Response to Optimal Selection of neo-adjuvant Chemotherapy in Operable breast cancer:

A randomised phase III, stratified biomarker trial of neo-adjuvant 5-Fluorouracil, Epirubicin and Cyclophosphamide vs Docetaxel and Cyclophosphamide chemotherapy

Sponsor: University of Birmingham

Version No.: 4.0a
Version Date: 29th October 2018

EudraCT Number: 2013-004307-39
Sponsor Number: RG_13-090
CRCTU Number: BR3044
IRAS Number: 129176
ISRCTN Number: ISRCTN15094808
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Enquiries
☎ 0121 414 3797  📞 0121 414 8392  ✉️ ROSCO@trials.bham.ac.uk

Randomisation
☎ 0121 414 3797 or 0121 414 2802 (9.00 am till 5.00 pm Monday to Friday)

Serious Adverse Event Reporting
☎ 0121 414 8392 or 0121 414 3700

CEP17/TOP2A Central Testing
HER2 Team, Cellular Pathology, Heart of England NHS Foundation Trust, Bordesley Green East Birmingham. B9 5SS
☎ 0121 424 2784

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This trial will be conducted in facilities funded through Birmingham Science City: Translational Medicine Clinical Research Infrastructure and Trials Platform, an Advantage West Midlands (AWM) funded project which forms part of the Science City University of Warwick and University of Birmingham Research Alliance.
ROSNO Trial Protocol version 4.0a, 29th October 2018

This protocol has been approved by:

Name: Prof Daniel Rea
Trial Role: Chief Investigator
Signature: [Signature]
Date: 31/09/2018

This protocol describes the ROSCO trial and provides information about procedures for patients taking part in the ROSCO trial. The protocol should not be used as a guide for treatment of patients not taking part in the ROSCO trial.
## Amendments

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

<table>
<thead>
<tr>
<th>Protocol Amendment Number</th>
<th>IRAS Amendment Number</th>
<th>Date of Amendment</th>
<th>Protocol Version Number</th>
<th>Type of Amendment</th>
<th>Summary of Amendment</th>
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<tr>
<td>1</td>
<td>1</td>
<td>05-Jan-2015</td>
<td>1.0</td>
<td>Substantial</td>
<td>Schedule of assessments updated to include vital signs. Eligibility criteria amended. Physical exam, biochemical, and haematological screens during treatment were included. Concomitant administration of live vaccines was prohibited.</td>
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<tr>
<td>2</td>
<td>8</td>
<td></td>
<td>2.0</td>
<td>Substantial</td>
<td>Several eligibility criteria have been reworded for the purpose of clarity. The wording of the outcome measures has been refined and an additional outcome measure for dose intensity of chemotherapy has been added. The use of subcutaneous trastuzumab has been included. The dose banding guidance has been updated. The requirement to perform the prothrombin time test has been removed throughout the protocol. A new section on the Quality Assurance of the Biomarker has been added. The requirements for tissue collection have been amended. The mechanism of assessing the primary outcome measure on central review has been amended. The data collection section has been amended to reflect amendments to the Case Report Form and the retrospective introduction of remote electronic data capture. The end of trial definition has been amended slightly. Other non-substantial amendments to update contact details or to provide clarity are also included.</td>
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<tr>
<td>3</td>
<td>12</td>
<td>07-Mar-2018</td>
<td>3.0</td>
<td>Substantial</td>
<td>Guidance on the use of pertuzumab whilst on trial treatment has been added. The addition of persons responsible for obtaining informed consent has</td>
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</table>
Clarification has been provided regarding reporting bilateral tumours.

Additional text clarifying interim biopsy collection for patient’s crossing over treatment arms.

The data collection text has been amended back to the version 2 protocol text and reference to electronic randomisation has also been removed from the protocol.

QoL collection time point has been amended post-surgery to the correct duration.

Other non-substantial changes to update contact details and amend typographical errors have been included.

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<th>N/A</th>
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<th>29-Oct-2018</th>
<th>4.0a</th>
<th>Notification</th>
<th>Change in Data Protection Regulations</th>
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Trial Synopsis

**ROSCO: Response to Optimal Selection of neo-adjuvant Chemotherapy in Operable breast cancer**

**Chief Investigator** Daniel Rea  
**IRAS No.** 129176

**Sponsor** University of Birmingham  
**EudraCT No.** 2013-004307-39

**Trial Design**
A multicentre, phase III, randomised (1:1) biomarker stratified, open-label, clinical trial designed to answer the following questions:

1. Is there a role for CEP17/TOP2A testing in selecting anthracycline or taxane chemotherapy as neo-adjuvant chemotherapy for early breast cancer? Specifically does CEP17/TOP2A status predict differential efficacy of anthracycline or taxane based therapy as assessed by measurement of a statistically significant treatment-biomarker interaction?

2. Is Sentinel Lymph Node Biopsy (SLNB) post neo-adjuvant chemotherapy in patients with biopsy proven ipsilateral axillary lymph node metastasis at diagnosis sufficiently sensitive to replace routine axillary node clearance?

**Primary Outcome Measure**
- Complete pathological response (pCR) rate

**Main Secondary Outcome Measures**
- Rates of breast conservation
- Radiological response in breast alone
- Sensitivity of SLNB following neo-adjuvant chemotherapy
- Quality of Life
- Clinical response in breast alone
- Tolerability and toxicity of treatment
- Survival
- Health economics

**Population and Sample Size**  
1050 patients with early operable breast cancer

**Main Eligibility Criteria**

**Inclusion**
- Patient with histological diagnosis of invasive breast cancer
- Suitable for neo-adjuvant chemotherapy
- Radiological size ≥20 mm by ultrasound
- Suitable for and fit to receive protocol specified trial chemotherapy
- Any Human Epidermal Growth Factor Receptor 2 (HER2) status
- Availability of embedded paraffin tumour blocks from pre-chemotherapy biopsy is required

**Exclusion**
- ‘Luminal A’ type tumours defined as tumours of low or intermediate grade (Grade 1 or 2), HER2 negative, which are also ER rich and progesterone receptor (PgR) rich or PgR unknown whatever the size or nodal status
- Previous breast cancer
- Unequivocal metastatic disease
- Uncontrolled hypertension, coronary heart disease, other significant cardiac abnormality
- Risk factors precluding co-administration of trastuzumab and FEC75

**Inclusion for the Sentinel Lymph Node Biopsy Protocol (in addition to the above)**
- Histopathological or cytopathological confirmation of involved nodes by biopsy/fine needle aspiration of ipsilateral axillary lymph nodes at diagnosis

**Exclusion for the Sentinel Lymph Node Biopsy Protocol (in addition to the above)**
- Negative nodes at diagnosis
- SLNB at diagnosis
- Allergy to patent blue dye
Trial Treatment:

Arm A (Control): 5-Fluorouracil 500mg/m², epirubicin 100mg/m², and cyclophosphamide 500mg/m² (FEC100)† 3 weekly x4 cycles ➔ surgery ➔ if pCR not achieved then docetaxel 75mg/m² and cyclophosphamide 600mg/m² 3 weekly x4 cycles.

Arm B: Docetaxel 75mg/m² and cyclophosphamide 600mg/m²† 3 weekly x4 cycles ➔ surgery ➔ if pCR not achieved then FEC100† 3 weekly x4 cycles.

After chemotherapy axillary node clearance +/- SLNB* will be mandatory in all patients with clinically or pathologically involved nodes prior to chemotherapy.

† All HER2 positive patients will receive Trastuzumab at 8mg/kg with first cycle of chemotherapy followed by 6mg/kg 3 weekly for 6-12 months. HER2 positive patients allocated to FEC will receive FEC75 (5-Fluorouracil 500mg/m², epirubicin 75mg/m², and cyclophosphamide 500mg/m² 3 weekly x4 cycles) to limit anthracycline exposure. * SLNB required for patients taking part in the SLNB Study.

Tumour Samples

The following tumour samples are required:

- Tumour biopsy block pre-treatment for CEP17/TOP2A analysis
- Interim biopsy block post-treatment (if applicable) but prior to surgery
- Tumour block at surgery

Optional Sub-studies

- Quality of Life and health economics
- Pharmacogenetics

Trial Duration

- Recruitment: 5 years
- Treatment duration: 12-33 weeks
- Follow-up: 5 years

Contact Details

ROSCO Trial Office
Cancer Research UK Clinical Trials Unit (CRCTU), Institute of Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham. B15 2TT
☎ 0121 414 3797 ☎ 0121 414 8392 ☮ ROSCO@trials.bham.ac.uk

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☎ 0131 777 3570 ☎ 0131 777 3520 ☮ carrie.cunningham@igmm.ed.ac.uk

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☎ 01223 348 083 ☎ 01223 348 071 ☮ caron.harvey@addenbrookes.nhs.uk
Trial Schema

**Identify Eligible Patients**
- Patient with histological diagnosis of invasive breast cancer
- Suitable for neo-adjuvant chemotherapy
- Radiological size ≥20 mm by ultrasound
- Suitable for and fit to receive protocol specified trial chemotherapy regimen
- Any HER2 status
- Availability of embedded paraffin tumour blocks from pre-chemotherapy biopsy is required

**Obtain Consent**

**Register Patient**
Call ROSCO Trial Office on 0121 414 3797/2802

**Tissue sent for CEP17/TOP2A Analysis**

**Randomisation**
Call ROSCO Trial Office on 0121 414 3797/2802
- CEP17/TOP2A status
- ER status
- HER2 status
- Nodal involvement

**Arm A: FEC**
- FEC100 3 weekly X4
  - All HER2+ve patients to receive trastuzumab and FEC75

**Arm B: Taxane**
- Docetaxel and cyclophosphamide 3 weekly X4
  - All HER2+ve patients to receive trastuzumab

**Surgery**
(to include Sentinel Lymph Node Biopsy and Axillary Node Clearance (if Fine Needle Aspirate or biopsy positive at presentation)

**Assessment of Pathological Response** (samples also sent for central review)

**Achieve Pathological Complete Response**

**Failure to Achieve Response on FEC**
- Docetaxel and cyclophosphamide 3 weekly X4
  - All HER2+ve patients to receive trastuzumab

**Failure to Achieve Response on Taxane**
- FEC100 3 weekly X4
  - All HER2+ve patients to receive trastuzumab and FEC75

**Follow-up For 5-years**
### Schedule of Events

<table>
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<tr>
<th>Assessment</th>
<th>Screening/ Baseline</th>
<th>Neo-adjuvant Chemotherapy Treatment</th>
<th>Protocol Defined Adjuvant Chemotherapy Treatment</th>
<th>Surgery</th>
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Notes:
1. Except C1
2. A biopsy should be performed after C4 for patients who do not respond to chemotherapy and require crossover neo-adjuvant treatment
3. Only if clinically indicated
4. Only if cycle 4 is not last cycle
5. Left Ventricular Ejection Fraction (LVEF) must be measured for all Human Epidermal Growth Factor Receptor 2 (HER2) positive patients by echocardiogram (ECHO) or Multi Gated Acquisition (MUGA) scan. Modality used should be consistent throughout the study.
6. HER2 positive patients who are receiving/have received trastuzumab require cardiac monitoring with ECHO or MUGA as per institutional standard
7. Radiological tumour measurement of the breast, and assessment of ipsilateral axilla: Magnetic Resonance Imaging (MRI) may be used in addition to ultrasound in accordance with local practice, or as the sole radiological technique if the tumour is not measurable by another method
8. Uninformative baseline radiological assessments do not have to be repeated at later time points
9. Required for CEP17/TOP2A analysis prior to randomisation
10. Only for those few patients where the neo-adjuvant aim of down staging to permit breast conservation has not been achieved and the investigator considers that further chemotherapy provides a realistic prospect of achieving a successful down staging effect and thus an interim biopsy is taken after cycle 4.
11. Liver function tests: Alanine Aminotransferase (ALT) or Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) and Bilirubin
12. Pregnancy test (Human Chorionic Gonadotropin urine or blood) should be performed if indicated at baseline for women of child bearing capacity
13. A full physical examination is not required at each clinic visit nor is clinical assessment of breast lesions unless there is concern or at discretion of the investigator
14. Only patients consenting to the Quality of Life and Health Economics Sub-study. Baseline QoL Questionnaire Booklets will handed out at clinic subsequent questionnaires will be sent from the ROSCO Trial Office
15. Only patients consenting to the Pharmacogenetics Translational Sub-study
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<td>ABPI</td>
<td>Association of the British Pharmaceutical Industry</td>
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<td>AE</td>
<td>Adverse Event</td>
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<td>ALP</td>
<td>Alkaline phosphatase</td>
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<td>Alanine transaminase</td>
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<td>Chromosome 17 Centromere</td>
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<td>Cancer Research UK Clinical Trials Unit</td>
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<td>CRF</td>
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<td>Clinical Trials Advisory &amp; Awards Committee</td>
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<td>CTCAE</td>
<td>Common Terminology Criteria for Adverse Events</td>
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<td>DCIS</td>
<td>Ductal carcinoma <em>in situ</em></td>
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<td>F</td>
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<td>FACT B</td>
<td>Functional Assessment of Cancer Therapy Breast Cancer</td>
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<td>Formalin-fixed paraffin-embedded</td>
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<td>Fine Needle Aspirate</td>
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<td>GCP</td>
<td>Good Clinical Practice</td>
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<td>White Blood Cell Count</td>
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1 Background and Rationale

1.1 Background

The incidence of breast cancer in the United States and Western Europe continues to rise and breast cancer remains a major healthcare problem. In 2010, 49,564 breast cancers were diagnosed in the United Kingdom (UK) (figures from Cancer Research UK). Stepwise improvements in treatment of the disease both in the adjuvant and in the metastatic setting have contributed to some recent reductions in mortality (1). However, worldwide considerable numbers of women still die of the disease, many at a young age. Although progress has been made through large adjuvant randomised treatment trials (2) this progress is by necessity slow because prolonged recruitment and follow up is required to measure adjuvant effects. By contrast, clinical trials in the neo-adjuvant setting promise faster progress, because the early endpoint of improved pathological Complete Response (pCR) at the time of surgery has been shown to correlate well with later improvement in disease free survival (DFS) for those in whom it is achieved. In addition, neo-adjuvant chemotherapy offers advantages to the individual patient, improving the rates of conservative surgery, and has therefore become a standard of care for higher risk or larger tumours for which mastectomy might otherwise be indicated (3-10).

