report Forms

Form	Summary of data recorded	Schedule for submission
Eligibility Checklist	Confirmation of eligibility, lists inclusion and exclusion criteria	Return paper form as soon as possible after registration
Registration	Patient details, registering Investigator and site optional consent for blood samples	As soon as possible after registration/ study entry
Baseline	ECOG performance status, clinical tumour measurements, and other study related assessments. Details of biopsy (with copy of diagnostic biopsy report) and details of planned surgery	Within 1 month of registration
Tumour Bed Core Biopsy - Diagnosis	To map original position of tumour	As soon as possible after registration
End of Treatment Cycle 3	Details of LVEF assessment and optional clinical assessment (caliper measurements) and MRI scan	As soon as possible following completion of cycle 3, or after registration if patient is registered after this point
End of Neoadjuvant Treatment	Summary of neoadjuvant chemotherapy and anti-HER2 treatment, results of LVEF, clinical tumour measurements, details of axillary US scan and FNA biopsy, details of reported adverse events and toxicity	Within 1 month of completion of neoadjuvant treatment
Radiology Assessment	Results of radiological assessment of tumour, radiological response and details of MRI sequencing.	Completed at baseline, end of neoadjuvant treatment and if patient is imaged mid-neoadjuvant treatment. Form to be returned within 1 month of assessment taking place
Neoadjuvant Treatment Discontinuation	Summary of neoadjuvant treatment and reasons and date for treatment discontinuation	As soon as possible after patient discontinues study treatment
Tumour Bed Core Biopsy	Summary of number or biopsies and location of biopsies	Within 1 month of trial tumour bed core biopsies
Tumour Bed Core Biopsy-Histopathology	Summary of histopathology of Tumour Bed Biopsies	Within 1 month of tumour bed core biopsies
Tumour Bed Core Biopsy Adverse Event	Details of reported AEs	As soon as possible after patient experiences an AE
Central Histopathology Review	Document pCR and RCB score and concordance between local and central histopathology review	N/A (to be completed in-house by Study Pathologist)

Tissue Sample Collection	Tissue sample collection details (type and timepoint of tissue collection, number of blocks/slides, date of biopsy and sample dispatch details e.g. date of dispatch)	To be sent with the tissue samples
ctDNA Blood Sample Collection	Blood sample collection details (timepoint of blood sample collection, date and time of collection and dispatch)	To be sent with the blood samples
Surgery	Full details of surgery and tumour histology if applicable	Within 1 month of completion of all surgery
Surgical Resection Histopathology Form	Details of surgical resection histopathology from the pathology report	Within 1 month of completion of all surgery
Adjuvant Treatment	12 month follow-up collecting late toxicity and survival data, summary of adjuvant trastuzumab treatment and radiotherapy treatment	Within 1 month of completion of 12 month follow-up and adjuvant treatment
Relapse- Distant	Details of distant relapse	Immediately upon discovering that a patient has relapsed
Relapse- First Locoregional	Details of local relapse	Immediately upon discovering that a patient has relapsed
New Primary Cancer	Details of new primary cancers	Immediately upon discovering that a patient has a new primary cancer
Death	Date and cause of death	Immediately upon notification of patient's death
Deviation	Completed in the event of a deviation from the protocol capturing date and type of deviation	Immediately upon discovering deviation
Withdrawal of Consent	Used to notify the NOSTRA-Feasibility Study Office of patient withdrawal from the study	Immediately upon patient withdrawal
Serious Adverse Event	See section 12	Immediately on discovering that the patient has experienced an SAE/AESI
Pregnancy Notification	See section 12	As soon as possible on discovering that the patient or their partner are pregnant

The CRF must be completed, signed and dated and returned to the Study Office by the Investigator or an authorised member of the site research team (as delegated on the SS&DL) within the timeframe listed above. Entries on the CRF should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

Data reported on each form should be consistent with the source data. Any discrepancies between the CRF and source data should be explained. If any information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

The completed originals should be sent to the Study Office and a copy filed in the ISF.

