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

**Tissue Collection Guidelines**

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Contact Details

Sponsor
University of Birmingham, Edgbaston, Birmingham. B15 2TT

Chief Investigator
Dr Daniel Rea
Cancer Research UK Clinical Trials Unit (CRCTU)
University of Birmingham
Edgbaston
Birmingham. B15 2TT

CEP17/TOP2A Central Testing
Biomarker Coordinator: Dr Jane Starczynski
HER2 Team
Cellular Pathology
Heart of England NHS Foundation Trust
Bordesley Green East
Birmingham. B9 5SS
☎ 0121 424 2784

Translational Coordinating Centre (Tumour Samples)
Translational Lead: Professor John Bartlett
Tissue Bank Manager: Ms Carrie Cunningham or Mrs Tammy Piper
Biomarkers and Companion Diagnostics
Edinburgh Cancer Research Centre
University of Edinburgh
Western General Hospital
Crewe Road South
Edinburgh. EH4 2XR
☎ 0131 651 8605/8694
☎ 0131 651 8711
✉ carrie.cunningham@igmm.ed.ac.uk or tammy.piper@igmm.ed.ac.uk

Central Pathology Review
ROSCO Pathology Lead Dr Jeremy Thomas
Western General Hospital
Edinburgh. EH4 2XU
☎ 0131 537 1961

ROSCO Trial Office
Cancer Research UK Clinical Trials Unit (CRCTU)
School of Cancer Sciences
University of Birmingham
Edgbaston
Birmingham. B15 2TT

Enquiries
☎ 0121 414 3797
☎ 0121 414 8392
✉ ROSCO@trials.bham.ac.uk
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1 Purpose of this Document
This document provides guidelines on the collection and shipment of histopathology tissue samples for the ROSCO trial.

2 Background
Please refer to the ROSCO trial protocol for detailed information about the background and rationale for the trial. In summary, the trial is designed to determine if there a role for CEP17 and TOP2A testing in selecting anthracycline or taxane containing chemotherapy as neo-adjuvant treatment for early breast cancer. Figure 1 shows a schematic of the trial.

Sites are requested to provide the following histopathology samples so that pathological Complete Response (pCR) (the primary outcome measure) can be determined:

- Diagnostic core biopsy
- "Interim" biopsy post-treatment but prior to surgery*
- Surgical sample

Provision of these blocks is essential to determine the primary objective of the trial.

* For those few patients who switch to the opposite treatment arm prior to surgery an "interim" biopsy is taken after cycle 4.
Figure 1. 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 0800 371 969

Tissue sent for CEP17/TOP2A Analysis

Randomisation
Call ROSCO Trial Office on 0800 371 969
- CEP17/TOP2A status
- ER status
- HER2 status
- Nodal involvement

Arm A: FEC
- FEC100 X 4
- 3 weekly
- All HER2 +ve patients to receive trastuzumab and FEC75

Arm B: Taxane
- Docetaxel and cyclophosphamide
- 3 weekly X 4
- 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 X 4
- All HER2 +ve patients to receive trastuzumab and FEC75

Failure to Achieve Response on Taxane
- FEC100 X 4
- 3 weekly
- All HER2 +ve patients to receive trastuzumab

Follow-up For 5-years
3  Lead Pathologist Responsibilities

A Lead Pathologist should be named for the trial at each participating site. This member of staff should:

1. Complete a Site Staff Registration Form and return to the ROSCO Trial Office.

2. Sign the Site Signature and Delegation Log which is provided in Investigator Site File. This record should be initialled by the Principal Investigator. The log will be returned to the ROSCO Trial Office by the main contact for the site research team prior to the commencement of recruitment.

3. Ensure samples are shipped for pathological review as detailed in this document and that copies of relevant pathological reports are provided to the Trial Office*.

4. Where possible attend site initiation visit or teleconferences where requested by the Trial Office.

Should the Lead Pathologist change, a new Site Staff Registration Form must be completed and the Signature and Delegation Log must be updated and forwarded to the ROSCO Trial Office as soon as possible.

* Please note: pathology reports should not be sent to the Translational Coordinating Centre, Edinburgh

4  Collection of Samples

4.1  Diagnostic Biopsy

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 HER2 Team, Heart of England NHS Foundation Trust using Kit 1 (see Section 5.0).

Central analysis of CEP17/TOP2A will usually be performed within 1 week, which can be calculated from the day the sample is received by the HER2 Team.

The blocks must be received and analysed before randomisation can proceed. The tissue block will then be sent for Central Review by the ROSCO Lead Pathologist, care of the Translational Coordinating Centre, Edinburgh, before being returned to Translational Coordinating Centre for storage.

Before being used for any additional research the ROSCO Pathology Lead will ensure that sufficient material remains for diagnostic purposes.

A copy of the associated pathology report for each patient should be anonymised and sent to the ROSCO Trial Office.*

* Please note: pathology reports should not be sent to the HER2 Team, Heart of England NHS Foundation Trust

4.2  “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, patients can cross over to the alternative arm pre-surgery. In these circumstances an “interim” biopsy must be taken after cycle 4 of neo-adjuvant chemotherapy.

The “interim” biopsy paraffin embedded tumour block should be sent to Translational Coordinating Centre, Edinburgh directly using Kit 2 (see Section 5.0).