1.2 Chemotherapy for Breast Cancer

Combination chemotherapy regimens administered as post-operative adjuvant therapy in appropriately selected patients result in a significant reduction in risk of recurrence and death from breast cancer. Anthracycline containing regimens are superior to non-anthracycline non-taxane combinations (2) and recent trials and meta-analyses indicate that the addition of adjuvant taxanes (docetaxel, paclitaxel) to anthracycline based regimes improves survival further but to a limited degree and at the cost of additional toxicity (11-16). In the light of these results with adjuvant treatment, and with the additional aim of improving breast conservation rates and cosmetic outcomes for larger tumours, pre-operative chemotherapy or neo-adjuvant chemotherapy has progressively been incorporated into the multidisciplinary treatment of early breast cancer. First demonstrated effective in rendering locally advanced disease operable, it has progressively been extended to stage 2 breast cancer not amenable to breast conserving therapy, or even to smaller tumours (1-3cms) when the cosmetic outcome of immediate surgery is expected to be poor. Neo-adjuvant chemotherapy has been evaluated in a number of prospective randomised studies and meta-analyses, and the following general principles can be derived:

- Chemotherapy used in the neo-adjuvant setting, with subsequent surgery, achieves the same results as adjuvant chemotherapy in terms of local recurrence rates, DFS and overall survival (3, 5, 8, 17, 18)
- pCR (absence of residual invasive carcinoma), is a good surrogate for prolonged DFS, and it is now used as an endpoint to compare the efficacy of different regimens (5, 8-10, 19-24). This relationship is weak for hormone receptor positive grade 1 and grade 2 Human Epidermal Growth Factor Receptor-type 2 (HER2) negative cancer (25).
- In HER2 negative disease best results in terms of pCR (20-30%) have been achieved with more prolonged regimens (26, 27) and especially with anthracycline based regimens and taxanes given sequentially (6, 28, 29) including docetaxel (30, 31) while simultaneous administration of anthracyclines and taxanes does not improve pCR rates (32-34). These improvements come at the cost of additional toxicity, and yield smaller benefits in terms of DFS. A means of selecting which patients benefit most from more prolonged therapy, and which benefit most from anthracyclines or from taxanes would markedly improve the clinical utility of the regimens.
- While a clinical response is observed in 50-70% of patients, the breast conservation rate reported can be as low as 34% among patients initially assessed as ineligible for breast conservation therapy (35).
- Improved clinical or pathological response rates remain desirable endpoints, but the incorporation of additional conventional chemotherapy agents into standard anthracycline/taxane containing regimens has not yet provided additional benefit (36).

These last points indicate that we may be nearing the limit of what can be achieved with conventional chemotherapy agents, and that significant further improvements in neo-adjuvant treatment outcomes may require incorporation of new targeted therapies (37, 38), or optimisation of conventional treatment selection on the basis of molecular characteristics of the individual patient’s tumour (39).
1.2.1 Standard Treatment

In Europe and the UK a widely used sequential anthracycline/taxane adjuvant regimen for high risk early breast cancer is based on the superior experimental arm of the influential PACS-01 adjuvant trial (16), consisting of three 3 weekly cycles of FEC (5-fluorouracil 500mg/m², epirubicin 100mg/m², cyclophosphamide 500mg/m²) and three 3 weekly cycles of docetaxel (100mg/m²). Direct evidence from formal phase II or III trials for the use of this particular regimen in the neo-adjuvant setting is lacking, and its widespread use in the neo-adjuvant setting in the UK is based on extrapolation from the adjuvant data, using the general principles of neo-adjuvant treatment described above. Nevertheless in practical terms this regimen has become an established standard of care for neo-adjuvant treatment in the UK. Similar regimens, though in some instances of slightly longer duration, are widely used throughout the world. The UK ARTemis trial is investigating the utility of adding bevacizumab to chemotherapy as neo-adjuvant treatment and utilising a chemotherapy schedule of 3 cycles of docetaxel and 3 cycles of FEC100 underscoring this regime as a widely recognised standard chemotherapy regimen.

1.2.2 Neo-adjuvant Trastuzumab

Adjuvant trastuzumab following chemotherapy is now a routine component of adjuvant treatment for HER2 positive early breast cancer. Findings from the N9831 adjuvant trial suggested the superiority of concomitant chemotherapy and trastuzumab (40).

Several trials have examined the potential benefits of concurrent trastuzumab in combination with anthracycline based or non-anthracycline based neo-adjuvant therapy in HER2 positive breast cancer, with reported pCR rates of up to 76%. Trastuzumab plus anthracycline based neo-adjuvant chemotherapy has been reported to be both effective and well tolerated, though on-going attention to the potential for cardiac toxicity is merited. The most recent data has confirmed earlier studies showing concurrent administration of anthracycline and trastuzumab in the neo-adjuvant context is safe but has also shown no difference in pCR rate when trastuzumab is administered across all cycles of a sequential taxane anthracycline regimen or delayed introduction with taxanes after anthracycline has been administered (41).

In the MDACC trial, patients with HER2 positive breast cancer received paclitaxel followed by FEC75, with or without concurrent trastuzumab (38). The pCR rate increased from 26% to 65% (p = 0.02) with trastuzumab. An expansion cohort in the experimental arm continued to show high rates of pCR (54.5%) without significant cardiac toxicity. The combined pCR rate for all patients who received trastuzumab was 60%. The Neo-adjuvant Herceptin (NOAH) trial evaluated the sequential administration of 3 cycles of doxorubicin and paclitaxel followed by 4 cycles of paclitaxel alone, then 3 cycles of cyclophosphamide, methotrexate, and 5-fluorouracil with or without concomitant trastuzumab (42). Patients with HER2 positive disease who received concurrent trastuzumab had a significantly higher pCR rate (43% vs 23%; p = 0.002), objective response rate (89% vs 77%; p = 0.02), and 3-year DFS (70.1% vs 53.3%; p = 0.007) compared with those who received chemotherapy alone. A pCR benefit with the addition of trastuzumab was also observed among inflammatory breast cancer patients (39% vs 20%; p = 0.002). In the phase III German Breast Group/Gynecologic Oncology Study Group trial (the GeparQuattro trial), patients with HER2 positive locally advanced breast cancer were treated with epirubicin/cyclophosphamide followed by docetaxel with or without capecitabine, and trastuzumab every 3 weeks. Neo-adjuvant trastuzumab plus chemotherapy demonstrated a significant increase in pCR rates (15.7% vs 31.7%; p <0.001) (43).
1.3 Biological Predictors of Response to Anthracycline or Taxane Treatment

Some biological predictors of pCR following conventional neo-adjuvant chemotherapy have been identified. In tumours with a lack of oestrogen receptor (ER) expression/null phenotype, a higher rate of pCR is reported (44), especially among triple receptor null/basal type breast cancer defined by gene expression profiling (45-48). The prognostic and predictive value of p53 has also been evaluated in a large trial (39). Gene expression profiling might also improve selection of specific chemotherapy drugs on the basis of properties of the individual patient’s tumour, with potential for further major improvement in pCR rates even with established conventional chemotherapy regimens (39). A complementary approach involves incorporation of new specifically targeted agents which lack the toxicity profile of conventional chemotherapy and can therefore be combined with conventional regimens without compromising dose. For example, the incorporation of trastuzumab into anthracycline/taxane based neo-adjuvant regimens for HER2 positive breast cancer dramatically improved pCR rates (37, 38). For HER2 negative disease no such tumour specific target has yet been identified but recently molecular signatures of tumour sensitivity to anthracycline and to taxane therapy have been identified in breast cancer trials or preclinical studies by accompanying translational science, with the aim of future optimal selection of patients most likely to benefit from treatment. Appropriate patient selection can markedly improve the cost effectiveness of expensive therapeutic agents.

Recent data from translational science using tumour samples within the NEAT study, which compared epirubicin (E) followed by cyclophosphamide methotrexate 5-fluorouracil (CMF) to CMF alone in women with early stage breast cancer, has produced striking data demonstrating that in the adjuvant context the presence of chromosome 17 centromere (CEP17) duplication is associated with benefit from epirubicin with non-amplified cases gaining no additional benefit. Data suggests assay for CEP17 duplication identifies 30% of breast cancers as anthracycline sensitive (49), this observation is confirmed by the MA5 trial (50) and a meta-analysis of similar trials with an undiluted hazard ratio (51). The assay is robust with less than 5% variation across centres in a National External Quality Assessment Scheme (NEQAS) study (52). The appropriate cut off point was validated prior to being applied to the reported analyses of clinical trial samples. This marker satisfies the appropriate criteria for a candidate diagnostic biomarker (53).

Chromosomal instability which may be the underlying mechanism for chromosomal amplification has been identified as a potential indicator of taxane resistance (54, 55). Other results suggest that a multiplex Fluorescent In Situ Hybridisation assay can predict response to taxanes. In silico modelling suggests the prospective application of such techniques could enhance pCR rates by 20-25%.

The relationship between anthracycline sensitivity and abnormal expression of topoisomerase enzymes in particular Topoisomerase 2A (TOP2A) has produced conflicting results across different trials however a recently presented more complex meta-analysis adjusted for the effect of CEP17 and other prognostic variables has now established that the presence of abnormal TOP2A gene copy in either reduced or amplified state is also associated with benefit from the use of anthracyclines (56).

Pharmacogenetic studies have also identified putative candidate genes as predictors of anthracycline and taxane sensitivity or toxicity with high levels of significance however these require further validation (57-59).
1.3.1 Pharmacogenetics and Studies Investigating Chemotherapy Toxicities

Pharmacogenetic studies have previously focused on pharmacodynamics/pharmacokinetic candidate genes. However these studies have been relatively underpowered and have relied on investigators selecting the correct candidates. A multitude of putative candidate genes have been suggested as a predictor of anthracycline and taxane response (57-59). The blood samples from ROSCO will be used to validate the findings of PGSNPS, a pharmacogenetics and prognosis genome wide association study, which is the largest study of its kind looking at the whole genome. Initial findings have revealed biomarkers associated with chemotherapy toxicities such as neurotoxicity and neutropenia reaching significance levels (p-value) of $10^{-7}$ and $10^{-8}$. The pharmacogenetic sub-study will contribute towards the development of profile of single nucleotide polymorphisms that improve prediction of treatment response and toxicity.

Recent, prospective cancer case-control studies indicate that telomere length (TL) attrition may occur post-diagnosis, possibly as a result of treatment (60). The samples from ROSCO may be used to investigate whether TL is an intermediate phenotype correlated with differences in chemotherapy side-effects and long-term prognosis.

There is increasing evidence that epigenetic changes may affect both chemotherapy toxicity and response. The samples from ROSCO may contribute to studies investigating germline epigenetic alterations and their role in these phenotypes (61).

1.4 Surgery Following Neo-adjuvant Chemotherapy

Surgical treatment following neo-adjuvant therapy aims to removal all residual viable invasive tumour and any associated pre-invasive disease. While baseline factors such as locally advanced disease, tumour location, or associated widespread pre-invasive change may impact on surgical choices. The extent of surgery also depends on the response to chemotherapy. Final surgical decisions are therefore only made after the impact of neo-adjuvant treatment has been assessed. A multidisciplinary discussion post neo-adjuvant treatment is therefore routine practice before surgical recommendations are finalised.

1.4.1 Axillary Staging and Surgery

In early breast cancers treated with surgery as the primary treatment, Sentinel Lymph Node Biopsy (SLNB) is the axillary surgical procedure of choice in patients who have a negative axillary ultrasound /biopsy (62). This is now routine practice in the UK with axillary node clearance (ANC) reserved for patients with clinically or imaging/biopsy proven nodal involvement. The false negative rate of SLNB is approximately 5% in all large series and the reported long term local recurrence appears to be as safe as ANC.

The majority of units with significant neo-adjuvant practice use SLNB to stage the axilla either before or after neo-adjuvant treatment but it is recognised that a high proportion of these women undergo ANC due to an uncertainty regarding the role of SLNB in this setting and there is great variability in practice. There is uncertainty regarding the sensitivity of SLNB post neo-adjuvant treatment in patients with clinical or biopsy proven evidence of nodal involvement at diagnosis who respond clinically to chemotherapy. These patients often currently undergo ANC, some unnecessarily, particularly given the high rates of pCR attained with current regimens.

Approaches to SLNB vary, with some authorities advocating a SLNB prior to neo-adjuvant treatment and some a post treatment neo-adjuvant biopsy. There is evidence from a large series (the ACOSOG Z1071 trial) (63) suggesting post neo-adjuvant chemotherapy SLNB may be sufficiently accurate to use as an axillary staging procedure where nodal involvement has been identified pathologically prior to chemotherapy but only if more than two sentinel nodes were recovered. If only one or two sentinel nodes were recovered the sensitivity of the procedure was substantially reduced resulting in an overall false negative rate of 12.6%. It is not clear how this data can be utilised in standard UK practice where there is biopsy evidence of nodal involvement before neo-adjuvant chemotherapy and additional data is needed. Generally current practice in this situation is to proceed to ANC. With increasing high pCR rates with modern neo-adjuvant regimens it is now necessary to determine if SLNB is sufficiently accurate for determining axillary node involvement post chemotherapy where there is biopsy proven axillary node involvement prior to initiation of neo-adjuvant treatment, thus allowing patients to avoid unnecessary axillary clearance with its associated morbidity.
1.5 Trial Rationale

The current standard practice in neo-adjuvant therapy is to expose all patients to both anthracyclines and taxanes. This means that all patients are exposed to the toxicities of both agents. It is unclear at present if this approach is needed for all patients, particularly those with tumours that express markers of sensitivity to anthracyclines such as CEP17 and TOP2A abnormalities. Similarly it is not known if tumours that do not exhibit CEP17/TOP2A abnormalities, where the added value of using an anthracycline is doubtful, would be better treated with non-anthracycline based chemotherapy. Although there is now evidence that different biological subgroups of breast cancer respond to taxanes and anthracyclines (49, 51, 64), there is no prospectively tested means of individualising chemotherapy selection. Therefore with the identification of an affordable diagnostic pathological assay for anthracycline sensitivity an opportunity exists to prospectively test if choice of chemotherapy agent based on validated biomarker assay will effectively select the patients most likely to respond optimally to an anthracycline or to a taxane. A trial to prospectively test the findings from adjuvant studies in a neo-adjuvant context provides an ideal platform to advance our understanding of how to optimise treatment in early breast cancer.

