OPTIMA Site Sample Collection Standard Operating Procedure

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Mary Falzon and Elaine Borg (Consultant Pathologists, University College London Hospitals) were consulted during the preparation of this SOP.

Revision chronology:	Effective date:	Reason for change:
Version 8.0	27 July 2021	Administrative amendments for consistency with Central Laboratory SOP and minor corrections.
Version 7.0	19 November 2020	Amendments for consistency with Protocol v7.0 and administrative changes.
Version 6.0	21 March 2019	Amendments for consistency with Protocol v6.0 and administrative changes.
Version 5.0	21 December 2016	Progression from the feasibility phase (OPTIMA prelim) to the main phase of the OPTIMA study. Amendments to reflect change of primary test used to allocate treatment to Prosigna.
Version 4.0	25 October 2013	Appendix 2 Pathology Sample Transit Form amended.
Version 3.0	29 January 2013	Additional detail to section 2 Bilateral and multifocal cancers.
Version 2.1	23 October 2012	Appendix 2 Pathology Sample Transit Form amended.
Version 2.0	03 September 2012	Appendix 2 Pathology Sample Transit Form amended.
Version 1.0	31 August 2012	

OPTIMA Site Sample Collection Standard Operating Procedure

This Standard Operating Procedure (SOP) describes the responsibilities and processes to be adopted by sites in relation to pathology tissue sample handling for OPTIMA. It provides additional guidance to that contained in the Protocol.

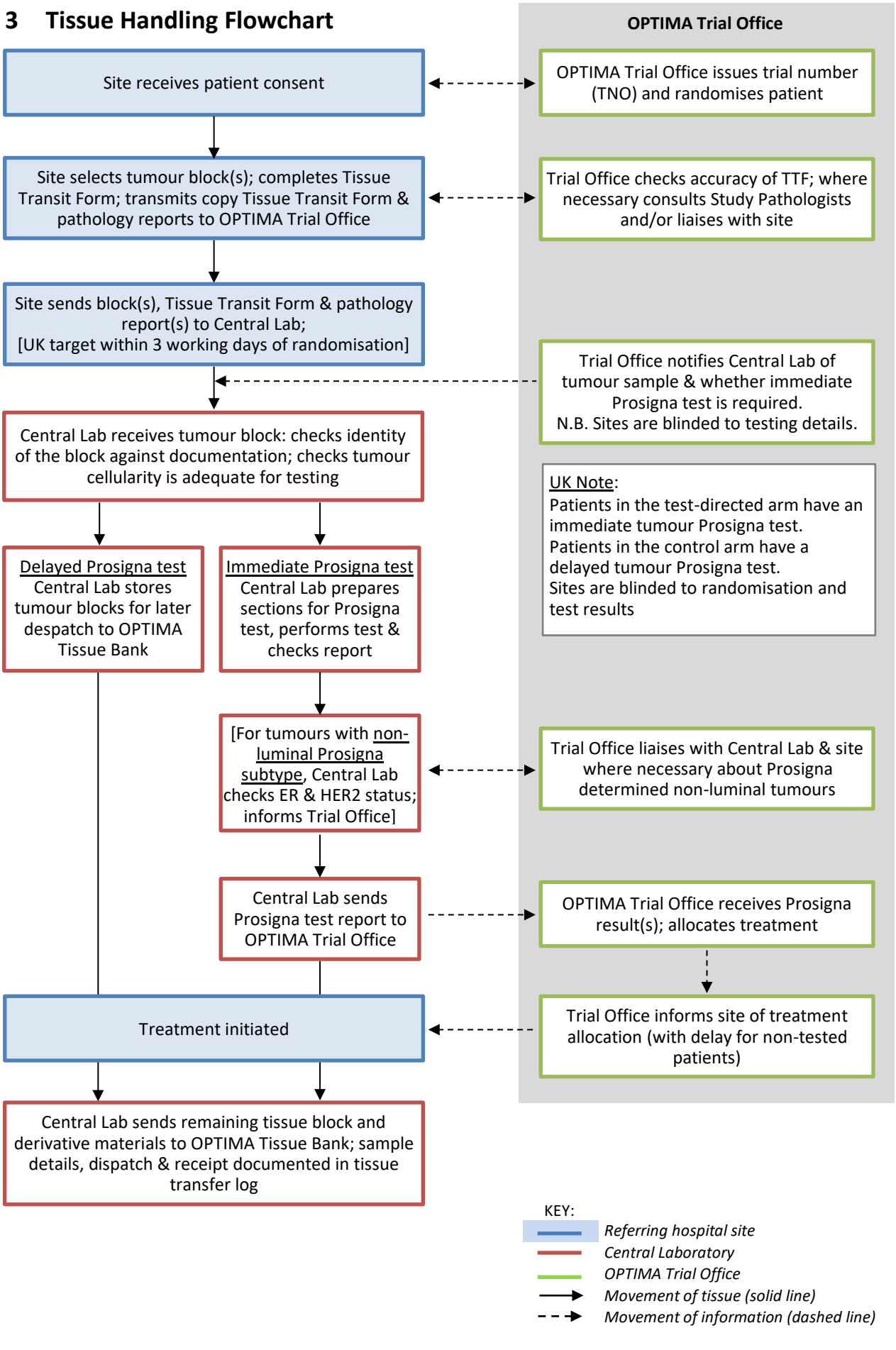
1 List of definitions:

