



LABMANUAL

Sampling instructions for DNA sequencing



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1 Introduction

The purpose of this laboratory manual is to specify all the instructions for the CPCT-02 trial considering the collection, processing, storage and transport of biological samples (blood and tissue) for DNA sequencing analysis.

Chapter 2 contains information on the procedures for blood collection and chapter 3 contains information on the procedures for biopsy collection.

2 Blood sampling for germline DNA and circulating tumor DNA sequencing

2.1 Sampling schedule

For all patients a baseline blood sample for next-generation sequencing (NGS) of germline and circulating tumor DNA (liquid biopsy) will be taken, preferably combined with other necessary blood draws.

In a sub study blood sampling for circulating tumor DNA analysis will take place in patients with breast and prostate cancer. These samples will be collected concurrent with tumor evaluation scans until the end of treatment (*i.e.* every two to three months after start of treatment and at the end of treatment).

2.2 Equipment and supplies

Please make sure that the blood sampling laboratory kits [a red medical envelope] (provided by Hartwig Medical

Foundation) are available at your site, the red medical envelope containing:

- CellSave® 10 mL blood collection tube with barcode label (check the expiration date before use)
- Barcode labeling (2 labels) and a Blood Sample Requisition Form
- Blister
- Safety bag

2.3 Processing instructions

- Collect 10 mL whole blood in a CellSave blood collection tube. These tubes contain a preservative to preserve the quality of the blood at room temperature for maximally 4 days.
- The tubes will be pre-labeled. Please paste the second label onto the Blood Sample Requisition Form¹. The 3rd label is optionally for your own registration.
- Complete all collection details on the Blood Sample Requisition Form and record any sample handling or quality issues.
- A copy of the Blood Sample Requisition Form will be saved for site filing.
- Place tube with whole blood in blister
- Place blister with tube in a safety bag
- Close safety bag

- Place safety bag with tube inside together with filled in Blood Sample Requisition Form in a red Medical Envelope.
- The envelope should be sent daily by mail (possible on every workingday) to Hartwig Medical Foundation. (the address is pre-printed on the envelope)



2.4 Transport of blood samples

Centers are requested to send the blood samples to the central laboratory **on the day of collection**. Samples should be kept at room temperature and send via regular mail. For this purpose, pre-addressed envelopes and transport blisters will be provided by the central laboratory.

The contact details of the central laboratory are:

Hartwig Medical Foundation
Sciencepark 408
1098 XH Amsterdam
E: x-lab@hartwigmedicalfoundation.nl
T: 020-2352640

If you have questions about the processing or transport please contact Hartwig Medical Foundation, E: x-lab@hartwigmedicalfoundation.nl T: +31 20 235 2640.

2.5 Resupply of blood sampling laboratory kits

In case resupply of blood sampling laboratory kits are necessary (either due to usage of supplied kits or to expiration of the CellSave tubes) please contact the central laboratory by email: x-lab@hartwigmedicalfoundation.nl.

3 Histological biopsy for DNA sequencing

3.1 Sampling schedule

Patients will be subjected to a histological biopsy of a locally advanced or metastatic and preferably radiological measurable lesion (a RECIST 1.1 target lesion).

The biopsy is obligatory at baseline (pre-treatment) and optional during and after treatment (on-treatment and post-treatment biopsies). In case sequential biopsies have limited feasibility, post-treatment biopsies are preferred above on-treatment biopsies.

Two to four (preferably) 18-gauge large-core needle (22 mm true-cut) or punch (skin) biopsies are required.

3.2 Equipment and supplies

Please make sure that the following supplies are available at your site.

Biopsy sampling laboratory kits [a safety bag] provided by Hartwig Medical Foundation, each containing:

- Three pre-labeled tissue tubes with screw cap
- Barcode labeling (2 labels)
- Biopsy Sample Requisition Formⁱⁱ
- Safety bags for shipment tissues

Please make sure that the following equipment is available at your site

- Large tweezers
- Liquid nitrogen container
- -70°C (or colder) freezer to store all samples until shipment;

A temperature control log should be filled in daily to ensure stable storage conditions as per the sites standard procedure.

3.3 Tumor biopsy collection and processing instructions

3.3.1 Tumor biopsy collection

Ultrasound or CT-guided large-core needle biopsy or a punch (skin) biopsy will be performed according to local guidelines.

Following guidelines are indicative:

- A body ultrasound (2-5 MHz) transducer is used to visualize the metastasis.
- An add-on guiding device is attached to the transducer and the appropriate needle path is selected (automatically).