It is widely accepted that maximisation of neo-adjuvant response in HER2 positive disease is achieved by the addition of trastuzumab and that docetaxel (the only National Institute for Clinical Excellence (NICE) endorsed taxane for the treatment of early breast cancer) or fluorouracil, epirubicin and cyclophosphamide can be safely combined with trastuzumab in appropriately selected cases. Trastuzumab is now licensed for use in combination with anthracyclines in the neo-adjuvant context. The inclusion of trastuzumab in HER2 positive cases is therefore both appropriate and necessary and will ensure that subgroup comparisons between HER2 positive and negative cases is clinically relevant.

Clinical trials provide high quality data and opportunities exist to explore supplemental questions. An important surgical question for women treated with neo-adjuvant therapies with high pathological complete responses is management of the axilla where there is pre-treatment evidence of lymph node involvement. The clinical utility of a negative sentinel node in these cases remains controversial with variable recommendations and practice. ROSCO provides an opportunity to prospectively explore this question and provide robust data on the false negative rate of a clear SLNB following contemporary neo-adjuvant treatment.

The ROSCO trial has been designed to investigate these issues.

Appropriate targeting of chemotherapy in future will improve cost effectiveness and data on quality of life (QoL) will be critical to any future health technology assessment of new chemotherapy regimens.

In addition, molecular signatures of response to anthracycline and to taxane based therapy can be tested or refined by analysis of prospectively collected paraffin and fixed tissues in the context of an appropriately randomised neo-adjuvant chemotherapy trial in early breast cancer.
2 Objectives and Outcome Measures

2.1 Objectives

2.1.1 Objectives of the ROSCO Trial

The objectives of this study are to determine:

1. Is there a role for CEP17 and TOP2A testing in selecting anthracycline or taxane chemotherapy as neo-adjuvant treatment for early breast cancer? Specifically, does CEP17/TOP2A biomarker status predict differential efficacy of anthracycline or taxane based therapy as assessed by measurement of a statistically significant treatment-biomarker interaction?

2. Is SLNB post neo-adjuvant chemotherapy in patients with biopsy proven ipsilateral axillary lymph node metastasis at diagnosis sufficiently sensitive to replace routine axillary node clearance?

2.1.2 Exploratory Translational Objectives

The primary translational science objective of the ROSCO trial is to validate the use of a predictive biomarker of anthracycline sensitivity for clinical use in neo-adjuvant breast cancer. A secondary exploratory objective will be to use a tissue, Deoxyribonucleic Acid (DNA) and serum/plasma sample repository from consented patients to extend current translational research into candidate biomarkers of chemotherapy response, predictive markers of response to chemotherapy without invasive testing and pharmacogenomics to investigate potential markers of toxicity.

The successful completion of a decade long process seeking to identify predictive biomarkers of response to anthracycline based chemotherapy provides the rationale for the ROSCO trial. However, the functional pathway underlying this response in clinical breast cancer remains elusive. On-going research which continues to build on a translational network of collaborative science has identified potential additional candidate biomarkers for both taxane based and anthracycline based chemotherapy. Novel diagnostic panels (John Bartlett personal communication) are currently undergoing testing which may – during the course of the ROSCO trial – provide viable alternatives or indeed add value to CEP17/TOP2A. The establishment of a tissue bio-repository from patients recruited into ROSCO will provide an important opportunity to progressively improve our ability to predict response to neo-adjuvant chemotherapy. Samples will be collected in accordance with current guidelines (65). We intend to collect serum and plasma sequentially during and after treatment to assess the role of non-invasive markers of cell death as potential candidates to predict chemotherapy response. A number of circulating markers including intact (global cell death) and cleave caspase (apoptosis marker) and circulating DNA have been proposed as candidates for predicting response to chemotherapy. Most remain unvalidated almost entirely due to a lack of appropriate sample collections. By collecting sequential serum/plasma during treatment we will be able to test the hypotheses that circulating markers of cell death or circulating tumour DNA may predict chemotherapy response within 1-2 cycles of treatment initiation. This element of the protocol is subject to a further funding application and will not commence until funding has been secured.

A tissue and DNA repository will be assembled to perform additional investigations of candidate markers to better understand the relationship between drug selection and tumour response.
2.1.2.1 Exploratory Objectives of the Pharmacogenetics and Studies of Chemotherapy Toxicity and Response

Further exploratory translational objectives of the ROSCO study include:

- Validation of identified germline biomarkers of chemotherapy toxicity and response
- Discovery of additional germline biomarkers of chemotherapy toxicity and response
- Investigation of the relationship between telomere length and chemotherapy toxicity and response
- Exploration of the role of epigenetics in predicting chemotherapy toxicity and response

The ROSCO trial will contribute to on-going investigations centred on the development of biomarkers predictive for chemotherapy toxicity and response. The availability of these patient samples will improve the overall power, of these studies, to detect biomarkers that are truly associated with these phenotypes. The samples collected will include one sample pre-commencement of chemotherapy and then one sample on completion of neo-adjuvant chemotherapy. The development of individualised treatments for patients will require an integrative approach incorporating the influence of tumour biology, inherited germline genetics and epigenetics.

2.2 Outcome Measures

2.2.1 Primary Outcome Measure

Complete pathological response (pCR) rate: pCR will be determined in patients following treatment with neo-adjuvant chemotherapy and will be defined as no residual invasive carcinoma within the breast (Ductal Carcinoma In Situ (DCIS) permitted) and no evidence of metastatic disease within the lymph nodes. The number of patients achieving pCR as a proportion of those randomised will be presented.

2.2.2 Secondary Outcome Measures

- pCR rate in breast alone: defined by the number of patients who have no residual invasive carcinoma within the breast (DCIS permitted) as a proportion of those randomised
- Clinical response in breast alone: defined as the number of patients with complete response (CR) or partial response (PR), measured by callipers at baseline and on completion of neo-adjuvant treatment, as a proportion of the number of patients randomised. Response will be assessed using Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1, see Appendix 5.
- Radiological response in breast alone: defined as the number of patients with CR or PR, measured by ultrasound (or MRI if used at baseline when ultrasound unavailable) at baseline and on completion of treatment, as a proportion of the number of patients randomised. Response will be assessed using RECIST version 1.1, see Appendix 5.
- Rates of breast conservation: defined as the number of patients treated with breast conservation as a proportion of the total number of patients receiving surgery. The number of patients who are assessed as requiring mastectomy prior to chemotherapy and subsequently are down staged to permit a breast conserving final surgical procedure as a proportion of the total number of patients assessed as requiring mastectomy prior to chemotherapy
- Tolerability and toxicity of treatment: Tolerability will be measured in terms of the mean number of cycles received and the proportion of patients receiving all 4 cycles. Toxicity will be measured in terms of the occurrence, severity, type and causality of Adverse Events (AEs) during the first 4 cycles of neo-adjuvant treatment, for any additional neo-adjuvant treatment and for additional adjuvant treatment
- Dose intensity of chemotherapy: defined as the amount of drug administered over time will be calculated for the first 4 cycles of neo-adjuvant treatment, for any additional neo-adjuvant treatment and for additional adjuvant treatment
- Sensitivity of SLNB following neo-adjuvant chemotherapy in patients who were node positive by biopsy at outset: defined as the sensitivity rate of SLNB, that is the number of patients correctly identified by SLNB as having lymph node involvement as a proportion of all patients with confirmed lymph node involvement following axillary clearance at surgery
- Correlation between SLNB at surgery and residual tumour burden in axilla: The correlation between false negative SLNB at surgery and residual tumour burden in axilla as measured by isolated tumour cells, micrometastases, macrometastases
• Time until loco-regional recurrence: defined in whole days, as the time from randomisation until loco-regional recurrence (ipsilateral recurrence within the breast, axillary and supraclavicular fossa nodes). Patients who withdraw or who are lost to follow-up will be censored at the date last known to be alive and relapse free. Patients not having an event will be censored at the date last seen alive and relapse free or date of death if death occurred without documenting a relapse.

• Disease Free Survival (DFS): defined in whole days, as the time from randomisation until disease recurrence or death from any cause, whichever comes first. Patients who withdraw or who are lost to follow-up will be censored at the date last known to be alive and disease free. Patients not having an event will be censored at the date last seen alive and relapse free.

• Distant Disease Free Survival: defined in whole days, as the time from randomisation until first documented distant disease or death from any cause, whichever is first. Patients who withdraw or who are lost to follow-up will be censored at the date last known to be alive and distant disease free. Patients not having an event will be censored at the date last seen alive and free of distant disease.

• Overall Survival: defined in whole days as the time from randomisation until death from any cause. Patients who withdraw or who are lost to follow-up will be censored at the date last known to be alive. Patients remaining alive throughout the duration of the study will have their survival time censored on the date last seen alive.

• Quality of Life (QoL): will be determined using the Functional Assessment of Cancer Therapy Breast Cancer (FACT B) and EuroQoL EQ-5D QoL questionnaires.

• Health Economics: cost per quality adjusted life year (QALY) will be calculated using the EQ-5D questionnaire and key resource use data from Case Report Forms (CRFs).

2.2.3 Exploratory Outcome Measures

• Utility of alternative molecular predictors of differential response to treatment with different drugs regimens will be explored.

• Pharmacogenetic analysis to identify differences in toxicity and efficacy in individuals with specific gene polymorphisms.

3 Trial Design

A multicentre, phase III, randomised (1:1), biomarker stratified, open-label, clinical trial designed to test the following hypotheses:

1. A significant treatment by biomarker interaction will exist, indicating that using anthracyclines (specifically epirubicin) in optimal doses in CEP17 duplicated and/or TOP2A gene deleted or amplified cancers (subsequently referred to as CEP17/TOP2A Abnormal) whilst using taxane based therapy in those with normal CEP17 and TOP2A (subsequently referred to as CEP17/TOP2A Normal) will result in a better pCR rate than might be obtained without using the CEP17 or TOP2A biomarkers to direct therapy.

2. A negative SLNB following neo-adjuvant chemotherapy will accurately predict sterilisation of lymph node involvement in patients with demonstrated lymph node involvement prior to chemotherapy.

Patients will be randomised to initial neo-adjuvant chemotherapy with FEC or docetaxel/cyclophosphamide. The Simon biomarker stratified randomised design, including patients with both CEP17/TOP2A Abnormal and CEP17/TOP2A Normal tumours, will be used thus testing treatment efficacy in both populations. Stratification of patients according to CEP17 status is more efficient than a ‘Marker based design’ because of the relatively low prevalence (30-40%) of CEP17/TOP2A abnormal cancers. The stratified approach has adequate power to test for the interaction between CEP17/TOP2A status and treatment effect. If a non-significant interaction test is observed the design has adequate power to detect for an overall treatment effect regardless of CEP17/TOP2A status. However if a significant interaction test is observed the design allows for the treatment effect within each strata to be tested separately.

Sub-studies include:

• Quality of Life and Health Economics

• Pharmacogenetics

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4 Eligibility

4.1 Inclusion Criteria

4.1.1 ROSCO Main Trial

- Patient with histological diagnosis of invasive breast cancer
- Suitable for neo-adjuvant chemotherapy in opinion of investigator
- Unifocal tumour:
  - Radiological size $\geq 20$ mm by ultrasound (unless the lesion cannot be measured by this method in which case measurement by Magnetic Resonance Imaging (MRI) will be acceptable, see Appendix 1)
  - T4 tumour of any size with direct extension to (a) chest wall or (b) skin or both (T4a, b, or c, see Appendix 2)
  - Inflammatory carcinoma (T4d) with tumour of any size

OR

Multifocal tumour:

- The sum of each tumour’s maximum diameter must be $\geq 20$ mm (total sum of multifocal deposits $\geq 20$ mm by ultrasound)

OR

Other locally advanced disease:

- Biopsy confirmed axillary lymph node involvement or large or fixed axillary lymph nodes (radiological diameter $\geq 20$ mm or clinical N2), or ipsilateral supraclavicular nodes and primary breast tumour of any diameter
- Involvement of large or fixed axillary lymph nodes (radiological diameter $\geq 20$ mm or clinical N2), or ipsilateral supraclavicular nodes without a primary breast tumour identified: in this case the presence of breast cancer in a lymph node must be histopathologically confirmed by lymph node biopsy (trucut or whole lymph node)
- Any HER2 status
- Patient fit to receive the trial chemotherapy regimen in the opinion of the responsible clinician. The following recommendations must be taken into account when making this assessment:
  - Patients with HER2 positive disease must not have clinically significant cardiac abnormalities. Cardiac function should be assessed by physical examination and baseline measurement MUST be made of Left Ventricular Ejection Fraction (LVEF) by Multi Gated Acquisition (MUGA) scan or echocardiogram (ECHO). LVEF must be within the normal range as defined locally by the treating centre (usually at least 50%)
  - Patients must have adequate bone marrow, hepatic, renal and haematological function*
- Eastern Co-operative Oncology Group (ECOG) performance status of 0 or 1 (see Appendix 3)
- Women of child-bearing potential, or men in a relationship with a woman of child-bearing potential, prepared to adopt adequate contraceptive measures if sexually active for at least 6 months after completion of trial medication
- 18 years or older
- Written informed consent for the trial
- Availability of embedded paraffin tumour blocks from pre-chemotherapy biopsy is required
- Patient willing and able to comply with scheduled visits, treatment plan and other study procedures

Please note:

- This trial is suitable for both male and female patients
- Patients with bilateral cancer are also eligible for this trial, if the histopathology criteria above are met for disease in at least one breast

* Defined as:

Hepatic function:
1. Alanine Aminotransferase (ALT) or Aspartate Aminotransferase (AST) $\leq 1.5 \times$ Upper Limit Normal (ULN)
2. Alkaline phosphatase (ALP) $\leq 2 \times$ ULN
3. 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

Bone marrow function:
1. Haemoglobin (Hb) > 100g/L;
2. White Blood Cell (WBC) > 3 x 10⁹/L;
3. Platelets > 100 x 10⁹/L

Patients with out of range blood tests are normally ineligible. The Chief Investigator or deputy should be contacted to discuss specific cases where identifiable reasons for abnormal test results can be demonstrated which will not impact on the safe administration of trial medication.