Site	Hospital that recruited the participant into the OPTIMA trial
OPTIMA Trial Office	Team based at Warwick Clinical Trials Unit who are responsible for the day-to day coordination of the trial
Central Laboratory	Laboratory contracted by the OPTIMA trial to conduct testing within the trial.
OPTIMA Tissue Bank	Laboratory responsible for the storage of tissue collected as part of the trial.

2 Contact details

OPTIMA Trial Office	Warwick Clinical Trials Unit Warwick Medical School University of Warwick Gibbet Hill Road Coventry CV4 7AL	Named contact: Georgi Dotchin Role: Clinical Trial Manager Tel: 024 7615 1948 Email: optima@warwick.ac.uk
UK Central Laboratory	HSL Advanced Diagnostics Ground Floor 60 Whitfield Street London W1T 4EU	Contact by sites to the Central Laboratory should be made via the OPTIMA Trial Office.
OPTIMA UK Tissue Bank	Biomarkers & Companion Diagnostics Group Edinburgh Cancer Research Centre Western General Hospital Crewe Road South Edinburgh EH4 2XR	Contact by sites with the Tissue Bank should be made via the OPTIMA Trial Office.

3 Tissue Handling Flowchart



4 Receptors

OPTIMA eligibility requires all tumours to be HER2-negative and ER-positive with greater than 10% staining for ER as determined by the local pathology service. ER low-positive tumours (i.e. with 1-10% staining for ER) are a patient ineligibility criterion.

To facilitate trial data collection, the OPTIMA Trial Office requests that sites which currently report only Allred/ Quick Scores or H-Score additionally report %staining for ER.

5 Tissue management within OPTIMA

This section is for information; it provides an overview of tissue management and the flow of information within OPTIMA.

The collection and gene expression testing of archival tumour blocks is integral to patient management in the OPTIMA trial. For each patient who consents to enter OPTIMA, sites will send a formalin fixed paraffin embedded (FFPE) block from the invasive breast cancer to the Central Laboratory.

Upon receipt of the tissue block, the Central Laboratory cuts a section for H&E staining to allow assessment for invasive tumour content.

For participants assigned to an immediate Prosigna test, the Central Laboratory will prepare the tissue block for macro-dissection and RNA extraction for Prosigna testing and will proceed with sample analysis. For participants randomised to a delayed test, the tissue block will be held at the Central Laboratory until onward transfer to the OPTIMA Tissue Bank.

UK patients randomised to the test-directed arm have an immediate tumour Prosigna test. UK patients in the control arm have a delayed tumour Prosigna test.

