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EORTC Radiation Oncology Group
EORTC Breast Cancer Group

A phase III randomized trial to assess the role of adjuvant chest wall irradiation in 'intermediate risk' operable breast cancer following mastectomy

EORTC 22051-10052

LABORATORY MANUAL

TRANS-SUPREMO

MRC SUPREMO (BIG2-04)

ISRCTN61145589

Version 5, 20/04/2016

Trans-Supremo sub-study

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CONTACT INFORMATION

TRANS-SUPREMO

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Tammy Piper – Senior Biomedical Scientist
Carrie Cunningham – Research Technician
Monika Sobol – Research Technician

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TELEPHONE
Office: +44 (0) 131-651-8605

FAX
+44 (0) 131-651-8711

When contacting, please include the following information:

- Your name, email address and telephone number
- Your centre details including centre name/ID number
- Patient trial number (if applicable)

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INTRODUCTION

TRIAL SUMMARY

MRC-SUPREMO is a MRC randomised phase III trial assessing the role of chest wall irradiation in women with intermediate risk of breast cancer following mastectomy, axillary surgery and appropriate systemic therapy. Patients will be randomly allocated to either receive chest wall irradiation or allocated to a control group.
The study will recruit 1600 patients.

TRANSLATIONAL SCIENCE SUB-STUDY SUMMARY

Within the context of the SUPREMO trial there exists a unique opportunity to expand our knowledge of the molecular mechanisms underlying the relapse of breast cancer and resistance to radiation therapy.
Standard prognostic factors do not define the 60-80% of patients in whom radiotherapy might be safely omitted. The aim of the present study is to identify molecular signatures and validate methods by which such patients can be identified in the clinic using the SUPREMO trial as a test system.

A 25ml blood sample will be taken from all patients who consent, at baseline and at disease recurrence (local and/or distant relapse).

Tissue blocks will be requested, following randomisation, from pathology departments. Blocks will be used for the extraction of small diameter cores of tissue for the production of tissue micro-arrays and will be returned after processing has been completed.

This manual contains general information about sample handling procedures as well as the storage and shipping of specimens and the use of the relevant forms.

PLEASE READ THIS LABORATORY MANUAL CAREFULLY TO ENSURE PROPER HANDLING OF THE SPECIMENS

THANK YOU

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GENERAL INSTRUCTIONS

Patients can be approached for inclusion to Trans-Supremo sub-study. A separate consent **must** be obtained for participation in this sub-study.

Patient tissue samples will also be used for the pathology audit as per the main trial protocol. A separate consent is **not** required for this as the patient has given consent to take part in the main trial. This consent covers any auditing that may be undertaken during the trial, under which the Pathology Audit is covered.

Consent Given For:	**Relevant pages in document**
Trans-Supremo	6-12
Trans-Supremo for tissue donation only	14-15

No additional consent required	**Relevant pages in document**
Pathology Audit (part of the main trial)	17-19

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INSTRUCTIONS

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FOR

TRANS-SUPREMO

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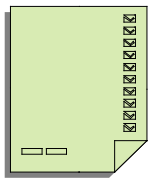
SUPPLIES FOR TRANS-SUPREMO

Each site will be supplied with kits containing the following items:

Laboratory Requisition Form (1 x NCR)



Tubes and coloured caps for processed specimens (5 x green; 5 x red; 5 x blue) plus labels and sample bag for freezing

Pasteur pipettes (x 4, including 1 spare)

Blood sample flow chart for Trans-Supremo – see page 9

When you have **2 blood sample kits remaining**, please **photocopy** the Blood Sample Kit Request Form (Appendix 1), **complete** and **fax** to Biomarkers & Companion Diagnostics Group, Edinburgh, UK (+44 (0) 131 651 8711).
5 kits will be despatched immediately. Please do not email requests to the clinical trials office as this may result in a delay in the kits being sent to you.

The following equipment is required but not provided:

- Venepuncture kit
- Plain tube with separator gel; 10 ml (SST Vacutainer or S-Monovette Serum *or equivalent*)
- EDTA tubes; 3 x 5 ml *or equivalent*
- Centrifuge
- Freezer; -80°C (or if not available, -20°C)

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USE OF LABORATORY REQUISITION FORMS

Please complete a Laboratory Requisition Form, in **black ball-point pen,** for each blood sample taken, i.e. at first visit and at disease recurrence (local and/or distant relapse).

Appendix 2 shows an example of the "Laboratory Requisition Form".

Please note that to ensure unique identification by the data matrix label system to be applied by the laboratory no copying or combined use of the form is allowed!

Please write the numbers and letters clearly and provide complete and accurate information.