4.1.2 Sentinel Lymph Node Biopsy Study (in addition to above)

- Histopathological or cytological confirmation of involved nodes by biopsy/fine needle aspiration of ipsilateral axillary lymph nodes at diagnosis

4.2 Exclusion Criteria

4.2.1 ROSCO Main Trial

- ‘Luminal A’ phenotype tumours defined as: tumours of low or intermediate grade (Grade 1 or 2), HER2 negative, which are also ER rich and Progesterone Receptor (PgR) rich or PgR unknown (see Appendix 4 for definition), whatever the size or nodal status
- Previous invasive breast cancer
- Unequivocal evidence of metastatic disease
- Previous diagnosis of other malignancy unless:
  - Disease-free for 5 years; or
  - Previous basal cell carcinoma, cervical carcinoma in-situ, superficial bladder tumour; or
  - Contralateral or ipsilateral DCIS of the breast treated by surgery alone
- Previous chemotherapy
- Prior extensive radiotherapy (as judged by the investigator) to bone marrow
- Previous neo-adjuvant endocrine therapy (unless less than 6 weeks duration)
- Concomitant hormonal therapies/chemotherapy or any other medical treatment in relation to treating the breast cancer with the exception of pertuzumab
- In HER2 positive patients risk factors precluding co-administration of trastuzumab and FEC75
  - Previous myocardial infarction during the 6 months prior to recruitment
  - LVEF below institutional lower limit of normal and no echocardiographic evidence of haemodynamically significant valvular heart disease or ventricular contractility
- Prior diagnosis of cardiac failure
- Uncontrolled hypertension, coronary heart disease other significant cardiac abnormality
- Bleeding diathesis
- Presence of active uncontrolled infection
- Any evidence of other disease which in the opinion of the investigator places the patient at high risk of treatment related complications
- Pregnant (female patients of child bearing potential should have a urine or blood Human Chorionic Gonadotropin test performed to rule out pregnancy prior to trial entry)
- Lactating females
- Patients who have received live vaccine within 4 weeks of the date of randomisation
- Any concomitant medical or psychiatric problems which in the opinion of the investigator would prevent completion of treatment or follow-up
4.2.2 Sentinel Lymph Node Biopsy Study (in addition to above)

- Negative nodes at diagnosis
- SLNB at diagnosis
- Allergy to patent blue dye

5 Screening and Consent

Potential patients will be identified at Multi-disciplinary Team meetings.

The majority of the screening tests defined in this protocol are standard practice and can be commenced prior to obtaining consent. However where tests do not form part of standard medical practice for this group of patients, at a specific site, consent (see Section 5.2) should be obtained prior to these tests being performed.

5.1 Screening

Screening procedures detailed below should be undertaken:

- Medical history
- Full physical examination including:
  - Height
  - Weight
- ER, PgR and HER2 status
- Radiological measurement of primary breast tumour(s) by standard practice ultrasound (unless the lesion cannot be measured by this method in which case measurement by MRI will be acceptable) and mammogram. Ultrasound should include assessment of axillary lymph node involvement 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 randomisation as close to (but before) cycle 1 as possible. For patients with bilateral disease a decision should be taken as to which side (left or right) to choose as the "Index Breast" for reporting purposes
- Fine Needle Aspirate (FNA) or core biopsy of clinically or radiologically enlarged/abnormal axillary nodes is required to define involvement
- Staging to exclude metastatic disease is in accordance with standard early breast cancer practice according to local site policy. However we recommend cross sectional imaging e.g. CT of the chest and abdomen/pelvis for patients with a significant risk of metastatic disease. Where staging is indicated this should be performed before randomisation if possible
- LVEF measured by Echocardiogram (ECHO) or MUGA within 12 weeks (provide there has been no documented cardiac event in which case the patient should be re-evaluated) prior to randomisation
- Biochemical screen to include:
  - ALT or AST
  - ALP
  - Bilirubin
  - Urea
  - Creatinine
  - Sodium
  - Potassium
- Pregnancy test: for females of child-bearing potential
- Full blood count to include:
  - Haemoglobin
  - WBC count including differential count
  - Platelets

Patients with out of range blood tests are normally ineligible. The Chief Investigator or deputy should be contacted to discuss specific cases where identifiable reasons for abnormal test results can be demonstrated which will not impact on the safe administration of trial medication.

For patients with an normal blood test result, locally advanced or inflammatory disease, or clinically involved axillary nodes, full staging is recommended and this should be performed prior to randomisation where possible. Patients can be randomised before complete staging results are available, however the results of these tests must be made available as soon as possible. If these patients are subsequently diagnosed with metastases they should continue with appropriate optimal therapy which may include completion of protocol therapy at the discretion of the investigator. Follow-up data will be collected on these patients (see Sections 7.10.9 and 11.0), and their results included in the intention to treat analysis.

The reason for screening failures should be captured on the Patient Screening/Enrolment Log which will be reviewed by the ROSCO Trial Office in order to monitor uptake and identify issues with eligibility. An Eligibility Checklist should 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. A copy of the patient's anonymised biopsy pathology report should be sent to the ROSCO Trial Office.

### 5.2 Informed Consent

It is the responsibility of the investigator to obtain written informed consent for each patient prior to performing any trial related procedure. Specialist trainees and non-training grade clinicians that have registered with the trial and have been delegated the task of consenting on the Site Signature and Delegation Log are permitted to obtain consent without the investigator's counter signature.

A Patient Information Sheet is provided to facilitate the consent process. Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time. 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 if they wish to. 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 trial without giving a reason must be respected. If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the Informed Consent Form. The investigator must then sign and date the form. 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 randomised into the trial the patient’s 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 ROSCO Trial Office for review.

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, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the patient should have the opportunity to ask questions about the trial 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 trial respected.

Electronic copies of the Patient Information Sheet and Informed Consent Form are available from the ROSCO Trial Office and should be printed or photocopied onto the headed paper of the local institution. Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log.
6 Trial Entry

6.1 Time Frame for Trial Entry
Ideally patients should be entered into the trial as soon as possible and within a maximum of 9 weeks (unless by patient choice) from the initial core biopsy result. Chemotherapy should ideally start within one week of randomisation.

6.2 Trial Entry
Entry into this trial is a three step process:
1. Registration
2. CEP17/TOP2A analysis
3. Randomisation

6.2.1 Registration
As soon as the patient is considered eligible the investigator should complete a Registration Form and call the ROSCO Trial Office on:

☎ 0121 414 3797 or 0121 414 2802
(9.00 am till 5.00 pm Monday to Friday)

The name of the investigator directly responsible for the patient’s care will be requested. Investigators must be registered with the ROSCO Trial Office before they are permitted to register patients into the trial (see Section 14.1).

The following information should be provided:

- Name of site and investigator
- Patient’s initials
- Date of birth
- Patient’s hospital number
- Date of consent
- For patients with bilateral disease, which side (left or right) will act as the “Index Breast” for reporting purposes

Registration will be performed on a paper-based system. At the end of the registration process patients will be allocated a unique TNO. The TNO will be used to identify the patient and should be recorded on the CRF and on any further correspondence with the ROSCO Trial Office. The TNO should also be documented on the original signed Informed Consent Form filed in the ISF.

Following registration (and before randomisation) patients opting to participating in the optional QoL and Health Economics Sub-study should be given a QoL Booklet (see Section 10.2). Prior to completing the baseline questionnaire a member of the Research Team should discuss the questionnaires with the patient and answer any questions they may have. Once the booklets have been completed by the patient the Site Research Team should check to make sure that all of the questions have been completed and that in particular that the date the QoL Booklet was completed has been accurately recorded.

The baseline questionnaire will be returned to the ROSCO Trial Office by the Research Nurse, using the pre-paid envelope provided, once the patient has been randomised into the trial.
6.2.2 CEP17/TOP2A Analysis

Central CEP17/TOP2A testing will be performed by the Cellular Pathology Laboratory, Heart of England NHS Foundation Trust, which is a UK NEQAS reference centre for HER2 testing. It is essential to the randomisation process that samples are provided for central CEP17/TOP2A testing in a timely fashion. Investigators should ensure that the Pathologist is made aware of the trial and included in the discussions relating to patient screening and entry.

Immediately following patient registration the investigator should arrange for a representative sample of the patient’s biopsy tissue to be sent for CEP17/TOP2A analysis to the address below:

HER2 Team
Cellular Pathology
Heart of England NHS Foundation Trust
Bordesley Green East
Birmingham B9 5SS

For patients with bilateral disease only the “Index Breast” should be sent for analysis.

Typically the sample will be analysed and the results supplied to the ROSCO Trial Office within 1 week (7 calendar days) of receipt of the sample. However if the initial CEP17/TOP2A analysis is unsuccessful the test may need to be repeated, which may cause a short delay in the communication of the result. There is a small possibility that it may not be possible to establish the patient’s CEP17/TOP2A status in which case they will not be eligible to enter the trial.

Following CEP17/TOP2A analysis samples will be transferred to the Translational Coordinating Centre, Edinburgh for long term storage in the ROSCO Biorepository.

6.2.3 Randomisation

On receipt of the patient’s CEP17/TOP2A status the ROSCO Trial Office will notify the investigator that the patient is ready to be randomised into the trial. The investigator (or a person delegated this responsibility) should randomise the patient into the trial by calling the ROSCO Trial Office on:

📞 0121 414 3797 or 0121 414 2802
   (9.00 am till 5.00 pm Monday to Friday)

The site will be asked to confirm the patient’s eligibility, and provide the following information over the telephone:

- Patient’s full name and address (if participating in the optional QoL and Health Economic sub-study)
- Patient’s National Health Service (NHS) number or in Scotland the Community Health Index (CHI)

Patients will be randomised 1:1 using a stratified block technique with variable block sizes. Stratification variables included:

- CEP17 and or TOP2A: normal, abnormal (this information will be held at the ROSCO Trial Office)
- ER status: positive, negative (as defined locally)
- HER2 status: positive, negative and unknown/equivocal (as defined locally)
- Nodal involvement: no, yes

Patients will be allocated to one of two pre-operative treatment groups:

- Arm A: 4 cycles of FEC at 3 weekly intervals
- Arm B: 4 cycles of TC at 3 weekly intervals

See Section 7.4 and 7.5 for treatment details.
On completion of the randomisation process the investigator will be notified of the patient's treatment allocation.

The ROSCO Trial Office will send the investigator formal confirmation of the patient's entry into the trial in the post and will fax confirmation of trial entry to the Responsible Pharmacist who will then be authorised to release trial mediation to the patient.

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

The completed and signed Eligibility Checklist, Registration Form, Randomisation Form, signed Informed Consent Form (if consented for copy to be sent to ROSCO Trial Office) and (if applicable) QoL Booklet should then be sent to the ROSCO Trial Office in the post.

Once patients have been randomised into the trial and prior to commencing neo-adjuvant chemotherapy a baseline blood sample should be taken for the Pharmacogenetics sub-study (see Section 10.1.3).

7 Treatment Details

Patients will be randomised to one of two pre-operative treatment groups:

- Arm A: 4 cycles of FEC at 3 weekly intervals
- Arm B: 4 cycles of TC at 3 weekly intervals

See Section 7.4 and 7.5 for details.

7.1 Investigational Medicinal Products

For the purposes of this study docetaxel (T), 5-fluorouracil (F), epirubicin (E), cyclophosphamide (C), and trastuzumab are regarded as Investigational Medicinal Products (IMPs).

Full details of the IMPs are contained in the Pharmacy Manual, which also lists the Pharmacists’ responsibilities, details of labelling, record keeping for prescribing, dispensing, and accountability of the IMPs. The ROSCO Pharmacy Manual will be sent to the responsible Pharmacist.

7.1.1 Description of Investigational Medicinal Products

7.1.1.1 Docetaxel

Docetaxel is an antineoplastic agent licensed by the European Medicines Agency (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 this 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 neo-adjuvant setting. The reverse sequence of docetaxel followed by FEC is often preferred in the neo-adjuvant context and forms the control arm of the ARTemis trial a recent National multicentre study.

Docetaxel solution for infusion was originally manufactured by Sanofi Aventis but is now off patent and is also supplied by Actavis UK Ltd and Hospira UK Ltd.

7.1.1.2 5-Fluorouracil

5-Fluorouracil is an antineoplastic agent licensed by the 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 ROSCO trial it is therefore being used within its licensed indication.

Fluorouracil is off patent and is available from a number of manufacturers.
7.1.1.3 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 ROSCO trial it is therefore being used within its licensed indication.

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

7.1.1.4 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 ROSCO trial it is therefore being used within its licensed indication.

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

7.1.1.5 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 neo-adjuvant chemotherapy followed by adjuvant trastuzumab, for locally advanced (including inflammatory) disease or tumours >20 mm in diameter. Its use in combination with FEC or with docetaxel based regimens in the specific context used in ROSCO is therefore within the licensed indication for this agent.

Trastuzumab is manufactured by Roche. Biosimilar compounds will become available during the conduct of this study.

7.1.2 Supply of Investigational Medicinal Product

Docetaxel, 5-fluorouracil, epirubicin, cyclophosphamide and trastuzumab should be prescribed from hospital pharmacy stocks. IMPs should be labelled with the Annexe 13 compliant labels supplied by the ROSCO Trial Office.

7.2 Tumour Positional Marking

An ultrasound visible marker clip should be inserted radiologically in the tumour before treatment commences. If the definitive surgical intention is mastectomy because of multi-focal disease, or inflammatory breast cancer, a marker clip is still necessary to assist the pathologist in identifying the tumour site (66).

7.3 Timing of Neo-adjuvant Treatment

Neo-adjuvant 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 1 week of randomisation. Where necessary, for local logistic reasons, chemotherapy appointments should be pre-booked in advance to minimise delay.
### 7.4 Treatment Summary

Trial treatment is summarised below and described in detail in the subsequent sections.

| **Arm A (Control):** 5-Fluorouracil 500mg/m², epirubicin 100mg/m², and cyclophosphamide 500mg/m² (FEC100)† | 3 weekly x4 cycles | surgery if pCR not achieved then docetaxel 75mg/m² and cyclophosphamide 600mg/m² 3 weekly x4 cycles. |
| **Arm B:** Docetaxel 75mg/m² and cyclophosphamide 600mg/m² ³ 3 weekly x4 cycles | surgery if pCR not achieved then FEC100 † 3 weekly x4 cycles. |

After chemotherapy axillary node clearance +/- SLNB* will be mandatory in all patients with clinically or pathologically involved nodes prior to chemotherapy.

† All HER2 positive patients will receive Trastuzumab at 8mg/kg with first cycle of chemotherapy followed by 6mg/kg 3 weekly for 6-12 months. HER2 positive patients allocated to FEC will receive FEC75 (5-Fluorouracil 500mg/m², epirubicin 75mg/m², and cyclophosphamide 500mg/m² 3 weekly x4 cycles) to limit anthracycline exposure.