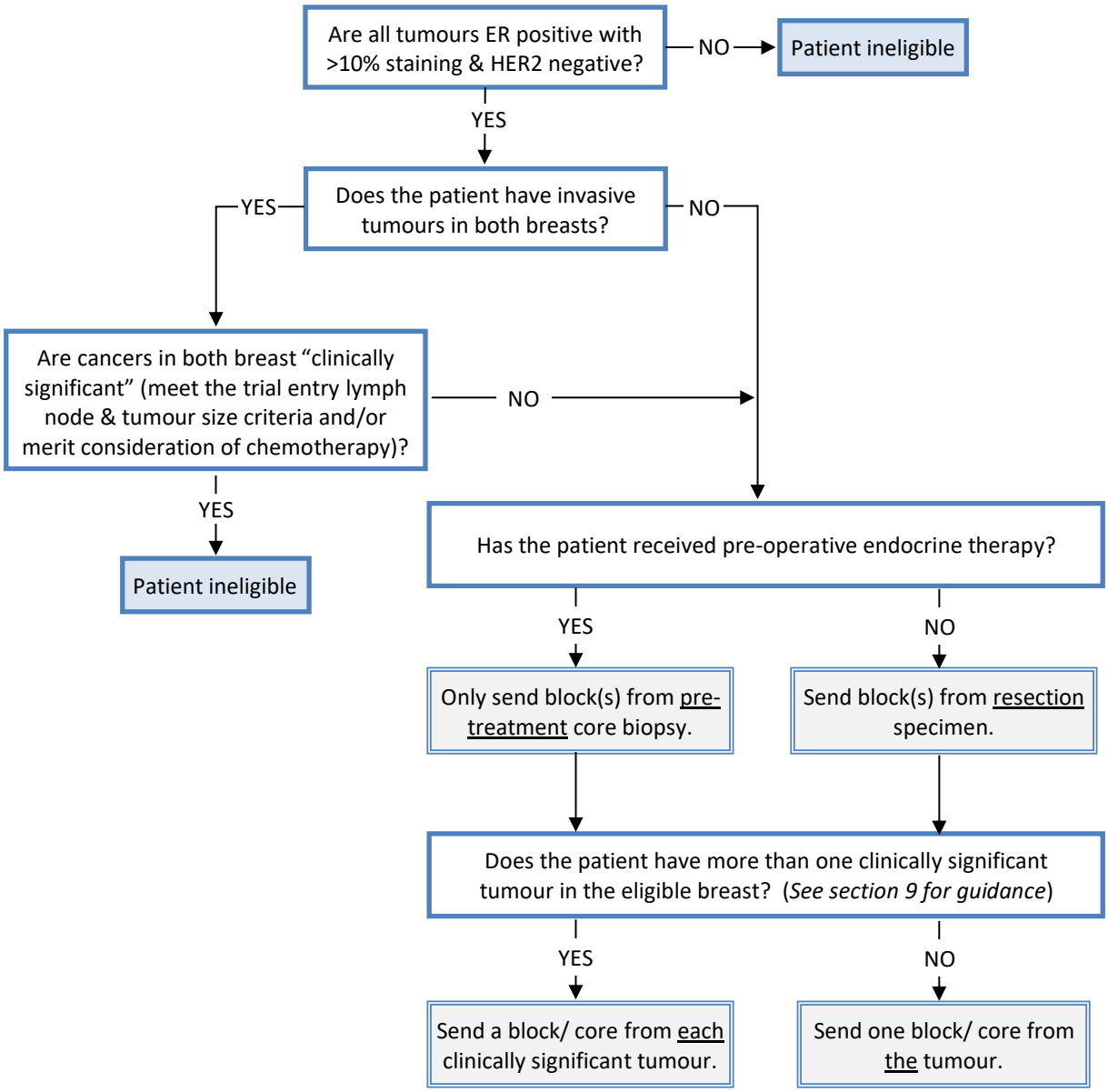
The Central Laboratory will not perform routine confirmation of ER and HER2 status: see section 10 on Ineligible Tumours.

The OPTIMA Trial Office will notify the site of patient treatment allocation as follows:

- For patients randomised to the control arm, after a delay equivalent to the time taken for the Prosigna test to be conducted, the site will be notified that the patient is to be treated with chemotherapy followed by endocrine therapy.
- For patients randomised to the test-directed arm, upon receipt of the Prosigna test results, the following rule will be applied by the Trial Office:
    - Risk of Recurrence score (Prosigna Score) greater than 60 – the site will be notified that the patient is to be treated with chemotherapy followed by endocrine therapy.
    - Risk of Recurrence score (Prosigna Score) of 60 or less – the site will be notified that the patient is to be treated with endocrine therapy alone.

Randomisation between the arms in OPTIMA is blinded. Sites will be aware that for patients allocated to endocrine therapy alone, the patient's tumour has undergone Prosigna testing and has a Risk of Recurrence score $\leq$60 but no further information about Prosigna testing at an individual patient level will be provided.

6 Tumour Block Assessment and Selection Flowchart



7 Provision of tumour blocks

For all patients who consent to enter OPTIMA, sites will send an FFPE block of tumour tissue to the Central Laboratory. Selection of tumour blocks depends on the treatment pathway and pathology findings as follows:

I. Participants who proceed immediately to surgery: a suitable block from the surgical resection should be selected.
II. Participants who have been treated with any pre-surgical endocrine therapy: a pre-treatment core biopsy should be selected.
III. Participants with multiple ipsilateral synchronous cancers: more than one block may be required. Refer to: Section 9: Bilateral and multiple ipsilateral cancers.