Trial forms may be amended by the NOSTRA-Feasibility Study Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

SAE reporting will be paper-based. Paper CRFs must be completed, signed and dated by the Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log) and returned to the NOSTRA-Feasibility Study Office within the timeframe listed above. The exceptions to this are the SAE Form, Deviation Form and Withdrawal of Consent Form which must be co-signed by the Investigator. Entries on the CRF should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

14.2 Archiving

It is the responsibility of the Principal Investigator to ensure all essential study documentation and source records (e.g. signed Informed Consent Forms, ISFs, Pharmacy Files, patients' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 10 years after the end of the study or following the processing of all biological material collected for research, whichever is the later. Do not destroy any documents without prior approval from the CRCTU Document Storage Manager.

15 QUALITY MANAGEMENT

15.1 Site Set-up and Initiation

All sites will be required to sign a Clinical Study Site Agreement and complete a Site Registration Form prior to participation. In addition, the Principal Investigator and all recruiting Investigators (consultant level doctors) will be asked to sign an Investigator Registration Form and supply a current signed curriculum vitae (CV) and proof of up to date Good Clinical Practice (GCP) training from within the last 3 years to the NOSTRA-Feasibility Study Office. Site staff who undertake key roles for the trial (e.g., main site contact, lead Research Nurse, Lead Pathologist, Lead Oncologist, Lead Surgeon, Lead Radiologist) or those who need access to the electronic Remote Data Capture System will be required to complete a Site Staff Registration Form. All members of the site research team will also be required to sign the Site Signature and Delegation Log. All documents should be returned to the Study Office as soon as possible to expedite site set-up.

Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend an initiation session (usually held remotely) which will cover aspects of the trial design, protocol procedures, Adverse Event reporting, collection of samples, collection and reporting of data and record keeping. Sites will be provided with an ISF and a Pharmacy File (if opening Pharmacy too) containing essential documentation, instructions, and other documentation required for the conduct of the study.

The NOSTRA-Feasibility Study Office must be informed immediately of any change in the site research team. The applicable Registration Forms and an updated Site Signature and Delegation Log should be supplied.

15.2 Site Monitoring Visits

Monitoring will be carried out as required following a risk assessment and as documented in the NOSTRA-Feasibility Study Quality Management Plan. In certain circumstances remote monitoring visits may be performed as an alternative to on-site monitoring visits. Additional on-site monitoring visits may be triggered for example by poor CRF return, poor data quality, low SAE reporting rates, excessive number of patient withdrawals or deviations. This may be at the request of the Trial Management Group or the Directors of the CRCTU. If a monitoring visit is required, the NOSTRA-Feasibility Study Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the NOSTRA-Feasibility Study staff access to source documents as requested.

15.3 Central Monitoring

Where a patient has given explicit consent, sites are requested to send in copies of signed Informed Consent Forms for in-house review.

Study staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Study staff will check incoming CRFs for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the TMG who may recommend that the Trial Steering Committee (TSC) is notified of the event in addition to the CRCTU Directors and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the study protocol to the Sponsor and REC.

15.4 Audit and Inspection

The Investigator will permit study-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Sites are also requested to notify the NOSTRA-Feasibility Study Office of any inspections.

15.5 Notification of Serious Breaches

In accordance with Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 and its amendments the Sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of:

- The conditions and principles of GCP in connection with that study or;
- The protocol relating to that study, within 7 days of becoming aware of that breach

For the purposes of this regulation, a “serious breach” is a breach which is likely to effect to a significant degree:

- The safety or physical or mental integrity of the subjects of the study or
- The scientific value of the study

Sites are therefore requested to notify the NOSTRA-Feasibility Study Office of a suspected study related serious breach of GCP and/or the study protocol. Where the NOSTRA-Feasibility Study Office is investigating whether or not a serious breach has occurred sites are also requested to cooperate with the study staff in providing sufficient information to report the breach to the Sponsor and REC where required and in undertaking any corrective and/or preventive action.

16 END OF STUDY DEFINITION

The end of study will be 6 months after the last patient's last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The NOSTRA-Feasibility Study Office will notify the Sponsor and REC that the study has ended and a summary of the clinical study report will be provided within 12 months of the end of study.

17 STATISTICAL CONSIDERATIONS

17.1 Definition of Outcome Measures

17.1.1 Primary Outcome Measures

The observed number of patients with false negative biopsies as a proportion of all evaluable patients; False negative biopsies are defined as the number of patients in which all core biopsies show no residual tumour but their surgical specimen does contain residual tumour.