* SLNB required for patients taking part in the SLNB Study.

See Section 7.6 for details of dose reductions and modifications and Section 7.7 for details of supportive care during neo-adjuvant treatment.

Surgery should proceed when the investigator is satisfied patients are sufficiently recovered from chemotherapy (see Section 7.9).

If pCR **is achieved** further FEC or TC, as allocated at randomisation, may be given at the discretion of the treating clinician (it is not recommended that more than 4 cycles of FEC are administered with concurrent trastuzumab).

Post-operatively those who have **not achieved** a pCR will cross over to the alternative chemotherapy treatment option, once wounds are healed to the satisfaction of the investigator. Those treated with FEC preoperatively will cross over to TC. Those treated preoperatively with TC will cross over to FEC.

Post-operative adjuvant chemotherapy (see Section 7.5.2) should start when wound healing and recovery from surgery is achieved to the satisfaction of the investigator.

See Sections 7.11.1 and 7.11.2 for details of adjuvant hormonal therapy and radiotherapy respectively.

### 7.5 Chemotherapy and Trastuzumab

#### 7.5.1 Neo-adjuvant Chemotherapy

##### 7.5.1.1 5-Fluorouracil Epirubicil Cyclophosphamide (FEC)

Patients will receive 4 cycles of iv 5-fluorouracil 500mg/m², epirubicin 100mg/m², and cyclophosphamide 500mg/m² (FEC100) at three weekly intervals. Drugs should be prepared and administered according to institutional standard practice (including any dose banding protocols delivering doses of within 6% of the calculated dose or the national dose banding tables developed by the NHS England’s Medicine Optimisation and Chemotherapy Clinical Reference Group – see [https://www.england.nhs.uk/commissioning/spec-services/npc-crg/group-b/b02/](https://www.england.nhs.uk/commissioning/spec-services/npc-crg/group-b/b02/) for further information). Supportive medication, dose reductions and delays are at the discretion of the investigator (see Section 7.7 and 7.6 respectively).

In all HER2 positive cases 5-fluorouracil and cyclophosphamide will be given at 500mg/m² and the epirubicin dose reduced to 75mg/m², (FEC75) and trastuzumab should be administered as described in Section 7.5.3. The doses of 5-fluorouracil and cyclophosphamide should remain unchanged in this regimen.
7.5.1.2 Docetaxel Cyclophosphamide (TC)

Patients will receive 4 cycles of intravenous (iv) docetaxel 75mg/m² and cyclophosphamide 600mg/m² at three weekly intervals. 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 or the national dose banding tables developed by the NHS England’s Medicine Optimisation and Chemotherapy Clinical Reference Group – see https://www.england.nhs.uk/commissioning/spec-services/npc-crg/group-b/b02/ for further information). Supportive medication, dose reductions and delays are at the discretion of the investigator (see Section 7.7 and 7.6 respectively).

In all HER2 positive cases trastuzumab should be administered as described in Section 7.5.3.

7.5.2 Adjuvant Chemotherapy

Cross-over to the alternative chemotherapy regimen post-operatively is protocol specified for patients who do not achieve a pCR.

For patients not achieving pCR, 4 cycles of the alternate chemotherapy schedule should be given such that patients exposed to TC in the neo-adjuvant phase are treated with FEC and those exposed to FEC in the neo-adjuvant phase will receive TC in the adjuvant phase.

Please Note: For patients not achieving pCR, who have already received 4 cycles of FEC and 4 cycles of TC prior to surgery (see Section 7.10.4) additional post-operative chemotherapy is not recommended.

For patients achieving pCR in both nodes and primary tumour further FEC or TC, as allocated at randomisation, be given at the discretion of the treating clinician. Please Note: It is not recommended that more than 4 cycles of FEC with concurrent trastuzumab are administered.

Post-operative adjuvant chemotherapy should ideally start within a 3-6 week window after surgery, when wound healing and recovery from surgery is achieved to the satisfaction of the investigator unless delayed by patient choice or by wound healing or other postoperative clinical issues. Delays of >12 weeks should be discussed with the ROSCO Trial Office. Patients should not embark on postoperative adjuvant chemotherapy unless and until they are fit to do so in the opinion of the local investigator.

7.5.3 Trastuzumab

Trastuzumab should be administered intravenously with a loading dose of 8mg/kg with the first cycle of neo-adjuvant chemotherapy and at 6mg/kg for all subsequent cycles. Dose banding of IV trastuzumab is acceptable and institutional standard practice should be followed. Sites may use subcutaneous trastuzumab as an alternative to intravenous formulation in which case fixed dosing of 600mg subcutaneously should be used throughout. Caution is advised in use of fixed dose subcutaneous dosing in patients with high body surface area due to the potential for under-dosing particularly over the early cycles of treatment. Trastuzumab should be continued following completion of trial chemotherapy at three weekly intervals to complete up to one year of treatment. Once trial chemotherapy has been completed trastuzumab may be administered as on-going intravenous therapy or as subcutaneous fixed dose schedule at sites where this mode of administration has been adopted. Note the lack of data supporting the use of subcutaneously trastuzumab for patients over 150 kilograms.

If the interval between delivery of last dose of preoperative chemotherapy and surgery is greater than 3 weeks, then a further dose of single agent trastuzumab may be given to avoid interruption of the 3 weekly trastuzumab schedule, and equally continued post-operative dosing of trastuzumab may also continue to maintain this schedule.

Cardiac monitoring of LVEF should be maintained throughout trastuzumab treatment according to local policy. If trastuzumab treatment is discontinued during chemotherapy then chemotherapy should continue uninterrupted if this is considered safe by the investigator. Trastuzumab 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 rescheduled to coincide with the next chemotherapy administration. If trastuzumab has been delayed by more than 4 weeks 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.
7.5.4 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 5%.

7.6 Dose Modifications and Delays
The chemotherapy schedules in ROSCO are in widespread use 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. Comprehensive and detailed guidance for the management of specific toxicity of individual agents is outside the remit of this protocol.

The decision whether to continue with trial 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 deputy but overall responsibility for patient care rests with the investigator.

7.6.1 Dose Modifications or Delays in Response to Toxicity

7.6.1.1 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

7.6.1.2 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

7.6.1.3 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 after poor emesis control and is recommended as standard treatment for all patients receiving FEC100
7.6.2 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

7.6.3 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 or deputy who can provide advice on dose and schedule for individual patients.

7.6.4 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 diarrhea associated with suspected dihydropyrimidine dehydrogenase (DPD) deficiency). Re-escalation after dose reduction is not recommended.

The maximum permitted dose-delay for chemotherapy is 4 weeks for recovery of severe toxicity or for unscheduled procedures (e.g. appendectomy). If longer delays are required, then the patient will be withdrawn from the trial, and alternative therapy considered by the responsible clinician.

7.7 Supportive Therapy and Concomitant Medication

Concomitant administration of live vaccines with trial chemotherapy is prohibited.

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 trial treatment, at the discretion of the local investigator provided the medication is not prohibited within the exclusion criteria for trial 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 each Summary of Product Characteristics (SPCs) and should be referred to for concerns relating to specific drug interactions.

7.7.1 Anti-emetic Therapy

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

7.7.2 Granulocyte–Colony Stimulating Factors

The use of primary neutropenia prophylaxis with G-CSFs is not mandated 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.

The Principal Investigator's primary prophylaxis intent for the first cycles of either treatment arm should be provided to the ROSCO Trial Office when they register the sites intent to participate in the trial.

7.7.3 Prophylactic Antibiotics

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

HER2 positive patients may also receive pertuzumab, where available, as a concomitant medication at 840mg IV with the first cycle of chemotherapy followed by 420mg IV for all subsequent cycles. Please note that if pertuzumab is administered, concurrent trastuzumab should also be given IV and not as subcutaneous injection.

For NHS England sites pertuzumab funding is currently accessed through the Current Cancer Drugs Fund Bluteq online application system. HER2 positive patients participating in ROSCO can access pertuzumab funding irrespective of the chemotherapy arm allocation in the trial.

7.8 Disease Progression While on Protocol Defined Chemotherapy Treatment

In the event of progression of disease on treatment the investigator can elect to change chemotherapy, revert to surgery, utilise radiotherapy or elect for an alternative treatment 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 Treatment Discontinuation Form (see Section 12).

7.9 Surgery

Definitive surgery should proceed when the investigator is satisfied that the patient is sufficiently recovered from neo-adjuvant chemotherapy, 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. Clear circumferential margins are required.

ROSCO addresses the surgical question of sensitivity of SLNB post neo-adjuvant chemotherapy in patients who have biopsy or FNA proven axillary node metastases at diagnosis. All patients require axillary ultrasound at diagnosis with biopsy or FNA of equivocal or suspicious nodes regardless of clinical assessment of the axilla.

Consenting patients who have biopsy proven nodal involvement pre-chemotherapy will undergo a SLNB and axillary clearance as a single procedure after neo-adjuvant chemotherapy at the same time as breast surgery, as in the ALMANAC trial audit phase (67).

The surgical management of patients with a normal axilla at diagnosis or with a negative ultrasound biopsy/FNA is according to local protocol.

7.10 Schedule of Assessments

7.10.1 Screening/baseline

See Section 5.1 for screening and baseline tests.

7.10.2 Prior to Each Cycle of Neo-adjuvant Chemotherapy

The following assessments should be made prior to day 1 of each cycle of neo-adjuvant chemotherapy (excluding cycle 1):

- Physical examination*
- 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
7.10.3 End of Cycle 2 of Neo-adjuvant Chemotherapy

A clinical assessment is recommended to confirm absence of early disease progression at the end of cycle 2 (i.e. prior to commencing cycle 3 treatment).

The following should also be performed at the end of cycle 2 of neo-adjuvant chemotherapy:

- For those patients receiving trastuzumab, LVEF measurement by ECHO or MUGA (as per the baseline assessment, as the modality used should be consistent throughout the study)

Patients participating in the optional QoL and Health Economics Sub-study will receive a QoL Booklet in the post from the ROSCO Trial Office after cycle 2 of neo-adjuvant chemotherapy.

7.10.4 End of Cycle 4 of Neo-adjuvant Chemotherapy

The following assessments should be made at the end of cycle 4 (within 28 days if cycle 4 is the last protocol defined treatment):

- Clinical measurement of breast and nodal lesions
- Radiological measurement of tumour(s) by ultrasound scan, which is mandatory unless the lesion cannot be measured by this method at baseline in which case measurement by MRI will be acceptable
- Physical exam to include:
  - Weight

Patients participating in the optional QoL and Health Economics Sub-study will receive a QoL Booklet in the post from the ROSCO Trial Office after cycle 4.

In some circumstances, where clinical response is sub-optimal and where the Investigator considers that further neo-adjuvant chemotherapy is indicated, presurgical crossover is permitted following completion of cycle 4. In these circumstances a biopsy (referred to as an “interim biopsy” for the purposes of this protocol) must be performed after cycle 4. If this biopsy demonstrates viable residual invasive cancer crossover to the alternative treatment arm before surgery is permitted. If the biopsy does not demonstrate residual invasive disease further chemotherapy should not be given until after surgery when the whole specimen has been examined.

If the patient crosses over to the alternative treatment arm before surgery the interim biopsy tissue sample will be requested as detailed in Section 9.1.2 and a copy of the patient's anonymised pathology report should be sent to the ROSCO Trial Office.

All other patients should be scheduled for surgery (see Section 7.9) after completing cycle 4 of neo-adjuvant chemotherapy.

7.10.5 On Completion of all Neo-adjuvant Chemotherapy

An AE review should be performed.

Clinical and radiological tumour measurement should be performed as detailed in Section 7.10.4. Please note these only need to be performed once if cycle 4 is the final cycle of neo-adjuvant chemotherapy. A Radiology Assessment Form should be completed to document the radiological response (see Section 12 and Appendix 5).
LVEF measurement by ECHO or MUGA (as per the baseline assessment, as the modality used should be consistent throughout the study) should also be performed for HER2 positive patients who are receiving/have received trastuzumab, as per institutional standard practice.

A blood sample should also be taken for the Pharmacogenetics Sub-study (see Section 10.1.3).

7.10.6 Prior to Each Cycle of Protocol Defined Adjuvant Chemotherapy
The following assessments should be made prior to day 1 of each cycle of protocol defined adjuvant chemotherapy:
- Full blood count within 3 days prior to day 1 of each 3 weekly treatment cycle
- Biochemical screen within 3 days prior to treatment cycle
- AE review

7.10.7 Surgery
SLNB and surgery should be performed in accordance with Section 7.9. Tumour samples will be requested as detailed in Section 9 and a copy of the patient’s anonymised pathology report should be sent to the ROSCO Trial Office.

7.10.8 Six Weeks Post-Surgery
Patients participating in the optional QoL and Health Economics Sub-study will receive a QoL Booklet in the post from the ROSCO Trial Office 6 weeks after the date of surgery.

7.10.9 Annual Follow-up
Long-term follow-up will include adjuvant hormonal therapy, non-protocol defined adjuvant chemotherapy and trastuzumab treatment data (year 1 only), an assessment of neuropathy, cardiac failure, lymphoedema, dates and sites of first relapse and date and cause of death. This is necessary for the trial’s secondary endpoints of survival and toxicity.

Patients participating in the optional QoL and Health Economics Sub-study will receive a QoL Booklet in the post from the ROSCO Trial Office 1 year and 2 years after the date of randomisation.

LVEF measurement by ECHO or MUGA (as per the baseline assessment, as the modality used should be consistent throughout the study) should also be performed for all patients 5-years post randomisation.

Patients should be followed-up annually in accordance with standard routine follow up policies (including mammography as applicable) for a total of five years after randomisation. The year 1 follow-up assessment should be carried out approximately 12 months post randomisation. Face to face or telephone 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.

The ROSCO Trial Office will request follow-up data annually for 5 years based on the randomisation date anniversary.

Where patients are not traceable by any other means the ROSCO Trial Office will contact the applicable Cancer Registry to collect overall survival data.
7.10.9.1 Relapse
See Table 1 for definitions of relapse.