Notes on block selection:

I. Pre-surgical endocrine therapy is known to affect the Prosigna subtype and Risk of Recurrence score. It is important that pre-treatment core biopsies rather than a block from the surgical resection is submitted to the Central Laboratory for such patients. Testing a resection specimen could result in a patient who should be treated with chemotherapy according to the Protocol being assigned endocrine therapy alone. For patients who proceed to surgery without receiving pre-surgical endocrine therapy, a block (or blocks) from the surgical resection should be submitted as the risk of test failure is increased by the small size of core biopsies. Prosigna testing has not been validated on lymph node metastases.

II. The Prosigna test requires a minimum tumour cellularity of 10% with a total surface area of 100mm^2, which may require more than 1 section to be cut. The block selected for submission should contain sufficient tumour material to allow more than one attempt at testing.

III. The target is for sites to despatch the tissue block to the Central Laboratory within 3 working days of patient randomisation. This is to minimise time taken to notify sites of treatment allocation. The OPTIMA Trial Office will provide all sites with pre-addressed, free-post envelopes for this purpose.

8 Clinical information and documentation

Each tumour block will be accompanied by:

I. partially anonymised copies of **all** pathology reports relevant to the diagnosis and management of breast cancer (including the core biopsy report which, it is anticipated is most likely to be the sample on which ER and HER2 status have been determined and reports of any axillary surgery when this has been performed separately from breast surgery) AND

II. a Tissue Transit Form.

Pathology reports should contain the following identifying information for verification purposes:

- Trial number (TNO)
- Participant initials and date of birth

All other personal identifying information including participant name and hospital/ NHS/ other national identification numbers etc should be redacted.

Note: pathology & block numbers are not considered to be PID and should be retained.

The Tissue Transit Form will contain the following information:

- Site name (and pathology hospital when different)
- Participant Trial number (TNO), initials and date of birth
- Signature and date by designated member of local research staff confirming
  a. that informed consent has been given by the participant and
  b. whether or not participant has given optional consent for use of tissue for non-obligatory research

  Note: Where the participant has only given initial Remote Verbal Consent, this field should be left blank and updated as soon as full written consent has been received from the participant; the **Site must transmit a copy to the OPTIMA Trial Office.**

- Information on number of tumours blocks to be submitted (unifocal /multiple ipsilateral synchronous)
- Information on whether the participant has received pre-surgical endocrine therapy
- Pathology number of the submitted FFPE sample, the block number assigned by reporting pathology department and the date of surgery or biopsy
- *Invasive* tumour size
- Number of lymph nodes involved (including both micro- and macro-metastases but not isolated tumour cell clusters)
- Tracking information for the tumour block
- Signature by designated member of staff and date of completion of form

The Tissue Transit Form must be completed and signed by an appropriately trained member of staff (trial investigator or pathologist who is a member of the breast multidisciplinary team or other competent individual delegated by the site PI) who can interpret the pathology reports to provide the correct clinical details.

Clinical information is required for the Central Laboratory to perform the Prosigna test correctly. Parameters are logged at the time the test is run and cannot be changed later. Incorrect information is likely to result in an incorrect Prosigna test result. The potential outcome of this would be a participant receiving an incorrect treatment allocation.

- Invasive tumour size is integral to the Prosigna test; the information required is the diameter of the *invasive* tumour. (Please note that if the *whole* tumour size, i.e. including the dimension of surrounding DCIS is supplied then this could result in a falsely high Prosigna test score.)
- The total number of lymph nodes that contain macrometastases and micrometastases is required; no distinction is made between these. (Please note that lymph nodes that contain only isolated tumour cell clusters, i.e. deposits ≤0.2mm diameter, should not be counted.) The lymph node status does not directly affect the Prosigna test score but is required for a secondary test parameter.

A copy of the completed Tissue Transit Form and all pathology reports will be transmitted to the OPTIMA Trial Office prior to sample dispatch. Any subsequent amendment to the Tissue Transit Form should be sent to the Trial Office who will communicate with the Central Laboratory.

N.B. The information to be entered onto the Tissue Transit Form is also required for the Baseline Pathology and Baseline Tumour Characteristics CRFs. Additional pathology details are required for the CRFs. Sites may find it helpful to complete these CRFs at the same time but should not delay sending the tumour block(s) and Tissue Transit Form to do this.

9 Bilateral and multiple ipsilateral cancers

Patients with more than 1 tumour are eligible to join OPTIMA provided no tumour is identified as either ER-negative/low positive or HER2-positive in which case the patient is deemed ineligible.

For separate synchronous primary cancers, whether ipsilateral or bilateral, it is anticipated that laboratories will assess ER and HER2 on the different lesions as required and according to UK guidelines (https://www.rcpath.org/resourceLibrary/g148-breastdataset-hires-jun16-pdf.html; pages 92 and 96).