Fill in a Laboratory Requisition Form with the following information:

1. Patient 7 digit trial number = 3 digit centre number and 4 digit trial number, e.g. E07 999R/C
2. Patient's initials = 3 letters; first-middle-last, if only 2 leave middle blank
3. Patient's date of birth = DD/MM/YY e.g. 09/05/65
4. Sample information = whether first visit or recurrence. If recording details of local or distant recurrence please include details of the site of recurrence.
5. Date of sampling = DD/MM/YY e.g. 23/02/04
6. Time of sampling = HH:MM e.g. 14:45 **NB USE 24 HOUR CLOCK**
7. Time sample centrifuged = HH:MM e.g. 15:15 **NB USE 24 HOUR CLOCK**
8. Time of freezing = HH:MM e.g. 16:30 **NB USE 24 HOUR CLOCK**
9. Sign and date the **'Informed Consent Checked'** box to indicate to the laboratory staff that the patient has given informed consent to participate in the biological sub-study – this section is to be completed by the person filling out the form, ***not*** the patient.
   Please do not include copies of the consent form with the sample as the central laboratory must not receive any paperwork with the patient's full name

Please note that the Laboratory Requisition Form is supplied in a "No Carbon Required" (NCR) format.

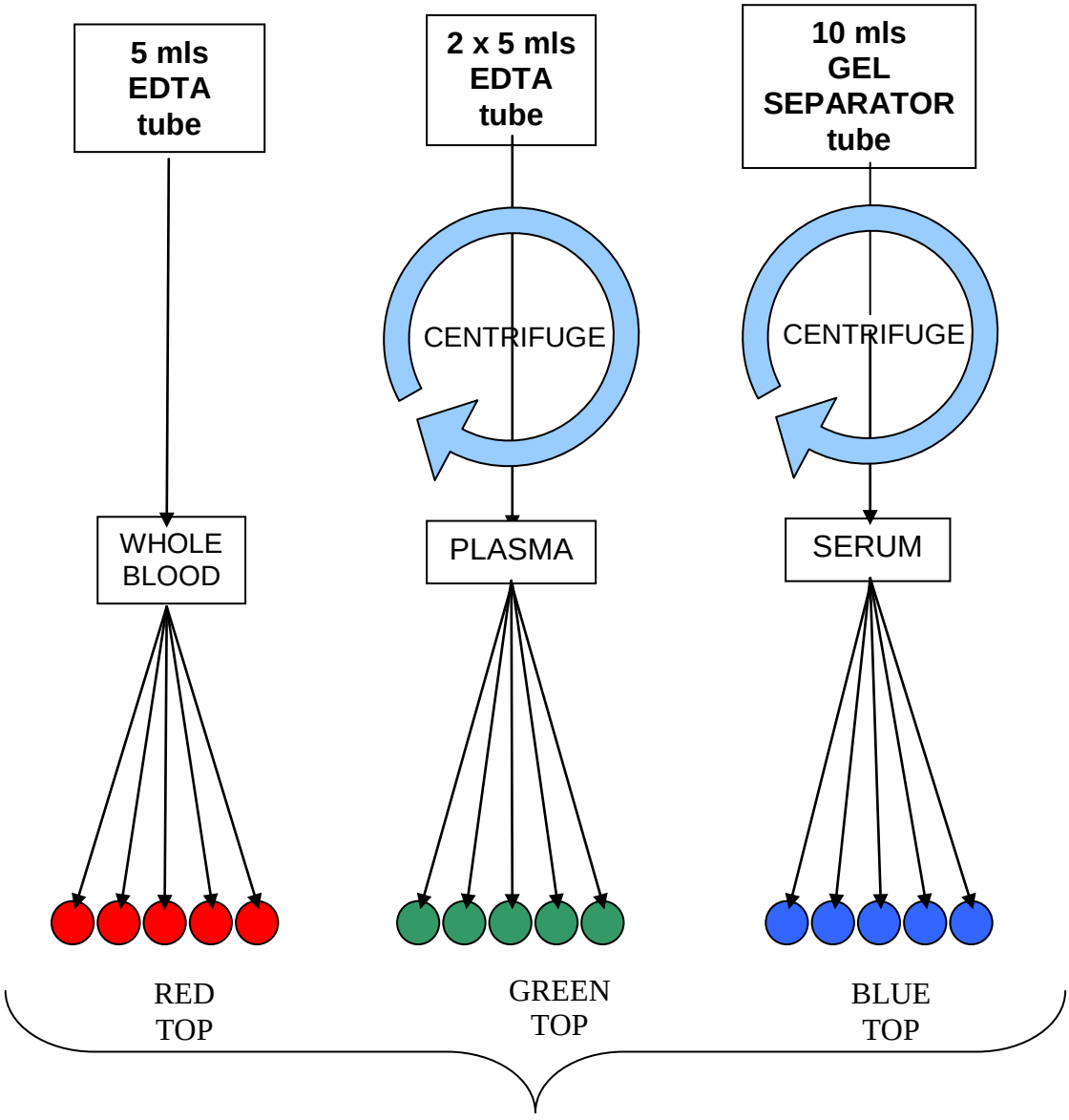
The **top copy (white)** is to be put into the pocket in the **FROZEN sample** bag and placed in the freezer at -80°C (or -20°C, if not available). This is to be despatched on request with the sample to the Biomarkers & Companion Diagnostics Group, Edinburgh.