Table 1: Definition of Relapse

<table>
<thead>
<tr>
<th>Type of Relapse</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loco-regional relapse</td>
<td>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</td>
</tr>
<tr>
<td>Distant relapse</td>
<td>Distant recurrence 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</td>
</tr>
<tr>
<td>New primary</td>
<td>Confirmation of the presence of a second unrelated and new cancer. For the purposes of ROSCO this will include contralateral malignant breast disease</td>
</tr>
</tbody>
</table>

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 ROSCO Trial Office.

Patients who relapse or develop a new second primary cancer should remain on follow-up.

7.10.9.2 Death
As soon as possible following notification that a patient has died a Death Form should be completed and returned to the ROSCO Trial Office. Every effort should be made to obtain a date and cause of death.

7.11 Additional Treatment

7.11.1 Endocrine Therapy

7.11.1.1 During Neo-adjuvant Chemotherapy
Concomitant endocrine therapy is not permitted during neo-adjuvant chemotherapy.
Hormone replacement therapy must be discontinued during chemotherapy.

7.11.1.2 Adjuvant Hormonal Treatment after Surgery
Following completion of chemotherapy women with ER-positive disease will be offered adjuvant hormonal therapy in accordance with local therapy protocols.

7.11.2 Adjuvant Radiotherapy
Radiotherapy will be given after definitive surgery according to local protocols Please Note: according to national guidelines adjuvant radiotherapy is mandated following breast conserving surgery. A radiotherapy boost may be used in keeping with local treatment practice.
Following mastectomy the indications for chest wall irradiation are according to local protocols. Decisions on the role of post-mastectomy radiotherapy should take into account pre-chemotherapy tumour characteristics, to avoid compromising potential gains achieved by neo-adjuvant treatment. Radiotherapy to the axilla and supraclavicular region may be utilised. The supraclavicular fossa should be treated to a radical dose if supraclavicular fossa nodes are deemed involved on radiological or clinical grounds prior to chemotherapy.
7.12 Trial Treatment Discontinuation and Withdrawal of Consent

7.12.1 Treatment Discontinuation

Patients should discontinue trial treatment in the following circumstances:

- If the patient experiences a dose delay of >4 weeks as a result of toxicity (see Section 7.6.4)
- On confirmation of disease progression
- 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 (see Section 7.12.2)

A Treatment Discontinuation Form should be completed to document the reason for treatment discontinuation.

7.12.2 Withdrawal of Consent

Patients may withdraw consent at any time during the study. For the purposes of this trial three types of withdrawal are defined:

- The patient would like to withdraw from trial treatment, but is willing to be followed up in accordance with the schedule of assessments (i.e. the patient has agreed that data can be collected and used in the trial analysis)
- The patient would like to withdraw from trial treatment and 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 trial analysis)
- The patient would like to withdraw from trial treatment and is not willing to be followed up for the purposes of the trial at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the trial 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 ROSCO Trial Office of the patient’s withdrawal from the trial.

7.13 Concurrent Studies

Investigators wishing to enrol patients into another trial should contact the ROSCO Trial Office in the first instance. The ROSCO Trial Management Group (TMG) will consider the enrolment of ROSCO 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 hormonal therapy, after completion of trial specific neo-adjuvant chemotherapy, surgery and adjuvant chemotherapy. The ROSCO Trial Office will maintain a contemporary record of trials approved by the TMG. Where a trial has not been considered the ROSCO Chief Investigator or deputy will provide advice based on the above principles.
8 Quality Assurance of Central Biomarker Testing

The categorisation of the CEP-17 and TOP2A biomarkers is critical to the primary endpoint of this trial. Quality assurance and cross validation between the Cellular Pathology Laboratory, Heart of England NHS Foundation Trust scoring and any additional reference centres used for biomarker analysis will be performed under the direction of Professor John Bartlett. This will require diagnostic samples to be made available for review in additional laboratories, including Professor Bartlett's own laboratory at Ontario Institute for Cancer Research, Toronto, Canada.

Samples sent outside NHS facilities will be identifiable only through unique TNO.

9 Tissue Collection and Definition of Primary Outcome

Principal Investigators are asked to nominate one individual at their site to act as a Pathology Lead Contact. This individual will be provided with information on the purpose of the study and shipment of samples. Pathology Departments will receive a per-patient payment for supplying the requested samples.

9.1 Mandatory Core Biopsy and Tumour Block Collection

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 randomising hospital.

9.1.1 Tumour Block Pre-treatment

Core biopsies will be performed on all patients to make the diagnosis of breast cancer.

The patient’s diagnostic paraffin embedded tumour block must be sent immediately after consent has been obtained for central CEP17/TOP2A assessment. Blocks should be sent to Cellular Pathology Laboratory, Heart of England NHS Foundation Trust, as detailed in Section 6.2.2. The blocks must be received and analysed before randomisation can proceed. The tissue block will then be sent to the Translational Coordinating Centre, Edinburgh for storage in the ROSCO Biorepository.

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 ROSCO Trial Office.

If diagnostic material is urgently needed, please contact the ROSCO Trial 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 tissue block to allow further diagnostic work up.

In cases where material is sparse the Central Pathology Reviewer(s) will ensure that sufficient material remains for diagnostic purposes.

9.1.2 Interim Biopsy

Where the neo-adjuvant aim of down staging to permit breast conservation has not been achieved and the investigator considers that further chemotherapy provides a realistic prospect of achieving a successful down staging effect, an interim biopsy must be taken after cycle 4 and prior to cycle 5. The interim biopsy tissue sample will be requested for central review of response and should be sent to the Translational Coordinating Centre, Edinburgh. Cross over to the alternative chemotherapy arm presurgery is also permitted for logistic reasons or where patient preference is for all chemotherapy to be delivered prior to surgery but only where an interim biopsy has been performed and demonstrated persistence of viable invasive disease. Where a biopsy has not been performed or has not shown invasive disease patients should proceed to surgery before any further chemotherapy. A copy of the associated anonymised pathology report will be requested for each patient and sent to the ROSCO Trial Office.
9.1.3 Tumour Blocks at Surgery

Representative Haematoxylin and Eosin (H&E) slides from the surgical resection tumour bed, and if applicable any positive lymph nodes, should be sent to the Translational Coordinating Centre, Edinburgh. A copy of the associated anonymised surgery pathology reports will be requested for each patient and should be sent to the ROSCO Trial Office.

Following review of the slides by the trial pathologists a representative tumour block will be requested by the ROSCO Trial Office for future translational research. The selected block should be sent to the Translational Coordinating Centre, Edinburgh. See the ROSCO Tissue Collection Guidelines for more information.

The surgical blocks will be retained at the ROSCO Biorepository for research purposes, material essential for diagnostic purposes can be returned as outlined in Section 9.1.1.

9.2 Central Review for pathological Complete Response

Pathological complete response will be determined by review of the relevant pathology reports by a Central Pathology Reviewer. A retrospective central pathological review of the surgical slides will be performed to quality assure this process (see Appendix 6). This will involve a retrospective central pathological review of selected patients for the primary endpoint of pCR. The Central Pathology Reviewer(s) will review all cases comparing the diagnostic core biopsy, with the surgical slides or interim biopsy provided. The grade of response will be defined for each patient. Cellularity will be compared with the original diagnostic core prior to neo-adjuvant chemotherapy.

10 ROSCO Sub-studies

10.1 ROSCO Translational Science

10.1.1 Biopsy and Tumour Tissue

Formalin-fixed, paraffin-embedded (FFPE) tissue will be collected from all patients for future research. Tissue will be sent to the Translational Coordinating Centre, Edinburgh for biobanking.

The diagnostic tumour biopsy block will initially be sent to the Cellular Pathology Laboratory, Heart of England NHS Foundation Trust, for CEP17/TOP2A analysis before being forwarded onto the Translational Coordinating Centre, Edinburgh (see Section 6.2.2).

In addition to the diagnostic tumour biopsy block, tissue will also be collected from the interim biopsy (collected at cycle 4 if applicable, see Section 7.10.4) and the surgical tumour specimen. Normal tissue blocks are also requested where available.

DNA and Ribonucleic Acid (RNA)/miRNA will be extracted from serial 4 micron sections cut from the core biopsy/resection samples and will be stored at -80°C for future analysis (this is subject to securing additional funding).

See the separate ROSCO Tissue Collection Guidelines for additional information.

10.1.2 Pharmacogenetics

A blood sample, consisting of 1x 9ml EDTA sample, should be obtained on two occasions from each patient who give consent for this optional sub-study and who are randomised into the trial. It is recommended that the blood sample is obtained at the same time as routine bloods are taken, where possible, to minimise the impact on the patient. The first blood sample should be taken prior to commencing all chemotherapy and the second blood sample should be taken on completion of neo-adjuvant chemotherapy. Blood sample kits will be provided.

See the separate ROSCO Blood Sample Collection Guidelines for additional information.
10.2 Quality of Life and Health Economic Sub-study

The QoL and Health Economic Sub-study is designed to assess the patient's well-being over a two-year period, by use of questionnaires collected at six specified time-points. This time-span allows data collection before, during and after neo-adjuvant chemotherapy and should provide insight into the impact treatment has on women’s health and the cost of the treatment interventions.

10.2.1 Questionnaires

The QoL and Health Economics Sub-study will utilise the following questionnaires:

- EQ-5D-5L™ (68) is a validated questionnaire used for the measurement of breast cancer specific patient reported outcome measures. It is a 44-item self-report questionnaire designed to measure multidimensional QoL in patients with breast cancer.
- FACT-B (69) consists of the FACT-General (FACT-G) plus the Breast Cancer Subscale. It is comprised of six domains (physical well-being, social/family well-being, relationship with doctor, emotional well-being, functional well-being, and additional concerns).

10.2.2 Target Accrual

Participation in this sub-study is optional for patients. Enrolment will be offered to all patients consented into the trial until the accrual target of 500 actively participating patients is met.

10.2.3 Time Points

Participating patients will be provided with a QoL Booklet comprised of the validated questionnaires at the following time points:

- Prior to commencement of neo-adjuvant chemotherapy (baseline)
- Following completion of cycle 2 of neo-adjuvant chemotherapy (approximately 6 weeks post-randomisation)
- Following completion of cycle 4 of neo-adjuvant chemotherapy (approximately 12 weeks post-randomisation)
- Six weeks after completion of surgery (approximately 21 weeks post-randomisation if the patient receives 4 cycles of neo-adjuvant chemotherapy)
- One year post-randomisation
- Two years post-randomisation

Sites will be provided with a supply of the baseline QoL Booklets at site initiation.

Prior to completing the baseline questionnaire a member of the Research Team should discuss the questionnaires with the patient and answer any questions they may have. The baseline questionnaire will be returned to the ROSCO Trial Office by the Research Nurse.

Subsequent QoL Booklets will be sent to patients in the post from the ROSCO Trial Office. The trials team will contact the site to confirm that it is appropriate to send the booklet to the patient. The booklet will be accompanied by a standard cover letter providing written instructions on how to complete the questionnaires. Patients will be instructed to return the questionnaires directly to the ROSCO Trial Office in the pre-paid envelopes provided.
11 Adverse Event Reporting

The collection and reporting of AEs will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. Definitions of different types of AE are listed in Appendix 7. The investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient with reference to the compendium of Summary of Product Characteristics for the IMPs used in the study.

11.1 Reporting Requirements

11.1.1 Adverse Events

AEs (see Appendix 7 for definition) are commonly encountered in patients receiving chemotherapy and the safety profiles of the IMPs used in this trial are well characterised. Therefore the focus of data collection will be AEs that are likely to be related to the IMPs being studied (i.e. Adverse Reactions (ARs) or toxicities). However sites should record all AEs experienced by patients in the source data not just those related to trial treatment and Serious Adverse Events (SAEs) should be reported as described in the following sections. It should be clear which events are thought to be related.

11.1.2 Serious Adverse Events

Investigators should report AEs that meet the definition of a SAE (see Appendix 7 for definition) and are not excluded from the reporting process (as detailed below) on a SAE Form as described in Section 11.3.3.

11.1.3 Events That Do Not Require Expedited Reporting

Patients receiving chemotherapy may require admission to hospital for appropriate medical intervention following development of some of the more severe known side effects of treatment. For this reason the following SAEs do not require expedited (immediate) reporting by site and are not regarded as unexpected for the purpose of this trial:

- Admissions for supportive treatment during an episode of febrile neutropenia, unless this proves fatal or requires admission to a high dependency or intensive care facility
- Admissions to control symptoms of vomiting unless the condition is life threatening or proves fatal
- Admissions for acute anaphylaxis following the administration of trial medication, unless this proves fatal or requires admission to a high dependency or intensive care facility
- Admissions for docetaxel related:
  - myalgia
  - arthralgia
  - fatigue

These events are regarded as expected Serious Adverse Reactions (SARs) for the purposes of this trial.

11.1.4 Events That Do Not Require Reporting on a Serious Adverse Event Form

The following events should not be reported on an SAE Form:

- Hospitalisations for:
  - Protocol defined treatment
  - Pre-planned elective procedures unless the condition worsens
  - Treatment for progression of the patient’s breast cancer
- Progression or death as a result of the patient’s breast cancer, as this information is captured on the Relapse or Death Form
- The expected SARs listed above, as this information will be captured on an Expected SAR Form (see Section 11.3.1.4)
11.1.5 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 or their partner becomes pregnant during the SAE reporting period please complete a Pregnancy Notification Form (providing the patient's details) and return to the ROSCO Trial Office as soon as possible. If it is the patient who is pregnant provide outcome data on a follow-up Pregnancy Notification Form. Where the patient's partner is pregnant consent must first be obtained and the patient should be given a Release of Medical Information Form to give to their partner. If the partner is happy to provide information on the outcome of their pregnancy they should sign the Release of Medical Information Form. Once consent has been obtained provide details of the outcome of the pregnancy on a follow-up Pregnancy Notification Form. If appropriate also complete an SAE Form as detailed below. Please note it is very important that pregnancies are followed up until the outcome is known.

11.2 Reporting period

Details of all AEs will be documented and reported from the date of commencement of protocol defined chemotherapy treatment until 30 days after the administration of the last protocol defined chemotherapy treatment.

11.3 Reporting Procedure

11.3.1 Site

11.3.1.1 Adverse Events

AEs will be reviewed using the Common Terminology Criteria for AEs (CTCAE), version 4.0 (see Appendix 8). Any AEs experienced by the patient but not included in the CTCAE should be graded by an investigator using a scale of (1) mild, (2) moderate or (3) severe. For each sign/symptom, the highest grade observed since the last visit should be recorded.