Guidance on submitting tumour blocks is contained in the OPTIMA Protocol and as follows.

Bilateral cancers – The rules on bilateral cancers are designed to permit a patient who has an eligible tumour in one breast and a small or clinically insignificant contralateral cancer to join OPTIMA provided this satisfies the receptor expression rules. In this context, the Protocol rules are that if a cancer enables the patient to join OPTIMA based on lymph node status and invasive tumour size, or if the site considers that chemotherapy should be considered on the basis of the tumour characteristic then the cancer is deemed clinically significant. Patients with clinically significant cancers in both breasts are ineligible to join OPTIMA. It should therefore never be necessary to submit blocks from the contralateral tumour to the Central Laboratory.

Multiple ipsilateral cancers – The Protocol requires that blocks from more than one lesion should be submitted for Prosigna testing when the lesions are considered to be:

I. clinically significant by the referring site AND
II. are interpreted as synchronous primary cancers (based either on the site of the lesions, i.e. in different quadrants, or if they are of differing morphology, i.e. histological type or grade).

It is for the site to decide whether individual tumour deposits are clinically significant, i.e. whether tumour characteristics additional to receptor expression could affect clinical decisions.

For instance, if a patient with node positive disease has a 2cm tumour in one quadrant and a 0.8cm tumour of the same grade in a different quadrant then the site may consider the smaller tumour to be not clinically significant so would only send one tumour block. If, however the 0.8cm tumour had a higher grade or significantly lower level of ER expression then both tumours could be considered clinically significant so blocks from both tumours should be sent.

Clinical management will be based on the highest Prosigna score for patients randomised to test-directed treatment where more than one block is tested.

The site should contact the OPTIMA Trial Office if unsure about eligibility and/or the necessity to submit more than one tumour block.

Each block is to be accompanied by a separate Tissue Transit Form.

10 Ineligible tumours

Receptor confirmation (ER and HER2 status) will be undertaken by the Central Laboratory where a tumour is identified as having a non-luminal subtype (i.e. HER2-enriched or basal-like) by Prosigna testing. It is anticipated that the majority of such tumours will be confirmed as ER-positive (>10% staining) HER2-negative. Where a tumour is found to be ineligible the site (both the local PI and the lead/ reporting pathologist) will be informed and the patient should be offered appropriate treatment as detailed in the Protocol.

11 Insufficient or unsuitable tumour samples

The Central Laboratory will notify the OPTIMA Trial Office if any tumour block is deemed as insufficient or unsuitable on review of H&E section or in the event of failure of Prosigna testing. A further tumour block will be requested from the recruiting site following discussion between the OPTIMA Trial Office and the site, for example if there is another representative and suitable block available.

12 Retention of tumour samples

Full analysis of the OPTIMA trial requires Prosigna testing to be performed on blocks from all patients randomised to the control arm (as well as the test arm); however, testing of the control arm blocks will be batched. This is considered to be obligatory research. Additional unspecified pathology research designed to develop and improve multi-parameter assays is integral to the OPTIMA study and will be performed using tumour blocks from patients irrespective of randomisation. Submitted blocks will therefore be retained in the OPTIMA Tissue Bank for each patient enrolled in the study for obligatory research (and if the patient consents, for unspecified non-obligatory research).

Surplus unstained slides and extracted RNA from Prosigna testing will be stored in the OPTIMA Tissue Bank.

The Protocol permits requests for further material as part of non-obligatory research where the original material has been exhausted or is otherwise unsuitable. These requests will ordinarily be made through the Trial Office, which will supply all required information.

13 Return of tumour samples to Site

Tumour blocks will be returned to sites if, at a future date, novel diagnostic tests are required and no suitable material is available locally. This is regarded, based on past experience, as an exceptional circumstance, since in most cases sufficient material is available locally to perform such tests. Requests for block return should be made to the OPTIMA Trial Office; blocks will be dispatched within 24-48 hours of the request being received. Blocks supplied to replace an inadequate sample will be retained but the inadequate sample will be returned.

Tumour blocks will also be returned in the following circumstances:

- The tumour sample has been deemed unsuitable for testing by the Central Laboratory.
- The tumour sample was surplus to the trial requirement.
- The participant has been identified as ineligible on the basis of secondary tumour ER testing (i.e. as ER-negative or ER-low positive), or HER2 testing (i.e. as HER2-positive).
- The participant has withdrawn from the trial and additionally has withdrawn consent for future research to be performed on stored tumour material.

Sites will be offered the option for the return of derivative materials (stained and unstained slides and RNA) where these are available; if declined these materials will be destroyed.