Lidocaine 1% is used for local anesthesia. If necessary systemic pain control using phentanyl and mild sedation using midazolam can be used.

- A 17-gauge guiding needle may be used, which is advanced under real-time ultrasound guidance into the tumor.
- After correct positioning of the guiding needle has been confirmed in 2D, a (preferably)
- 18-gauge large-core needle (22 mm true-cut) is advanced through the guiding needle and two to four biopsy specimens are obtained

In case the tumor cannot be accurately visualized with ultrasound, CT could be selected as guiding modality. If preferable, biopsies can be taken by GI endoscopist under imaging guidance, in which cases mild sedation (midazolam, combined with the opioid phentanyl) is allowed and biopsy will be performed according to the following protocol:

- An echo-endoscope is used to visualize the metastasis or locally advanced lesion.
- A (preferably) 19-gauge EUS histology needle is used, which is advanced under real-time ultrasound guidance into the tumor.
- After correct positioning of the needle, two to four passes through the tumor are made to obtain the biopsy specimens for DNA sequencing.
- All patients should be observed after biopsy according to local protocol for full recovery.

3.3.2 Processing instructions

Please comply with the following processing instructions:

- o Biopsy specimens and appropriate storage material should be handled wearing nonsterile gloves.
- o Only supplied pre-labeled tissue tubes will be used, **one for each biopsy specimen** (maximum of 3 tissue in 3 different tubes).
- o Place each of the biopsy specimens directly into the appropriate tissue tube.
- o Close each of the tissue tubes and “snap-freeze” immediately.
- o Use large tweezers to pick up the tissue tubes. **Do NOT tilt the tubes.**
- o Snap-freeze each of the tissue tubes by placing the bottom of the tube in the surface of the liquid nitrogen for 10 seconds. **Do NOT tilt the tubes.**
- o Release each of the tissue tubes in the liquid nitrogen container for at least 20 seconds to secure proper freezing of the specimen.
- o Complete all relevant information on the CPCT-02 Biopsy Sample Requisition Form
- o Make a copy of the completed Biopsy Sample Requisition Form
- o Place the tissue tubes in a safety bag (**per patient, per moment**), place original completed Biopsy Sample requisition form in the Safety bag.
- o Place the safety bag (with tubes and requisitionform) in the -70°C (or colder) freezer for storage until shipment.
- o Complete all relevant information in the appropriate section of the eCRF.
- o A copy of the Biopsy Sample Requisition Form will be saved for site filing.
- o Ship biopsy samples in safety bags **per patient per moment** (together with the biopsy sample requisition form) on sufficient dry ice to assure that they remain frozen for at least two days, **compare more biopsy samples of different patients**

to one shipment to reduce courier costs. The shipment should be done at least ones per two weeks to Hartwig Medical Foundation.

- Ship samples **by courier** to:

Hartwig Medical Foundation
Sciencepark 408
1098 XH Amsterdam
E: x-lab@hartwigmedicalfoundation.nl
T: 020-2352640

3.4 Transport of biopsy samples

For the transport of biological specimens from local sites to the Hartwig Medical Foundation sequencing facility and CPCT biobank, arrangements have been made with:

SDX BV
Damzigt 14B
3454 PS De Meern
Tel.: 030 – 241 01 06
Fax*: 030 – 241 33 11
Email*: info@sdx.nl

Packages for transportation are expected to be 3-5 kg, with an average size of 30 x 30 x 30 cm. Materials for transport (e.g. boxes, dry ice) will upon request on the Courier Shipment Request Formⁱⁱⁱ be provided by the courier (min. 1 day in advance).

Shipments can be requested until 3 days before the day of pick-up by fax, email or website. The courier will contact the site for the pick-up time.*
For pick-up requests the Courier Shipment Request Formⁱⁱⁱ may be used.
Centres are requested to ship samples in a maximum frequency of once every two weeks.

* No shipment on Fridays or the day before public holidays, if delivery is planned the next day.

If you have questions about the processing or transport please contact Hartwig Medical Foundation, E: x-lab@hartwigmedicalfoundation.nl T: +31 20 235 2640.

3.5 Resupply of biopsy sampling kits

In case resupply of biopsy sampling kits is necessary, please contact the central laboratory by email: x-lab@hartwigmedicalfoundation.nl.

4 Related forms

- i CPCT-02 Blood Sample Requisition Form
- ii CPCT-02 Biopsy Sample Requisition Form
- iii Courier Shipment Request Form

provided in the laboratory kit
provided in the laboratory kit
provided separately