AEs thought to be related to the IMPs (i.e. ARs) will be captured on the Treatment Form.

11.3.1.2 Serious Adverse Events

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

AEs defined as serious and which require reporting as an SAE (excluding events listed in Section 11.1.3 and 11.1.4) 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, the investigator (or delegate) must complete, date and sign an SAE Form. The form should be faxed together with a SAE Fax Cover Sheet to the ROSCO Trial Office using one of the numbers listed below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, fax the SAE Form with an SAE Fax Cover Sheet to -

☎ 0121 414 8392 ☎ 0121 414 7989

On receipt the ROSCO Trial Office will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Fax Cover Sheet which will then be faxed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the ROSCO Trial Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE. The SAE Fax Cover Sheet completed by the ROSCO Trial Office should 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 ROSCO Trial 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.

11.3.1.3 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).

11.3.1.4 Expected Serious Adverse Reactions
The expected SARs defined in Section 11.1.3 should be reported on an Expected SAR Form rather than an SAE Form. SAR Forms should be completed and returned in the post as soon as possible.

11.3.2 Trial Office
On receipt of an SAE Form seriousness and causality will be determined by a Clinical Coordinator (clinical member of the TMG). An SAE judged by the investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a SAR. The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

Events received on an Expected SAR Form will not be assessed by a Clinical Coordinator as they are by definition expected.

11.3.3 Reporting to the Competent Authority and Research Ethics Committee
11.3.3.1 Suspected Unexpected Serious Adverse Reactions
The ROSCO Trial Office will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC) within 7 days. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as SUSARs will be reported within 15 days.

11.3.3.2 Serious Adverse Reactions
The ROSCO Trial Office will report details of all SAEs, SARs (including SUSARs) to the MHRA and REC annually from the date of the Clinical Trial Authorisation, in the form of a Development Safety Update Report (DSUR).

11.3.3.3 Adverse Events
Details of all AEs will be reported to the MHRA on request.

11.3.3.4 Other Safety Issues Identified During the Course of the Trial
The MHRA and REC will be notified immediately if a significant safety issue is identified during the course of the trial.

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

11.3.5 Data Monitoring Committee
The independent Data Monitoring Committee (DMC) will review all SAEs.
12 Data Handling and Record Keeping

12.1 Data Collection

The CRF will comprise the following forms:

Table 2: ROSCO Trial Case Report Form

<table>
<thead>
<tr>
<th>Form</th>
<th>Summary of data recorded</th>
<th>Schedule for submission to Trial Office</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration</td>
<td>Minimal patient identifiers and hospital number</td>
<td>Return paper form as soon as possible after registration</td>
</tr>
<tr>
<td>Eligibility</td>
<td>Confirmation of eligibility and satisfactory staging investigations where necessary;</td>
<td>Return paper form as soon as possible after randomisation</td>
</tr>
<tr>
<td>Randomisation</td>
<td>Patient details hormone receptor status, nodal involvement</td>
<td>Return paper form as soon as possible after randomisation</td>
</tr>
<tr>
<td>Baseline</td>
<td>Patient characteristics, biopsy histology, details of planned surgery, concomitant disease</td>
<td>Within 1 month of randomisation</td>
</tr>
<tr>
<td>Radiological Assessment</td>
<td>Results of radiological assessment of tumour and radiological response</td>
<td>Completed at baseline and end of cycle 4 or on completion of neo-adjuvant treatment as applicable. Form to be returned within 1 month of assessment taking place</td>
</tr>
<tr>
<td>Treatment</td>
<td>Actual chemotherapy doses and dates given; details and reasons for dose reductions and delays; details of supportive treatment; details of reported toxicity, clinical tumour measurements, results of LVEF (if applicable)</td>
<td>Within 1 month of completion of relevant protocol defined chemotherapy cycle</td>
</tr>
<tr>
<td>End of Neo-adjuvant Chemotherapy</td>
<td>Results of protocol defined assessments completed at the end of neo-adjuvant chemotherapy</td>
<td>Within 1 month of completion of protocol defined neo-adjuvant chemotherapy</td>
</tr>
<tr>
<td>End of Treatment Discontinuation</td>
<td>Summary of protocol defined chemotherapy treatment and reasons for treatment discontinuation.</td>
<td>Within 1 month of completion of protocol defined chemotherapy or if patient discontinues trial treatment</td>
</tr>
<tr>
<td>Surgery</td>
<td>Full details of surgery and whether the patient will have further chemotherapy.</td>
<td>Within 1 month of completion of all surgery</td>
</tr>
<tr>
<td>Adjuvant Radiotherapy Summary</td>
<td>Summary of adjuvant radiotherapy treatment</td>
<td>Within 1 month of completion of adjuvant radiotherapy</td>
</tr>
<tr>
<td>Annual Follow-up</td>
<td>One form per year completed on the anniversary of randomisation collecting late toxicity and survival data. Year 1 only: details of adjuvant hormone therapy, non-protocol defined adjuvant chemotherapy and trastuzumab treatment</td>
<td>Within 1 month of relevant anniversary of randomisation</td>
</tr>
<tr>
<td>Relapse – First Locoregional, Relapse – First Distant</td>
<td>Details of local and distant relapse</td>
<td>Immediately upon discovering that a patient has relapsed</td>
</tr>
<tr>
<td>New Primary Cancer</td>
<td>Details of new primary cancers</td>
<td>Immediately upon discovering that a patient has a new primary cancer</td>
</tr>
<tr>
<td>Death</td>
<td>Date and cause of death</td>
<td>Immediately upon notification of patient’s death</td>
</tr>
<tr>
<td>Deviation</td>
<td>Completed in the event of a deviation from the protocol</td>
<td>Immediately upon discovering a deviation</td>
</tr>
<tr>
<td>Withdrawal of Consent</td>
<td>Used to notify the ROSCO Trial Office of patient withdrawal from the trial</td>
<td>Immediately upon patient withdrawal</td>
</tr>
<tr>
<td>Serious Adverse Event</td>
<td>See Section 11</td>
<td>Return paper form immediately on discovering that the patient has experienced an SAE</td>
</tr>
<tr>
<td>Expected Serious Adverse Reaction</td>
<td>See Section 11</td>
<td>Return paper form as soon as possible after patient experiences an expected SAR</td>
</tr>
<tr>
<td>Pregnancy Notification</td>
<td>See Section 11</td>
<td>As soon as possible on discovering that the patient or their partner are pregnant</td>
</tr>
</tbody>
</table>
The CRF must be completed, signed/dated and returned to the ROSCO Trial Office by the investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log) within the timeframe listed above. The exceptions to this are the SAE Form and Withdrawal of Consent Form which must be co-signed by the investigator.

Entries on the paper 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 or the discrepancies should be explained. If 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. QoL and Health Economic Evaluation questionnaires will be regarded as source data for this trial, data will be recorded directly onto the relevant questionnaire and sent to the ROSCO Trial Office.

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.

Where paper forms are completed the originals should be sent to the ROSCO Trial Office and a copy filed in the ISF.

Trial forms may be amended by the ROSCO Trial 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.

13 Archiving

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients’ hospital notes, copies of CRFs etc.) at their site are securely retained for at least 25 years after the end of the trial 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 Cancer Research UK Clinical Trials Unit (CRCTU) Document Storage Manager.
14 Quality Management

14.1 Site Set-up and Initiation
All sites will be required to sign a Clinical Study Site Agreement prior to participation. In addition all participating investigators will be asked to complete and sign a Registration Form and supply a current CV and proof of up to date GCP training to the ROSCO Trial Office. All members of the site research team will also be required to sign the Site Signature and Delegation Log which should be returned to the ROSCO Trial Office. Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, AE reporting, collection and reporting of data and record keeping. Sites will be provided with an ISF and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The ROSCO Trial Office must be informed immediately of any change in the site research team.

14.2 On-site Monitoring
Monitoring will be carried out as required following a risk assessment and as documented in the ROSCO Quality Management Plan. 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. If a monitoring visit is required the ROSCO Trial 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 ROSCO trial staff access to source documents as requested.

14.3 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. Pathology reports received will also be checked to ensure patient eligibility.
Trials staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trials staff will check incoming CRF 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 Good Clinical Practice (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 and DMC are notified of the event in addition to the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the REC and the MHRA.

14.4 Audit and Inspection
The investigator will permit trial-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 ROSCO Trial Office of any MHRA inspections.

14.5 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 trial or;
- The protocol relating to that trial,
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 trial; or
- The scientific value of the trial

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

15 End of Trial Definition

The end of trial will be 6 months after the last patients’ last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The ROSCO Trial Office will notify the Sponsor, the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.
16 Statistical Considerations

16.1 Definition of Outcome Measures
See Section 2.2.

16.2 Analysis of Outcome Measures

There are two key research questions to be addressed:

1. Does treating patients according to CEP17/TOP2A status improve pCR rates?
2. Does SLNB accurately predict nodal involvement in patients with known nodal involvement prior to chemotherapy?

All analyses comparing treatment effects will be carried out on an intent to treat basis.

Question 1

The aim of the primary research question is to test the hypothesis that treating patients according to CEP17/TOP2A status improves pCR rates in the neo-adjuvant setting. Emerging evidence suggests that patients with abnormal CEP17/TOP2A status may have a better pCR rate if treated with anthracyclines (49) whereas patients with normal CEP17/TOP2A status are better treated with a taxane.

Null hypothesis: Treatment effect in CEP17/TOP2A Abnormal patients is not different to the treatment effect in CEP17/TOP2A Normal patients.

Alternative hypothesis: Treatment effect in CEP17/TOP2A Abnormal patients is different to treatment effect in CEP17/TOP2A Normal patients.

The primary analysis will assess the interaction between the treatment effect and CEP17/TOP2A status to determine if a differential treatment effect exists between CEP17/TOP2A Normal and CEP17/TOP2A Abnormal patients. Complete pathological response will be analysed using a logistic regression model including co-variates for treatment, CEP17/TOP2A status and an interaction term of the two effects. The subsequent analysis depends on the outcome of this primary analysis. Given a significant interaction effect the treatment effect will be explored in the CEP17/TOP2A normal and abnormal patient populations separately. Given a null interaction effect the treatment effect will be evaluated overall in the whole patient population.

Response rates in breast alone, radiological response rates after 4 cycles of chemotherapy, loco-regional recurrence, disease free survival, overall survival, tolerability, toxicity, dose intensity of chemotherapy, and QoL will be compared between treatments as outlined above.

Disease free survival and overall survival will be calculated for all patients and time to event data presented using methods of Kaplan and Meier. Hypotheses will be tested using appropriate survival modelling techniques for the distribution of the data. The pCR rates in breast alone, radiological response after 2 and 4 cycles and the rate of breast conservation surgery will be compared across treatments using the chi-square statistic. Tolerability and toxicity will be analysed using either parametric or non-parametric tests for numeric data as appropriate and either a chi-square or test of trend for categorical data. QoL will be analysed using standardised area under the curve and Mann-Whitney tests. Health economic data will be used to provide a cost-utility analysis and sensitivity analysis will be performed. Logistic regression and survival models will be undertaken to determine prognostic and predictive factors and their influence on response rates and survival whilst providing an adjusted treatment effect for important prognostic, stratification and molecular factors.

Question 2

A key secondary outcome will be to determine whether a negative SLNB following neo-adjuvant chemotherapy will accurately predict clearance of lymph node involvement in patients with demonstrated lymph node involvement prior to chemotherapy. The sensitivity rate of SLNB; that is the number of positive SLNB as a proportion of all positive lymph node involvement following axillary clearance at surgery will be presented with the 95% confidence interval. Prevalence, sensitivity, specificity, positive and negative predictive values will all be presented to assess the correlation between SLNB and other informative outcomes (isolate tumour cells, micrometastases, macrometastases).
16.3 Planned Interim Analysis

The only planned interim analysis will be for the purpose of the DMC. The data will be presented to the DMC after the first year and then annually thereafter (see Section 17.5).

16.4 Planned Final Analyses

The primary and available secondary analyses will be performed once the last recruited patient has completed surgery. This is expected to be approximately 5 ½ years after the trial opens to recruitment. Analysis of DFS and overall survival will be performed after 5 years of follow up.

16.5 Exploratory analyses

Exploratory analysis of clinically relevant subgroups will be reported these will include response by hormone receptor expression, HER2 expression, tumour size, grade, nodal involvement, histological type, patient age, treatment with trastuzumab.

16.6 Power Calculations

The sample size is based on the primary outcome measure of pCR rate measured at surgery after completing neo-adjuvant chemotherapy. The intent is to recruit 1050 patients overall into the trial with a 1:1 allocation between taxane and anthracycline randomisation. This allows for a small number of patient withdrawals and loss to follow-up. Sample size adjustments may be performed following interim analyses if any of the assumptions about the patient population participating in the trial differ significantly from those used in the initial calculation.

Question 1

Sample size calculations are based on methods proposed by Simon's design for a prospective, phase III, biomarker stratified trial with a binary endpoint, testing for a differential treatment effect between CEP17/TOP2A Normal and CEP17/TOP2A Abnormal patients. The primary outcome measure is pCR.

Null Hypothesis: Treatment effect in CEP17/TOP2A Abnormal patients is not different to the treatment effect in CEP17/TOP2A Normal patients.

Alternative Hypothesis: Treatment effect in CEP17/TOP2A Abnormal patients is different to treatment effect in CEP17/TOP2A Normal patients.

Assumptions: Prevalence of abnormal CEP17/TOP2A is thought to be in the range 30-40% and a conservative 30% estimate has been used in the calculations, TOP2A status is associated with HER2 status and it is estimated that HER2 positive patients will account for up to 15% of patients randomised, CEP17/TOP2A Abnormal within HER2 positive population will be at least 37%, pCR rate in CEP17/TOP2 Normal, HER2 negative patients is 20%, HER2 positive patients is 35%, CEP17/TOP2A Abnormal, HER2 negative patients treated with anthracyclines is 50% and CEP17/TOP2A Abnormal, HER2 positive patients treated with anthracyclines is 65%.