17.1.2 Secondary Outcomes Measures

- Concordance between local and central histopathology reporting of pCR; is defined as the number of patients whose initial local pathological assessment of pCR is confirmed by Central Histopathology Review.
- Compliance with neoadjuvant and adjuvant treatment; will be assessed by calculating relative dose intensity taking into account of both reductions in dose and delays to treatment.
- Time to local recurrence; defined as time in whole days from date of registration to local recurrence or death from any cause. Patients who are alive and without local recurrence at the time of analysis will be censored at the date last seen.
- Time to distant recurrence; defined in whole days from date of registration to distant recurrence or death from any cause. Patients who are alive and without distant recurrence at the time of analysis will be censored at the date last known to be alive.
- Overall survival; defined in whole days as the date of registration to death from any cause. Patients alive at the time of analysis will be censored at the date last seen.
- Re-evaluation of the primary outcome using the Central Histopathological Review determination of RCB to define false negative biopsies as RCB-0 or 1 (no tumour or minimal residual disease) in the core biopsies but RCB-2 or 3 in the surgical specimen. This will be reported as a proportion of all recruited patients
- Ability of the axillary lymph node assessments post-neoadjuvant chemotherapy to identify definitive axillary lymph node involvement determined by surgery histopathology. Sensitivity, specificity and false negative rates will be reported.

17.2 Analysis of Outcome Measures

17.2.1 Primary Outcome Measures

To be considered evaluable for the inclusion in the primary outcome analysis registered patients must meet the following criteria

a. Patient completed at least 2 cycles of chemotherapy
b. US scan directed tumour bed core biopsies taken and pathology reported locally
c. Surgery conducted and pathology reported locally

The primary research question aims to establish whether multiple US scan directed tumour bed core biopsies can detect residual tumour following dual-targeted neoadjuvant treatment with a proportion of false negative biopsies of no greater than 10%. The proportion of false negatives will be reported with 95% CI calculated using the Clopper-Pearson exact methodology. To conclude that the proportion of false negative biopsies is less than 10% the upper limit of the CI needs to be less than 10%.

17.2.2 Secondary Outcome Measures

Concordance between local and central histopathology reporting of pCR will be assessed using the Cohen's Kappa measure of agreement with 95% confidence intervals presented.

Time to local recurrence, time to distant recurrence and overall survival will be calculated for all patients and presented using Kaplan Meier methods. One year rates for all time to event outcomes will be presented with 95% confidence intervals.

Re-evaluation of the primary outcome using RCB and lymph node assessments will be reported as proportions using the same methodology described for the primary outcome.

Compliance with neoadjuvant and adjuvant treatment will be assessed using relative dose intensity. This will be calculated for each patient regardless of which treatment regimen they receive and will be reported using means with standard deviations, median with inter quartile ranges and range. Relative dose intensity will be presented both separately for each regimen and for the trial population as a whole.

17.3 Planned Final Analyses

Analysis of the primary and available secondary outcomes will be performed once the last patient has completed surgery. Analysis of compliance with treatment will be performed once all patients have completed treatment and time to event analysis will be conducted once all patients have completed follow-up.

17.4 Sample Size Calculation

The sample size is based on the primary outcome measure of proportion of false negative biopsies. Sample size tables for clinical studies software was used to calculate the number of patients needed to assess the prevalence of false negative tumour bed core biopsies with a reasonable degree of accuracy, based on the calculation of a single proportion in samples from a finite population(59).

The sample size calculates how many patients are required to access both that the estimate of the proportion of false negative biopsies is less than 10% and that the upper limit of the 95% confidence interval (CI) of the estimate is less than 10%. It is expected that 30% of patients will have residual disease following neoadjuvant treatment and that a maximum level of 10% for false negative tumour bed biopsies would be clinically acceptable to proceed to a phase III trial. Given these criteria and prevalence, this equates to the proportion of false negative biopsies being 4%.

Three assumptions were made within the sample size calculation; (1) the estimate of the false negative proportion, (2) the width of the CI and (3) the level of certainty required. The estimate of the false negative proportion has been specified at 4%. The width of the CI has been set at 7% ensuring that that the upper bound of the CI is less than 10% and the level of certainty is set at 95%. 135 patient are required and allowing for the possibility that 10% of patients may be unevaluable for the primary outcome the feasibility study aims to recruit 150 patients.

18 TRIAL ORGANISATIONAL STRUCTURE

18.1 Sponsor

The study is sponsored by the University of Birmingham.

