



NERO – Niraparib Efficacy in patients with unResectable Mesothelioma: A randomised phase II trial of Niraparib versus active symptom control in patients with previously treated mesothelioma

LABORATORY MANUAL

SPONSOR: University Hospital Southampton NHS Foundation Trust

COORDINATING CENTRE: Southampton Clinical Trials Unit

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Table of Contents

1	INTRODUCTION	3
1.1	Funded Costs	3
1.2	Summary of NERO translational sample collection	4
2	COLLECTION AND TRANSPORTATION OF TISSUE SAMPLES TO THE CENTRAL LABORATORY	5
2.1	Sample Collection	5
3	COLLECTION, PREPARATION, STORAGE AND TRANSPORTATION OF TRANSLATIONAL BLOODS	6
3.1	Sample collection and processing	6
4	Stool Sample Collection	8
5	CONTACT DETAILS	8
6	SUMMARY OF VERSION CHANGES	9

1 INTRODUCTION

The purpose of this manual is to describe the collection and transportation of blood and tissue samples for the NERO **translational study**. All NERO participants consent to the provision of an archival or fresh tissue biopsy at baseline and those randomised to the niraparib arm may consent to an OPTIONAL biopsy post-progression. In addition, all participants will have a translational blood sample collected at baseline, prior to starting treatment. Those that are randomised to the niraparib arm may also consent to a further translational blood sample following progression/treatment discontinuation. The blood and tissue samples will be stored and analysed at the Mesothelioma Research Lab in the Robert Kilpatrick Clinical Sciences Building at the University of Leicester. Southampton Clinical Trials Unit (SCTU) will oversee the processing, transport, and tracking of these translational samples.

In order to preserve the integrity of the patient samples (and thus comply with the clinical trials regulations), we request that clinical staff at participating centres collect, handle, process and store patient samples at their clinical centre in accordance with these instructions.

1.1 Funded Costs

All other consumables associated with sample collection, processing and shipping should be paid for from per patient costs outlined in the NERO site agreement.