These are all composite estimates based upon the anticipated profile of patients to be entered into the trial. The assumptions will be monitored throughout the course of the trial and estimates of the required sample adjusted where the patient population is significantly different to that expected at the outset. Patients randomised to a taxane treatment are expected to have a pCR rate of around 21%, taking into account the differential response rates according to their CEP17/TOP2A and HER2 status. Whereas it is hypothesized that patients randomised to anthracycline treatment will have an increased pCR rate in the region of 30% due to the increased pCR rate seen in CEP17/TOP2A Abnormal and HER2 positive patients. The sample size is based on the difference between the treatment arms in overall response rate, the extent of which the anthracycline treated patients do better is further influenced by the prevalence of the biomarker abnormality in the randomised population. The response rate is expected to be in the region of 21% in the taxane treated patients and a sample size of 1050 patients has the ability to detect at least a 9% improvement in response to 30% in the anthracycline treated patients.

A sample size of 1050 patients gives 90% power at the 10% significance threshold for detecting an interaction. The power to test for an overall treatment effect regardless of CEP17/TOP2A status is greater than 90%. Allowing for up to a 10% loss to follow-up/non-adherence still ensures greater than 87% power at the 10% significance threshold to test for a treatment by CEP17/TOP2A interaction. The proposed stratified approach has adequate power to test for the interaction between CEP17/TOP2A status and treatment effect.
If a non-significant interaction test is observed the design has adequate power to detect for an overall treatment effect regardless of CEP17/TOP2A status.

**Question 2**

The sensitivity rate of SLNB at identifying macrometastases will need to be at approximately 90% to be acceptable to surgeons and patients and similar to that in patients unexposed to systemic therapy.

Using conservative estimates 24% of patients randomised will be able to be identified upfront as having lymph nodal involvement and will therefore go on to receive a SLNB as well as axillary clearance after neo-adjuvant chemotherapy. This takes into account the current characteristics of patients entered into the ARTemis trial and false negative rates of radiologically guided biopsy prior to chemotherapy. Approximately 252 patients will be available for analysis to determine the sensitivity of SLNB compared to an axillary clearance, of which approximately 202 (80%) will have nodal involvement as assessed by an axillary clearance based on an overall pCR rate of 20%.

The sensitivity, that is the proportion of patients with lymph node involvement correctly identified by SLNB, will be calculated and the associated 95% confidence interval computed. SLNB will be considered as a robust alternative to axillary clearance if it correctly predicts nodal involvement in at least 90% of cases. If the true sensitivity was around 95%, 202 patients would provide enough patients to ensure the lower confidence limit did not fall below the lowest acceptable level of sensitivity (90%), approximately 90% of the time.

### 17 Trial Organisational Structure

#### 17.1 Sponsor

The trial is sponsored by the University of Birmingham.

#### 17.2 Coordinating Centre

The trial is being conducted under the auspices of the CRCTU, University of Birmingham according to their standard operating procedures.

#### 17.3 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). The TMG will be responsible for the day-to-day conduct of the trial. They will be responsible for the clinical set-up, promotion, on-going management of the trial, the interpretation of the results and preparation and presentation of relevant publications.

The TMG will meet regularly (usually by teleconference).

#### 17.4 Trial Steering Committee

The Trial Steering Committee will be set up to oversee the trial. Membership will be composed of the TMG, independent clinicians, invited Principal Investigators, representatives from the funders and at least one patient advocate. The Trial Steering Committee will meet shortly before commencement of the trial and then annually (usually by teleconference), they will supervises the conduct of the trial, 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 trial.
17.5 **Data Monitoring Committee**

Data analyses pertaining to trial conduct, data quality and patient safety will be supplied in confidence to an independent DMC, which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group. During the recruitment phase of the trial the DMC is scheduled to meet after the first year of recruitment and then annually thereafter. Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMC will report directly to the TMG. The DMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety.

17.6 **Finance**

ROSCO is an investigator-initiated and investigator-led trial funded through a project grant from the Clinical Trials Awards and Advisory Committee (CTAAC) and an educational grant from Celgene. The trial has been independently peer reviewed and has been adopted by the NIHR Clinical Research Network (CRN) Portfolio.

No payments will be made to patients participating in this trial. NHS Trusts will receive a per patient payment for provision of tissue blocks.

18 **Ethical Considerations**

The trial 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 (see Appendix 9).

The trial will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the Data Protection Act 1998 and Human Tissue Act 2008) and the EU GCP Directive (2005/28/EC). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by the REC prior to circulation.

Before any patients are enrolled into the trial, 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 approval is received by the ROSCO Trial 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.
19 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 General Data Protection Regulation and the Data Protection Act (2018). With the patient’s consent, their full name, date of birth, address, post code, NHS number (or in Scotland the CHI) and hospital number will be collected at trial entry in order for the ROSCO Trial Office to send the QoL Booklet directly to patients at their home address, to allow tracing through the Cancer Registries and the Health and Social Care Information Centre, Data Linkage Service, and to assist with long-term follow-up via other health care professionals (e.g. patient’s GP) Patients will be identified using only their unique TNO, initials, and date of birth on the CRF and correspondence between the ROSCO Trial Office and the participating site. However patients are asked to give permission for the ROSCO Trial 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.

The investigator must maintain documents not for submission to the ROSCO Trial 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 trial records, provided that patient confidentiality is protected.

The ROSCO Trial 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 ROSCO trial 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.

20 Insurance and Indemnity

University of Birmingham employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University’s employment.

In terms of liability at a site, NHS Trust and non-Trust hospitals have a duty to care for patients treated, whether or not the patient is taking part in a clinical trial. Compensation is therefore available via NHS indemnity in the event of clinical negligence having been proven.

The University of Birmingham cannot offer indemnity for non-negligent harm. 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 patient compensation.

21 Publication Policy

Results of this trial 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.

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 trial was performed with the support of the University of Birmingham. Intellectual property rights will be addressed in the Clinical Study Site Agreement between Sponsor and site.
22 References


20. Pinder SE, Provenzano E, Earl H, Ellis IO. Laboratory handling and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy. Histopathology2007


54. Munro AF, Twelves C, Thomas JS, Cameron DA, Bartlett JM. Chromosome instability and benefit from adjuvant anthracyclines in breast cancer: British Journal of Cancer. 107 (1) (pp 71-74), 2012. Date of


Appendix 1: Clinical and Radiological Monitoring

In the neo-adjuvant setting, radiology has a role in estimating chemotherapy effect both in terms of tumour size and activity. Mammography in this situation shows poor correlation, as many of the tumours are irregular and lie in dense breasts. Candidates for neo-adjuvant chemotherapy are more commonly younger women, and mammographic density is associated with both age and high tumour grade (more common in candidates for neo-adjuvant chemotherapy). Ultrasound also has limitations again due to the often irregular and multifocal nature of these tumours and is operator dependent. MRI has the potential to view tumours in three dimensions, and may give a better estimate of volume. Moreover, the behaviour of the contrast gives some information on tumour activity.

However, as ultrasound remains the most widely available tool across the UK, it is a pragmatic robust radiological assessment which fulfils the RECIST criteria (see Appendix 5), for consistency of radiological measurement and in order not to compromise the analysis, ultrasound measurements are required for all ROSCO patients. Mammography is also required before treatment and may be helpful after cycle 4 for optimal surgical planning. MRI may be used in addition to ultrasound and mammography in accordance with local practice, or as the sole radiological technique if the tumour is not measurable by another method at baseline. If MRI is used as the baseline measurement it should then be used as the radiological modality for that patient throughout the course of the trial.

Radiological measurement of the breast, and assessment of axillary nodes should be performed.

Variation in timing of scans across tumours or patients can compromise the analysis. Therefore, applicable radiological measurement must be performed, as closely as possible, at the time points below:

- Before the first cycle of chemotherapy: ultrasound should be performed as close to the start of treatment as possible. Mammography should also be performed at baseline.
- After completion of the cycle 4 of neo-adjuvant chemotherapy (before surgery): ultrasound will be repeated to assess radiological response. The date of measurement should be after day 10 of final cycle of chemotherapy.
- After completion of all neo-adjuvant chemotherapy: ultrasound will be repeated to assess radiological response and to plan surgery.

The sum of the longest single diameter of all ultrasound measurable breast tumours must be recorded on the Radiology Assessment Form (see Appendix 5). Comment should also be made on axillary or supraclavicular fossa nodes if enlarged, including their response to treatment or otherwise.

It should be noted that the accurate excision of tumour sites when tumours have undergone significant pathological and radiological response to preoperative chemotherapy can be difficult and will be aided by clear communication of the original sites of the tumour, on the CRF and histology request form and especially by marking the sites with radiologically inserted clips prior to neo-adjuvant chemotherapy.
Appendix 2: TNM Staging System for Breast Cancer, Stage Grouping and Eligibility

Definitions of TNM Staging System for Breast Cancer

Primary Tumour (T)

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

Note

* Paget’s disease associated with a tumour is classified according to the size of the tumour.
## Regional Lymph Nodes (N)

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed (for example, previously removed)</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis to movable ipsilateral axillary lymph node(s)</td>
</tr>
<tr>
<td>N2</td>
<td>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</td>
</tr>
<tr>
<td>N2a</td>
<td>Metastasis in ipsilateral axillary lymph nodes fixed (or matted) to one another or to other structures</td>
</tr>
<tr>
<td>N2b</td>
<td>Metastasis only in clinically apparent* ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis</td>
</tr>
<tr>
<td>N3</td>
<td>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</td>
</tr>
<tr>
<td>N3a</td>
<td>Metastasis in ipsilateral infraclavicular lymph node(s)</td>
</tr>
<tr>
<td>N3b</td>
<td>Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)</td>
</tr>
<tr>
<td>N3c</td>
<td>Metastasis in ipsilateral supraclavicular lymph node(s)</td>
</tr>
</tbody>
</table>

**Notes**

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

## Distant Metastasis (M)

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Presence of distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis*</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Stage Grouping</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>T0</td>
<td>N1*</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>N1**</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIB</td>
<td>T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIIA</td>
<td>T0</td>
<td>N2*</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>N2**</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N2</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N2</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>T4</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIIC</td>
<td>Any T</td>
<td>N3</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>

Rows shaded blue are ineligible for the ROSCO trial.

**Note**
- TO and T1 tumours are eligible in presence of axillary node >20mm
- TO N1, N2 tumours must be histopathologically confirmed by lymph node biopsy (trucut or whole lymph node)
## Appendix 3: Eastern Cooperative Oncology Group (ECOG) Performance Status

<table>
<thead>
<tr>
<th>Grade</th>
<th>ECOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
</tr>
<tr>
<td>1</td>
<td>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</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self-care, confined to bed or chair more than 50% of waking hours</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
</tr>
</tbody>
</table>

(70)
Appendix 4: Hormonal Receptor Definitions

Oestrogen Receptor Rich Definition

Different laboratories use different scoring methodologies (Allred, Histoscore/Quick score and simple percentage assessment). These systems do not match exactly for a particular score. For the purposes of this trial we have defined “ER-rich” carcinomas as those tumours that have Allred scores of 7 and 8 with a minimum of intermediate (on average) intensity nuclear staining. We have then set a cut point for the two other systems that best matches these Allred scores – a Histoscore of ≥120 and a simple proportion score of ≥50% of cells staining. We anticipate that if the different scoring methodologies were applied to individual cases there would be complete concordance of >90% of cases. Allred and Histoscores are illustrated in the figure below with the ER-rich scores in blue.

<table>
<thead>
<tr>
<th>% Staining</th>
<th>Allred Proportion</th>
<th>Intensity</th>
<th>Histoscore Proportion (%)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
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<td>95</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>95</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>

Progesterone Receptor Negative Definition

PgR-negative will be accepted as per local protocols. Cut points are normally the same as those for ER.
Appendix 5: Response Evaluation Criteria In Solid Tumours (RECIST) Quick Reference

Eligibility

Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary outcome measure.

**Measurable disease:** the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

**Measurable lesions:** lesions that can be accurately measured in at least one dimension with longest diameter \( \geq 20 \text{ mm} \) using conventional techniques or \( \geq 10 \text{ mm} \) with spiral CT scan.

Response Criteria

Evaluation of Target Lesions

<table>
<thead>
<tr>
<th>Response Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response (CR)</td>
<td>Disappearance of all target lesions</td>
</tr>
<tr>
<td>Partial Response (PR)</td>
<td>At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD</td>
</tr>
<tr>
<td>Progressive Disease (PD)</td>
<td>At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. In addition to this, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more lesion is also considered progression.</td>
</tr>
<tr>
<td>Stable Disease (SD)</td>
<td>Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started</td>
</tr>
</tbody>
</table>

Please Note: If there is evidence of progressive disease in nodal sites, then the overall radiological response will be characterised as progressive disease independent of the characterisation of the breast lesions arrived at above.
Appendix 6: Histopathology and Assessment of Histopathological Response

Histopathological Examination

Histopathological assessment will be carried out at diagnosis, on the interim biopsy 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 prior to neo-adjuvant 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 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 and the tumour bed dimensions (x x y mm) should be included in the pathology report. Histopathological response will also be checked 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) or as an interim biopsy, see Section 9.0. The primary endpoint for the trial is pCR of all invasive disease in the tumour and nodes following neo-adjuvant treatment, and is defined below:

pCR rates (tumour and lymph nodes) after neo-adjuvant chemotherapy defined as no residual invasive carcinoma within the breast (DCIS permitted) AND no evidence of metastatic disease within the lymph nodes (20).

Histopathological tumour response following neo-adjuvant chemotherapy will be evaluated by the Central Pathology Reviewer(s) from the pathology reports. Surgical and interim biopsy pathology reports will be reviewed by one pathologist and categorised as follows.

Breast:

1. pCR
2. Minimum residual disease (MRD)
3. Significant residual disease (invasive only)

Axillary Lymph Nodes:

1. No tumour, no evidence of response
2. Tumour present but evidence of response
3. Tumour not present but evidence of response
To achieve the primary outcome definition of pCR the following must be achieved:

- a pCR in the breast; and
- either ‘no tumour, no evidence of response’ or ‘tumour not present but evidence of response’ must be recorded for the axillary lymph nodes.

In addition, a retrospective central pathology review of response will be performed. The central pathology review will be restricted to relevant slides from the tumour bed area and positive axillary lymph nodes.

**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 neo-adjuvant therapy 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 (71). RCB index and class can be calculated using an on line tool ([www.mdanderson.org/breastcancer_RCB](http://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 (24). RCB index and class will be recorded where provided.
Appendix 7: 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) 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 8: Common Toxicity Criteria Gradings
Toxicities will be recorded according to the CTCAE, version 4.0. The full CTCAE document is available on the National Cancer Institute website, the following address was correct when this version of the protocol was approved:

Appendix 9: 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 fulfillment 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, 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.
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