18.2 Coordinating Centre

The study is being conducted under the auspices of the CRCTU, University of Birmingham according to their local procedures.

18.3 Trial Management Group

The Chief Investigator, Co-investigators, Trial Statistician, Senior Trial Manager and Trial Coordinator will form the TMG (members are identified in the introductory pages). Other key trial personnel may be invited to join the Trial Management Group as appropriate to ensure representation from a range of professional groups.

The Trial Management Group will operate in accordance with a trial specific charter based upon the template created by the Damocles Group.

Notwithstanding the legal obligations of the sponsor and Chief Investigator, the Trial Management Group will be responsible for the day-to-day running and management of the trial, such as clinical set-up, promotion, on-going management of the study, the interpretation of the results and preparation and presentation of relevant publications. Day-to-day decisions will be made by two or more members of the Trial Management Group, as pertinent to their role within the trial and the decision to be taken, on behalf of the Trial Management Group.

The Trial Management Group will meet formally every 3 months (usually by teleconference) during recruitment. Thereafter the formal Trial Management Group meetings may become less frequent, but it is anticipated that a formal report will be sent to Trial Management Group members at least annually. Meetings may be face-to-face or remote.

18.4 Trial Steering Committee

An independent Trial Steering Committee will be set up to oversee the conduct of the study. The Committee will be chaired by an independent Chair. Membership will include of the TMG, independent clinicians, invited Principal Investigators, representatives from the funders, an independent chair and at least one patient advocate. Selected members of the Trial Management Group including the Chief Investigator and Trial Biostatistician will report to the Trial Steering Committee. A secretariat will be provided by the Trial Coordinator.

The Trial Steering Committee will operate in accordance with a trial specific charter based upon the template created by the Damocles Group.

The Trial Steering Committee will meet shortly before commencement of the study and then every 6 months (usually by teleconference).

The Trial Steering Committee will supervise the conduct of the study, monitoring progress including recruitment, data completeness, losses to follow-up, and deviations from the protocol. They will make recommendations about conduct and continuation of the study to the sponsor.

19 FINANCE

NOSTRA-Feasibility is a clinician-initiated and clinician-led study funded by Roche and endorsed by Cancer Research UK Clinical Research Committee. The study has been independently peer reviewed and has been adopted by the National Institute for Health Research (NIHR) Clinical Research Network (CRN) Portfolio. No individual payment will be made to participants taking part in this trial and NHS Trusts will receive a per participant payment for research costs incurred while taking part in this trial and as in detailed in the Model Agreement for Non-Commercial Research.

20 ETHICAL CONSIDERATIONS

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964, amended at the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996.
(website: http://www.wma.net/en/30publications/10policies/b3/index.html)

The trial will be conducted in accordance with: the UK Policy Framework for health and social care research, 2017; the applicable UK Statutory Instruments (which include the Medicines for Human Use (Clinical Trials) regulations 2004 and subsequent amendments; and the Data Protection Act (2018)) and Good Clinical Practice (GCP). The protocol will be submitted to and approved by the Research Ethics Committee and Health Research Authority prior to circulation.

Before any patients are enrolled into the study, the Principal Investigator at each site is required to obtain local Research and Development (R&D) approval. Sites will not be permitted to enrol patients until written confirmation of R&D permission is received by the NOSTRA-Feasibility Study Office.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

21 CONFIDENTIALITY AND DATA PROTECTION

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act (2018). The University of Birmingham will act as the Data Controller for this trial.

Further information about how data is handled by the University of Birmingham and the CRCTU can be found within the CRCTU Privacy Notice (https://www.birmingham.ac.uk/crctu).

With the patient's consent, their initials, ethnicity, hospital number and date of birth will be collected at registration. Patients will be identified using only their unique TNO, initials, and date of birth on the CRFs and correspondence between the NOSTRA-Feasibility Study Office and the participating site. However, patients are asked to give permission for the NOSTRA-Feasibility Study Office to be sent a copy of their signed Informed Consent Form which will not be anonymised. This will be used to perform in-house monitoring of the consent process and may also be forwarded to other health care professionals involved in the treatment of the patient (e.g. patient's GP).