A copy of the associated pathology report for each patient should be anonymised and sent to the ROSCO Trial Office.*

* Please note: pathology reports should not be sent to the Translational Coordinating Centre, Edinburgh
4.3 Tumour Blocks at Surgery

Haematoxylin and Eosin (H&E) slides from all blocks taken at surgery (tumour and lymph nodes where applicable) will also be requested for all patients participating in the trial. Where slides are unavailable, these can, on request, be made from the paraffin-embedded blocks by Translational Coordinating Centre, Edinburgh.

Representative tumour blocks (tumour tissue and normal tissue) obtained from surgical specimen will also be requested. Where a complete clinical or pathological response is observed the following are requested:

- 1 block of the tumour (or the original site of the tumour)
- 1 block of adjacent normal tissue, from the same quadrant as the carcinoma

Blocks and slides should be sent to the Translational Coordinating Centre, Edinburgh directly using Kit 3 (see Section 5).

A copy of the associated pathology report for each patient should be anonymised and sent to the ROSCO Trial Office. *

* Please note: pathology reports should not be sent to the Translational Coordinating Centre, Edinburgh

4.4 Retention of Tissue Samples for ROSCO Bio-repository

The tissue samples for the trial will be retained at the Translational Coordinating Centre, Edinburgh for research purposes. 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.
5 Sample Shipment

5.1 Tissue Sample Shipment Kits

Three Tissue Sample Collection Kits will be provided by the ROSCO Trial Office for shipment of tissue samples:

- **Kit 1: Diagnostic Block for CEP17/TOP2A Testing**
  - SafeBOX™ pre-addressed with a blue coloured label to the HER2 Team, Heart of England NHS Foundation Trust
  - ROSCO Tissue Sample Collection Form – Diagnostic Block

- **Kit 2: “Interim” Biopsy**
  - Pre-paid padded jiffy bag addressed to the Translational Tissue Bank (Biomarkers and Companion Diagnostics, Edinburgh Cancer Research Centre)
  - ROSCO Tissue Sample Collection Form – “Interim” Biopsy

  * For those few patients who switch to the opposite treatment arm prior to surgery

- **Kit 3: Surgical Samples**
  - Pre-paid padded envelope addressed to Translational Tissue Bank (Biomarkers and Companion Diagnostics, Edinburgh Cancer Research Centre)
  - ROSCO Tissue Sample Collection Form – Surgical Sample
  - White slide box for slides

Kits should be stored at room temperature.

A stock of kits and a fine permanent pen (for labelling purposes) will be provided at site initiation. The stock will be replenished as patients are registered into the trial; however if additional supplies are required please contact the ROSCO Trial Office.

5.2 Shipping Samples

5.2.1 Shipping Blocks

5.2.1.1 Diagnostic Biopsy

1. Select Kit 1.

2. Package the samples by following the SafeBOX™ step by step instructions.

3. Complete the Tissue Sample Collection Form – Diagnostic Biopsy (one form per SafeBOX™). Attach the small sender sticker number (highlighted with the blue circle in Figure 2 below) on to the space provided for the Royal Mail Tracking Number.
4. Take two photocopies of each completed Tissue Sample Collection Form.
5. Enclose the original form in the relevant compartment next to the samples.
6. Close the SafeBOX™ lid and wrap the label around the box as directed.
7. Affix the tracking barcode from the receipt to the SafeBOX™ as displayed in Figure 3.
8. Post the samples on the same day. Tumour samples should preferably not be posted on a Friday.
5.2.1.2 Interim Biopsy and Tumour Blocks at Surgery

1. Select the appropriate Kit (2 or 3 as applicable for the type of sample to be shipped).

2. Label the jiffy bag as illustrated in the example below choosing from the following sample type options codes:
   - Interim biopsy: IB
   - Surgical sample: SS

Figure 4. Example of How to Label Jiffy Bag

<table>
<thead>
<tr>
<th>Trial Number:</th>
<th>123</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type:</td>
<td>SS</td>
</tr>
</tbody>
</table>

3. Ensuring the patient’s name is not included on the block (initials are permissible).

4. Place the blocks inside the labelled jiffy bag.

5. Complete the appropriate Tissue Sample Collection Form (one form per patient per time point):
   - Tissue Sample Collection Form – Interim Block (see Figure 5)
   - Tissue Sample Collection Form – Surgical Sample (see Figure 6)
Figure 5. Example of the “Interim” Biopsy Tissue Collection Form

![Tissue Sample Collection Form](image)
Figure 6. Example of the Surgical Sample Tissue Collection Form

![Figure 6. Example of the Surgical Sample Tissue Collection Form](image)
6. Take two photocopies of the completed form.

7. Staple the completed original form to the jiffy bag and place inside the padded postage pre-paid envelope.

8. Ensure samples are posted on the same day.

9. Post a photocopy of the Tissue Sample Collection Form to the ROSCO Trial Office.

10. Ensure that a photocopy of the Tissue Sample Collection Form is filed in Section 8 of the Investigator Site File.

5.2.2 Surgical Resection Slides

1. Ensure the patient’s name is not included on the slides (initials are permissible).

2. Label the white box with the patient(s) Trial Number(s).

3. Place the slides inside the white box. Fill any space with tissue to minimise slide movement during transit and tape the edges of slide box together to make ensure the lid does not open or come off during transit.

4. Complete the Tissue Sample Collection Form – Surgical Sample (one form per patient).

5. Take two photocopies of the completed form.

6. Place the labelled white box together with the completed original form inside the padded postage pre-paid envelope.

7. Ensure samples are posted on the same day.

8. Post a photocopy of the Tissue Sample Collection Form to the ROSCO Trial Office.

9. Ensure that a photocopy of the Tissue Sample Collection Form is filed in Section 8 of the Investigator Site File.