1.2 Summary of NERO translational sample collection

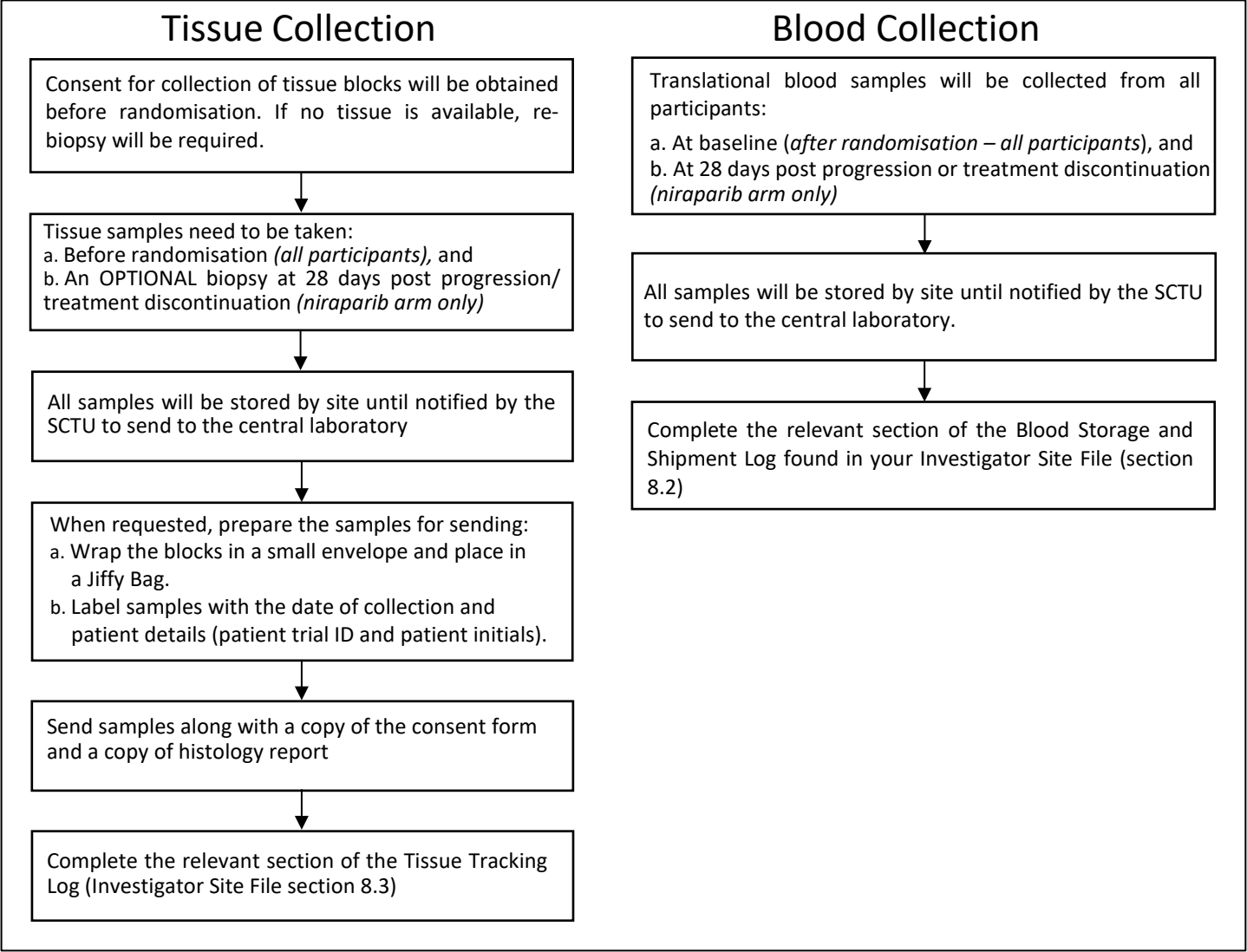


Figure 1 Summary of NERO translational sample collection

2 COLLECTION AND TRANSPORTATION OF TISSUE SAMPLES TO THE CENTRAL LABORATORY

2.1 Sample Collection

All patients will be asked for consent for collection of blood samples and tissue blocks before randomisation. Patients in the niraparib arm will also be asked to donate an optional tissue sample upon disease progression. Samples will be retrieved by the central laboratory when required, as requested by the SCTU. Do not send samples until requested.

- 2.1.1 Request paraffin block and a copy of histology report.
- 2.1.2 On receipt, complete the Translational Tissue eCRF and write the trial subject number on block. Additionally, record the date that the biopsy was taken on the eCRF.
- 2.1.3 Anonymise the histology report and consent form, adding the trial subject number, month and year of birth and initials and retain to be sent with the tissue sample when requested.
- 2.1.4 For patients who donate an optional biopsy at progression, follow the same guidelines.
- 2.1.5 When requested, prepare the samples for sending:
 - a) Wrap the blocks in a small envelope and place in a Jiffy Bag.
- 2.1.6 Send samples along with a copy of the consent form and histology report to the central lab:

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Leicester Cancer Research Centre
Office 402, Level 4, Mesothelioma Lab
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3 COLLECTION, PREPARATION, STORAGE AND TRANSPORTATION OF TRANSLATIONAL BLOODS

3.1 Sample collection and processing

All patients will be asked for consent for collection of blood samples and tissue blocks before randomisation.

Translational blood samples will be collected at baseline from all participants. Additionally, translational blood samples will be collected at 28 days post disease progression/treatment discontinuation from participants in the niraparib arm of the trial.