The Investigator must maintain documents not for submission to the NOSTRA-Feasibility Study Office (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

The NOSTRA-Feasibility Study Office will maintain the confidentiality of all patients' data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent for data transfer (e.g. Cancer Registries, laboratory staff). Representatives of the NOSTRA-Feasibility Study team may be required to have access to patient's notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

22 INSURANCE AND INDEMNITY

The University of Birmingham has in place Clinical Trials indemnity coverage for this trial which provides cover to the University for harm which comes about through the University's, or its staff's, negligence in relation to the design or management of the trial and may alternatively, and at the University's discretion provide cover for non-negligent harm to participants.

With respect to the conduct of the trial at site and the clinical care of the participant, responsibility for the participant remains with the NHS organisation responsible for the site and is therefore indemnified through the NHS Litigation Authority.

The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for participant compensation.

23 PUBLICATION POLICY

Results of this study will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the TMG and authorship will be determined by mutual agreement. A lay summary of the results will be published on ISRCTN, Cancer Research UK website, and the CRCTU Trials website.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the study was performed with the support of University of Birmingham. Intellectual property rights will be addressed in the Clinical Study Site Agreement between Sponsor and site.

24 REFERENCE LIST

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APPENDIX 1: EASTERN CO-OPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

APPENDIX 2: TNM STAGING SYSTEM FOR BREAST CANCER, STAGE GROUPING AND ELIGIBILITY

Definitions of TNM Staging System for Breast Cancer Primary Tumour (T)

Category	Description
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma *in situ*: intraductal carcinoma, lobular carcinoma *in situ*, or Paget's disease* of the nipple with no tumour
T1	Tumour ≤2 cm in greatest dimension
T1mic	Micro-invasion ≤0.1cm in greatest dimension
T1a	Tumour >0.1 but ≤0.5 cm or less in greatest dimension
T1b	Tumour >0.5 cm but ≤1 cm in greatest dimension
T1c	Tumour >1 cm but ≤2 cm in greatest dimension
T2	Tumour >2 cm but ≤5 cm in greatest dimension
T3	Tumour >5 cm in greatest dimension
T4	Tumour of any size with direct extension to (a) chest wall or (b) skin, only as described below.
T4a	Extension to chest wall, not including pectoralis muscle
T4b	Oedema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
T4c	Both (T4a and T4b)
T4d	Inflammatory carcinoma

Note

* Paget's disease associated with a tumour is classified according to the size of the tumour.

Regional Lymph Nodes (N)

Category	Description
NX	Regional lymph nodes cannot be assessed (for example, previously removed)
N0	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral axillary lymph node(s)
N2	Metastasis to ipsilateral axillary lymph node(s) fixed or matted, or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident* axillary lymph node metastasis
N2a	Metastasis in ipsilateral axillary lymph nodes fixed (or matted) to one another or to other structures
N2b	Metastasis only in clinically apparent* ipsilateral internal mammary nodes *and* in the absence of clinically evident axillary lymph node metastasis
N3	Metastasis to ipsilateral infraclavicular lymph node(s) with or without axillary lymph node involvement, or in clinically apparent* ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary or internal mammary lymph node involvement; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

Notes

* Clinically apparent is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination or grossly visible pathologically.

Distant Metastasis (M)

Category	Description
MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis*
M1	Distant metastasis

Notes

* Asymptomatic patients with no clinical sign of distant metastases should be regarded as MO unless staging investigations have been performed

Stage and Grouping and Eligibility

Stage Grouping	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0	N1*	M0
	T1	N1**	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2*	M0
	T1	N2**	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Note:
T1c to T4d are eligible for the NOSTRA-Feasibility Study.

APPENDIX 3: HISTOPATHOLOGY AND ASSESSMENT OF HISTOPATHOLOGICAL RESPONSE

Histopathological Examination

Histopathological assessment will be carried out at diagnosis and repeated on the definitive surgical specimens; either wide local excision or mastectomy, and axillary clearance. These specimens will be handled and reported according to national guidelines for non-operative procedures and for pathology specimen handling (NHS BSP Publication No 50. 2001 and NHS BSP Publication No 58, 2005). It should be noted that the macroscopic search for tumours which have undergone significant pathological response will be aided by clear communication of the original sites of the tumour, on a copy of each of the Radiology Assessment Forms, and histology request form and especially by marking the sites with clip(s) prior to neoadjuvant chemotherapy. The latter approach is recommended. Pathologists should sample this area thoroughly and record the macroscopic measurement of the tumour bed in two dimensions to enable subsequent calculation of residual cancer burden (see below).