The **bottom copy (blue)** is to be **retained** for your records.

The **second copy (yellow)** is an additional copy and can be sent with the samples or retained for your records.

BLOOD SAMPLE FLOW CHART: TRANS-SUPREMO

A 25 ml blood sample should be taken at the following time points: first visit (baseline) and at disease recurrence (local and/or distant relapse). The samples should be processed as follows:-



PIPETTE 0.8 - 1ml INTO EACH TUBE
APPLY AN ADHESIVE LABEL (provided) TO EACH TUBE

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PLACE USED *AND* UNUSED TUBES IN BAG PROVIDED
PLACE LAB REQUISITION FORM INTO SLEEVE
FREEZE AT -80°C (OR -20°C, IF NOT AVAILABLE)

Biomarkers & Companion Diagnostics Group: email tammy.piper@igmm.ed.ac.uk or tel +44 (0) 131 651 8605
Cancer Clinical Trials Team: email nss.isdsupremo@nhs.net or tel +44 (0) 131 275 7058

BLOOD SAMPLE INSTRUCTIONS: TRANS-SUPREMO

The samples need to be separated within 1 hour and frozen immediately after processing (up to a maximum of 30 minutes).

- A 25 ml blood sample to be taken at baseline (first visit) and at disease recurrence, (local and/or distant relapse) - Collect 10 ml of blood in a plain tube with separator gel and 15 ml of blood in 3 x 5 ml **plastic** EDTA tubes.
- Complete the 15 small labels provided with the patient trial number, patient initials and the date – these will be used to label the small cryovials.
- Complete Laboratory Requisition Form as described on page 8.

WHOLE BLOOD SAMPLING:

- Do not centrifuge one of the EDTA tubes (whole blood sample).
- Using the pipette provided, transfer **0.8 ml** of **whole blood** into each of the **5 red-topped** cryovials* and apply small labels.
-

PLASMA SAMPLING:

- Centrifuge the 2 remaining EDTA tubes at 1300g (approximately 2,500 rpm) for 15 minutes.
- Using the pipette provided, transfer **0.8 ml** of **plasma** into each of the **5 green-topped** cryovials*. ***Take care not to disturb the cells with the pipette.*** Apply small labels.

SERUM SAMPLING:

- Allow blood in the plain tube with gel separator to clot for 30 minutes at room temperature. Centrifuge at 1300g (approximately 2,500 rpm) for 15 minutes.
- Using the pipette provided, transfer **0.8 ml** of **serum** from the gel separator tube into each of the **5 blue-topped** cryovials* and apply small labels.

(* If insufficient sample is obtained, please indicate on the Laboratory Requisition Form the number of cryovials containing samples.)

- Put the filled and any empty, unused cryovials into the small zip topped sample bag provided.
- Fold and place **top copy (white)** into the pocket in the rear of the **sample** bag.
- The **bottom copy (blue)** of the Laboratory Requisition Form is to be **retained** for your own records.
- The **second copy (yellow)** is an additional copy and can be sent with the samples or retained for your records.
- Place samples into your freezer at -80°C (-20°C acceptable for up to 6 months if you do not have access to -80°C).
- Larger centres will be contacted once per year to arrange collection of frozen samples by courier; however centres that are recruiting faster or those with a -20°C freezer are encouraged to contact EORTC sooner if the available storage space is filling up.
- Smaller centres should contact EORTC directly to arrange a courier collection when they have collected 5 or more patient samples (i.e. 5 or more packs of 15 tubes).
- All transport packaging and dry ice will be provided.
- On arrival at the central laboratory the frozen samples will be stored at -80°C until analysis.