- 3.1.1 Samples are to be obtained aseptically by venepuncture or from a venous port and collected into 1 x 10ml EDTA Vacutainer Tube (plasma) and 1 x 10ml red top Vacutainer Tube (serum). It is advised that samples be taken at the same time as the routine bloods to avoid additional venepunctures.
- 3.1.2 After blood draw, perform tube inversions to ensure mixing of clot activator (serum) or anticoagulant (plasma) with blood.
- 3.1.3 Using a permanent marker pen label 1.5ml Eppendorf tubes with the following details:
 - Patient's trial number
 - Trial time point e.g., baseline
 - Date sample taken
 - Sample type ('Serum', 'Plasma' or 'Buffy coat')
- 3.1.4 Centrifuge the EDTA Vacutainer blood tube at 1000g (+/-10% to allow for difference in centrifuge models) with brake off for 10 minutes at 4°C.
- 3.1.5 From the EDTA Vacutainer tube transfer plasma to a 15 ml Falcon tube using sterile Pasteur pipettes. Aliquot 0.5 ml buffy coat into a labelled Eppendorf tube—using sterile Pasteur pipettes (see Figure 2). Place the buffy coat tubes on ice until step 3.1.9.
- 3.1.6 Centrifuge the 15 ml Falcon tube of plasma and the red top Vacutainer (serum) at 2000g (+/-10% to allow for difference in centrifuge models) with brake off for 10 minutes at 4°C.
- 3.1.7 Remove plasma from the 15 ml falcon after the second centrifugation using sterile Pasteur pipettes and transfer 1ml aliquots into labelled 1.5 ml Eppendorf tubes. Place the plasma tubes on ice until step 3.1.9.
- 3.1.8 From the red top Vacutainer tube remove serum using sterile Pasteur pipettes and transfer 1ml aliquots into labelled 1.5 ml Eppendorf tubes. Place the serum tubes on ice until ready to freeze in step 3.1.9.
- 3.1.9 Transfer all plasma, serum and buffy coat immediately after separation to a -80°C freezer. A storage temperature tolerance of +/- 10°C is acceptable to allow for changes in external temperature and door opening.
- 3.1.10 On the day of storage, complete the relevant section of the NERO Blood Storage and Shipment Log found in your Investigator Site File (section 8.2).
- 3.1.7 SCTU will request collection of these samples using cryogenic transportation. **Do not use the Royal Mail Safeboxes.**

Figure 2: Process for centrifuging whole blood to isolate buffy coat and plasma.