Receptor Assays

ER and HER2 receptor assessment will be carried out routinely on all tumours at diagnosis. Assessment of ER and HER2 status should, if possible, be repeated on the definitive surgical specimen if adequate numbers of tumour cells remain. Where PgR assays are carried out routinely this information will also be collected at diagnosis and again at definitive surgery. ER, PgR and HER2 will be reported as per UK National Guidelines (NHS BSP Publication No 58, 2005).

Grading of Pathological Response

Ideally, a comment as to the degree of chemotherapy effect should be included in the pathology report. However histopathological response will also be assessed centrally in blinded fashion (i.e. blinded to treatment assignments) by the Central Pathology Reviewer(s) using H&E slides from blocks taken at surgery (tumour +/- lymph nodes), see Section 7.0. The primary endpoint for the trial is pathological complete response of all invasive disease in the tumour and nodes following neoadjuvant treatment, and is defined below:

pCR rates (tumour and lymph nodes) after neoadjuvant chemotherapy defined as no residual invasive carcinoma within the breast (DCIS permitted) AND no evidence of metastatic disease within the lymph nodes (14).

Histopathological tumour response following neoadjuvant chemotherapy will be evaluated by the Central Pathology Reviewer(s) using the following system (60):

Grade 1: some alteration to individual malignant cells but no reduction in overall numbers compared with the pre-treatment core biopsy.
Grade 2: a mild loss of invasive tumour cells but overall cellularity still high
Grade 3: a considerable reduction in tumour cells up to an estimated 90% loss
Grade 4: a marked disappearance of invasive tumour cells such that only small clusters of widely dispersed cells could be detected
Grade 5: no invasive tumour cells identifiable in the sections from the site of the previous tumour, i.e. only *in situ* disease or tumour stroma remain.

In addition, Residual Cancer Burden (RCB) will be calculated (61).

Residual Cancer Burden

The concept of residual cancer burden has been developed and validated as a complementary system of defining the extent of disease after neoadjuvant treatment and takes into account multiple variables and using a mathematical algorithm arrives at a score which is related to prognosis. The RCB index is a continuous numerical variable but can be categorised into distinct classes of response, RCB-0 to RCB-3. RCB-0 corresponds to pCR with no *in situ* disease, (ypT0 ypN0), RCB-I minimal residual disease, RCB-II moderate residual disease, and RCB-III extensive residual disease. The distant DFS for RCB-0 and RCB-1 is similar and favourable with poor outcomes for RCBIII and intermediate with RCBII (61). RCB index and class can be calculated using an on line tool

(www.mdanderson.org/breastcancer_RCB). The parameters required are, extent of disease as two dimensional tumour bed measurements. Overall cellularity as a percentage, percentage of *in situ* component, number of lymph nodes with residual disease and the diameter of the largest nodal metastasis. Details for the methodology for obtaining these parameters are published in the online appendix (13). RCB index and class will be calculated for all cases and secondary analysis of long term outcomes performed using RCB class.

APPENDIX 4: DEFINITION OF ADVERSE EVENTS

Adverse Event
Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
Comment:
An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.

Adverse Reaction
All untoward and unintended responses to an IMP related to any dose administered.
Comment:
An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event
Any untoward medical occurrence or effect that at any dose:

- Results in death unrelated to original cancer
- Is life-threatening*
- Requires hospitalisation** or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

Comments:
The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.
* Life threatening in the definition of an SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.
*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Serious Adverse Reaction
An Adverse Reaction which also meets the definition of a Serious Adverse Event.

Suspected Unexpected Serious Adverse Reaction
A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information.
A SUSAR should meet the definition of an AR, UAR and SAR.

Unexpected Adverse Reaction
An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product).
When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.

APPENDIX 5: COMMON TOXICITY CRITERIA GRADINGS

Toxicities will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. The full CTCAE document is available on the National Cancer Institute (NCI) website, the following address was correct when this version of the protocol was approved: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

APPENDIX 6: WMA DECLARATION OF HELSINKI

World Medical Association Declaration of Helsinki

Recommendations guiding physicians in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly Helsinki, Finland, June 1964 and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, 35th World Medical Assembly, Venice, Italy, October 1983, 41st World Medical Assembly, Hong Kong, September 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the WMA binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the WMA has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.
12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE

(Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.
4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

NOSTRA-Feasibility

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