SAMPLING PRIORITIES

- We appreciate that it can sometimes be difficult to collect several blood samples from cancer patients. If this is the case please prioritise the blood samples as follows:

  Serum (10 ml gel separator)
  Plasma (2 x 5 ml EDTA)
  Whole blood (1 x 5 ml EDTA)

- For each of the individual sample types please put 0.8 ml into each of the cryovials provided. If not enough sample is obtained, it is better to put a larger volume into fewer cryovials than to put a smaller volume in more cryovials i.e. put 0.8 ml into 2 cryovials rather than 0.3 ml into 5 cryovials.
- Complete the form with the number of samples filled in the appropriate boxes
- Place all filled and unfilled tubes into the sample bag.

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COURIER COLLECTIONS OF FROZEN SAMPLES

Centres will be contacted by the EORTC to arrange collection of the frozen samples by courier.
If samples need to be collected before this time then please contact EORTC (22051@eortc.be) to arrange collection. Please **do not** contact the Clinical Trials Team as this may result in a delay in processing your request.

In order to arrange the courier collections please provide EORTC with the following information:

- How many samples need to be collected
- The name and direct telephone number of the main contact at the centre to liaise with the courier company
- The exact address of where the samples are to be collected from.

The courier company will liaise with the centre directly to arrange uplift of samples.

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INSTRUCTIONS

FOR

TISSUE BLOCK

PROCESSING

FOR

TRANS-SUPREMO

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INSTRUCTIONS FOR TISSUE BLOCK PROCESSING

- Written consent is required from patients who wish to donate tissue for research.
- Tissue blocks will be requested for all patients who consent to the Trans-Supremo sub-study, at baseline and again at times of local and/or distant recurrence or at first diagnosis of contra-lateral breast cancer, if available.
- Small 0.6 mm cores will be extracted from the blocks for tissue micro-array construction.
- Patient randomisation confirmation details and a lab kit will be supplied for each block requested consisting of;
  - Pathology Request Form (Appendix 3)
  - Small sample bag
  - Addressed, padded envelope
- Complete the Pathology Request Form with all the information indicated.
- **Please note that to ensure unique identification by the data matrix label system to be applied by the laboratory no copying or combined use of the form is allowed!**
- Sign and date, **'Informed Consent Checked'** box to indicate to the laboratory staff that the patient has given informed consent for their tissue to be used for the biological sub-study – this section is to be completed by the person filling out the form, ***not*** the patient.
  Please do not include copies of the consent form with the sample as the central laboratory must not receive any paperwork with the patient's full name
- After completion of the Pathology Request form, please fold and place **top copy (white)** into the pocket in the rear of the **sample** bag. The **bottom copy (blue)** is to be **retained** for your records.
- Please send **one** representative tumour block from the mastectomy or wide local excision for each patient. Please do not send needle or core biopsies.
- Return the block and form in the addressed, padded envelope.
- Blocks will be stored in a secure facility and all data will be held in a confidential manner. Blocks will be returned to the source pathology department upon completion of processing. Should you require the block back sooner, then please contact Tammy Piper and the block will be returned within 48 hours where possible.

If you experience any difficulties or have any questions, please contact the Biomarkers & Companion Diagnostics Group.

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INSTRUCTIONS

FOR THE

PATHOLOGY AUDIT

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INSTRUCTIONS FOR THE PATHOLOGY AUDIT

- All patients who have consented to the main MRC Supremo trial will have a tissue block requested for participation in the quality assurance pathology audit.
- A separate consent **is not** required as the audit is part of the main trial which the patient has already consented to.

Note 1: ***Blocks sent for Trans-Supremo patients are automatically included in the pathology audit so a separate request for these samples will not be made.***

- A kit will be supplied for each block requested consisting of;
  - Pathology Request Form (Appendix 4) - clearly labelled with 'PATHOLOGY AUDIT'
  - Small sample bag
  - Addressed, padded envelope
- Complete the Pathology Request Form with all the information indicated.
  - The 'Informed Consent Checked' box will be scored through as separate consent is not required for the pathology audit. Please do not write anything in this box – this is used solely to indicate consent given for Trans-Supremo
- After completion of the Pathology Request form, please fold and place **top copy (white)** into the pocket in the rear of the **sample** bag. The **bottom copy (blue)** is to be **retained** for your records.
- Please send **one** representative tumour block for each patient
- Return the block and form in the addressed, padded envelope.
- Blocks will be stored in a secure facility and all data will be held in a confidential manner.
- Sections will be taken from the tissue block and stained with haematoxylin and eosin at the central laboratory using standardised methods. These slides will be circulated to 3 trial pathologists for blinded review. They will assess the type of tumour, grade and presence of lymphovascular invasion.