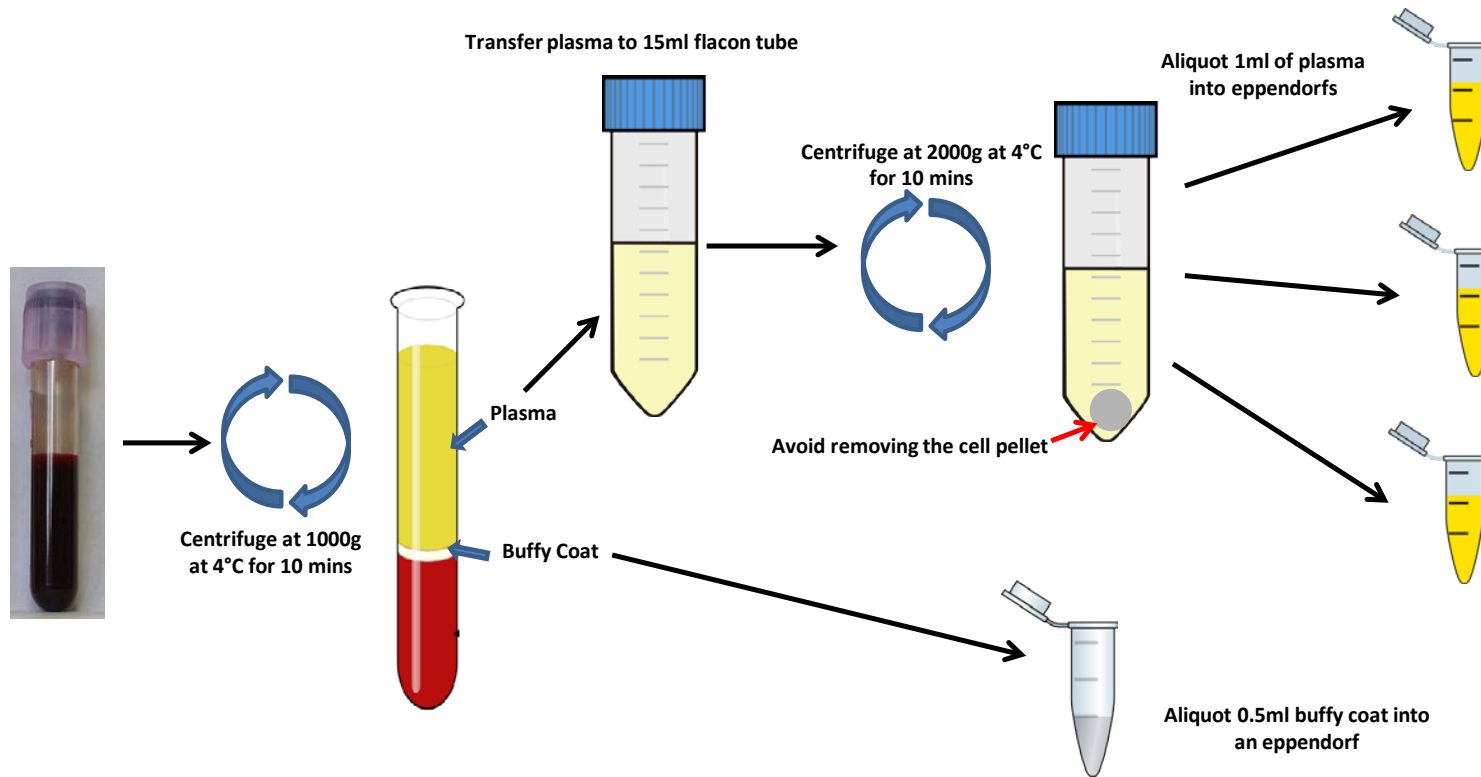


Figure 2: Process for centrifuging whole blood to isolate buffy coat and plasma

4 Stool Sample Collection

Patients will be asked to consent to an optional stool sample at screening, for gut microbiome analysis. The sample will be collected using Atlas Biomed Microbiome Test Kits.

Ordering of the test kits will be done through the SCTU. Please contact the NERO trial team if you require more kits. Delivery will occur within 2 working days, providing we are informed before midday when the kits are requested.

Once a patient is deemed eligible, they have been registered in Rave, and they have provided consent for a microbiome test, a collection kit will need to be registered on the Atlas Biomed Pro account (please request access to this through the NERO inbox). Kits **SHOULD NOT BE** used for patients prior to eligibility being confirmed.

Login to the Atlas pro account (<https://atlasbiomed.co.uk/pro/>) and select 'Add new client' to begin registering the kit to the patient. The name of the patient should be the patients trial ID (NE2-XXXXXXX), no patient identifiable information should be entered on the Atlas platform. The barcode number you will need to enter will not be the barcode number on the box, you will need to enter the barcode number on the tube inside the kit. The box does not need to be opened for this barcode number. Instead, a list of tube barcode numbers, relating to the barcode on the box, will be provided to site with each order.

Once the kit has been registered, the patient should perform the stool collection as per the instructions in the box. The sample will then be ready for delivery and should be sent back to Atlas Biomed by the research nurse or other delegated individual. No payment for postage will be required, the return labels will already be on the box.

5 CONTACT DETAILS

All NERO trial enquiries should in the first instance be directed to the Trial Manager:

NERO Trial Manager
Southampton Clinical Trials Unit,
MP 131 Southampton General Hospital,
Tremona Road,
Southampton, SO16 6YD.

Email: NERO@soton.ac.uk
Telephone: 023 8120 4307

6 SUMMARY OF VERSION CHANGES

Version No.	Date Effective	Revision History
1	19-May-2022	First issue
2	06-Feb-2023	Clarification of sample collection on the Niraparib vs ASC only arm Added in details of stool sample collection