Note 2: ***These blocks will be returned to the department of origin as soon as the pathology audit has been performed and the results have been received for the sample.***

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ALTERNATIVE INSTRUCTIONS FOR THE PATHOLOGY AUDIT

- If the pathology department refuses to send blocks for the audit we will accept two H&E slides they have prepared locally.
- If you experience any difficulties or need any advice please contact the Biomarkers & Companion Diagnostics Group

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Appendix 1

BLOOD SAMPLE KIT REQUEST FAX

TO ORDER 5 KITS:

When you have **2 blood sample kits remaining**, please photocopy this form, complete and fax to:-

FAO: Tammy Piper, Carrie Cunningham, Monika Sobol

Fax no: +44 (0) 131 651 8711

(PLEASE PRINT DETAILS)

Contact name _____ Date _____

Email address_____

Centre number_____

Hospital _____

Address _____

_____

_____

_____

For Laboratory Use: - Kits despatched

Comments

Signature Date

If you experience any difficulties or have any questions, please contact the Biomarkers & Companion Diagnostics Group by emailing tammy.piper@igmm.ed.ac.uk; or telephoning +44 (0) 131 651 8711.

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Appendix 2

Affix Data matrix label (barcode)

LABORATORY REQUISITION FORM (EXAMPLE)

PATIENT TRIAL NUMBER ☐☐☐☐☐☐☐

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

PATIENT INITIALS **(First:Middle:Last)** ☐☐☐

PATIENT DATE OF BIRTH **(DD/MM/YY)** ☐☐ / ☐☐ / ☐☐

SAMPLE INFORMATION
(**Please tick** √)

First visit (screening) ☐ Post chemo/preRT ☐ Post R[illegible]therapy ☐

1 yr visit ☐ 5 yr visit ☐ 10 yr visit ☐ Local recurrence ☐ Distant recurrence ☐

Site _____

DATE OF SAMPLING **(DD/MM/YY)** ☐☐ / ☐☐ / ☐☐

TIME SAMPLE TAKEN **(HH:MM)** ☐☐ : ☐☐

TIME CENTRIFUGED **(HH:MM)** ☐☐ : ☐☐

TIME SAMPLE FROZEN **(HH:MM)** ☐☐ : ☐☐

N° of 0.8ml samples stored:-

SERUM ☐

PLASMA ☐

WHOLE BLOOD ☐

White copy with frozen samples
Yellow copy with BNP sample
Blue copy to file

NAME OF STUDY NURSE / TECHNICIAN:
(Capital letters)

Sign Date

Trans-Supremo sub-study

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Appendix 3

Affix Data matrix label (barcode)

PATHOLOGY REQUEST FORM (EXAMPLE)

FAO:
Research nurse (Name) _____

Pathologist (Name) _____

Centre ID _____

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

Please forward paraffin block(s) relating to the patient below, to the Biomarkers & Companion Diagnostics Cancer Group, Edinburgh. Blocks **must** be from the surgical resection and **not** core biopsies.

PATIENT TRIAL NUMBER | | | | | | | |

PATIENT INITIALS
(First:Middle:Last) | | |

PATIENT DATE OF BIRTH
(DD/MM/YY) | | / | | / | |

Full Pathology number (invasive primary tumour)

Block despatched to Biomarkers & Companion Diagnostics Cancer Group, Edinburgh UK
Pathology Number:

Type of sample/origin:

Primary ☐ Local relapse ☐ Distant relapse ☐ Contralateral breast cancer ☐

Comments:

Name of person completing this form (Capital letters)

Signature Date

Block returned to Pathology Dept:-

Signature Date

White copy with block

Blue copy to file

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PATHOLOGY AUDIT

PATHOLOGY REQUEST FORM (EXAMPLE)

FAO:
Research nurse (Name) _____

Pathologist (Name) _____

Centre Name/ ID _____

Informed Consent (Trans-Supremo) checked:
Name

Sign

Date

Please forward one representative paraffin embedded tumour block relating to the patient below, to the Biomarkers & Companion Diagnostics Cancer Group, Edinburgh. Blocks MUST be from the surgical resection and NOT core needle biopsies.

PATIENT TRIAL NUMBER | | | | | | |

PATIENT INITIALS
(First:Middle:Last) | | | |

PATIENT DATE OF BIRTH
(DD/MM/YY) | | | / | | / | | |

Full Pathology number (invasive primary tumour)

Block despatched to Biomarkers & Companion Diagnostics Cancer Group, Edinburgh UK
Pathology Number:

Type of sample/origin:

Primary ☐ Local relapse ☐ Distant relapse ☐ Contralateral breast cancer ☐

Comments:

Name of person completing the form: (Capital letters)

Signature Date

Block returned to Pathology Dept:-

Signature Date

White copy with block / slides

Blue copy to file