**Cancer Proteomics Tumor Analysis Consortium**
**Prospective Biospecimen Collection Protocol**
**Ovarian Cancer**
**V 2.0**

## Overview

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) sponsored by the NCI's Office of Cancer Clinical Proteomics Research is a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust, quantitative, proteomic technologies and workflows. The overarching goal of CPTAC is to improve our ability to diagnose, treat and prevent cancer. To achieve this goal in a scientifically rigorous manner, the NCI launched CPTAC to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provide this data with accompanying assays and protocols to the public.

CPTAC consists of a network of Proteome Characterizations Centers (PCCs) and a Data Coordinating Center (DCC) serving as a hub and central repository for CPTAC data. CPTAC will be expanded to include a 1) network of Tissue Source Sites (TSSs) to procure clinical specimens for proteomic and genomic analysis, 2) Biospecimen Core Resource (BCR) to serve as a repository for tissue and associated, de-identified clinical data submitted to the program, and 3) Genomic Characterization Center (GCC) dedicated to the genomic analysis of CPTAC specimens.

## Purpose

The purpose of this protocol is to establish the minimum procurement parameters for high-grade serous ovarian, fallopian tube, and peritoneal cancer specimens to be submitted to the CPTAC for proteomic and genomic analysis. The tissue source will be from newly diagnosed, untreated patients undergoing definitive surgery for ovarian cancer.

The protocol builds on CPTAC experience with human tissues obtained from The Cancer Genome Atlas (TCGA) program and specifically aims for:

- Minimized specimen processing and ischemia time with the ischemia time recorded.
- Sufficient total material from each patient divided into multiple, homogeneous samples suitable for independent processing for proteomic and genomic analysis.
- Histological assessment and quality assurance by the BCR (through frozen sectioning) of all tissue specimens utilized for each analytical platform.
- Improved determination of weights of individual samples for improved estimates of protein yield.

## Scope

The protocol applies to any samples submitted by a Leidos Biomedical Research, Inc. subcontractor to the CPTAC BCR.

## Requirements

***Patient Inclusion Criteria***

- Newly diagnosed, untreated patients undergoing primary cytoreductive surgery for high-grade serous ovarian cancer.
- Tumor from ovary, pelvic mass or omentum only (other anatomic sites not acceptable).

***Patient Exclusion Criteria***

- Prior history of other malignancies within the past 12 months except non-melanomatous skin cancer and in situ cervical cancer.
- Other malignancies at the time of surgery.
- Any prior systemic chemotherapy or biological therapy for any cancer.
- Prior hormonal therapy within the last five years for any cancer.
- Prior radiation therapy for any prior malignancy that involves treatment to the abdomen or pelvis.
- Patients who are found to have low-grade (grade 1) or low stage (stage I or II) serous ovarian, fallopian tube, or peritoneal cancer based on final pathology (typically 5-10 days after surgery).

***Regulatory (before procurement)***

- IRB approval received and documented with the CPTAC BCR.
- MTA/DUA agreement received and documented with the CPTAC BCR.

***Tissue Procurement and Shipping***

- Signed patient consent (maintained at the tissue source site, copy to CPTAC BCR not required).
- Cancer tissue per protocol.
- Normal fallopian tube fimbrae if possible (per Crum protocol).
- Blood per protocol.
- Shipping Manifest completed and accompanying tissue shipment.
- CPTAC Tissue Submission Form (contains details regarding procurement such as ischemia time along with minimal patient information) completed and electronically submitted within 1-2 working days after tissue procurement. Secure access to the electronic clinical data management system with the form to be provided by the CPTAC BCR.
- Adherence to BCR shipping instructions (the BCR will provide the shipping cryoport and cover the cost of shipping).

***Patient Data***

- CPTAC Baseline Case Report Form (CRF) containing the patient's history and status at surgery along with diagnostic information completed and electronically submitted prior to tissue

shipment. Secure access to the electronic clinical data management system with the CRF to be provided by the CPTAC BCR.

- Pathology Report (de-identified, following the guidelines from to the 7th Edition 2013 AJCC) should be submitted within 5 days after overall (pathology + molecular) qualification.
- At least one representative image of a hematoxylin/eosin-stained slide from a formalin-fixed piece of the carcinoma in SVS, JPG, or TIFF format. FFPE H&E diagnostic slides/images representative of the diagnosis in the pathology report should be submitted within 5 days after overall (pathology + molecular) qualification. Slides will be returned.
- CPTAC One-Year CRF with updated history and status one year after completion of the initial treatment regimen. Secure access to the electronic clinical data management system with the CRF to be provided by the CPTAC BCR.

***Tumor Specimen Inclusion Criteria***

- Greater than 300 mg total of all segments obtained from a patient.
- Greater than 60% tumor cell nuclei.
- Less than 20% necrosis.
- Equal to or less than 30 minutes total ischemia time.

## Tissue Procurement Procedure

The ovarian specimens should be collected in a very rapid fashion to avoid changes associated with warm ischemia. Since ovarian tumors are generally quite large, the approach should be to remove a portion of tumor that is part of a larger tumor mass. If at all possible, the portion of tumor for proteomics will be weighed and frozen immediately (< 5 minutes) directly in the operating room. An adjacent portion of the same tumor should then be resected and sent to pathology for diagnostic purposes. Since these tumors (omental cake or adnexal masses) are large, a portion removed for the CPTAC study should not interfere with standard and necessary diagnostic procedures since plenty of tumor will remain for this purpose and only a small portion of these large tumors are generally evaluated by diagnostic pathologists.

***Tumor Tissue***

- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue embedding and freezing. Label each cryomold with an appropriate ID and "T" to indicate tumor tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.
- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Identify a 1-2 cc nodule that appears to be mostly tumor, with little or no intervening normal tissue.

- Dissect free from surrounding attachments leaving main blood supply intact for as long as possible unless specimen is to be excised immediately from within a larger tumor mass.

- Start timer when blood supply is transected.

- Take nodule off operating field onto back table, bisect to confirm apparent tumor.

- Divide the tumor tissue which is procured for research purposes and not needed for clinical management into segments that are no larger than 1 cm x 1 cm x 0.5 cm. Depending upon the size of the tumor, 2-4 such tumor tissue segments may be procured, embedded, and frozen.

- Gently place each tissue segment in the well of an "Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx). The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6). Place the tissue flat in the cryomold, with its largest two dimensions sitting parallel to the bottom of the mold. Avoid placing the tissue in the mold in any other orientation (Fig. 7). Each tissue segment should be placed in a different cryomold.

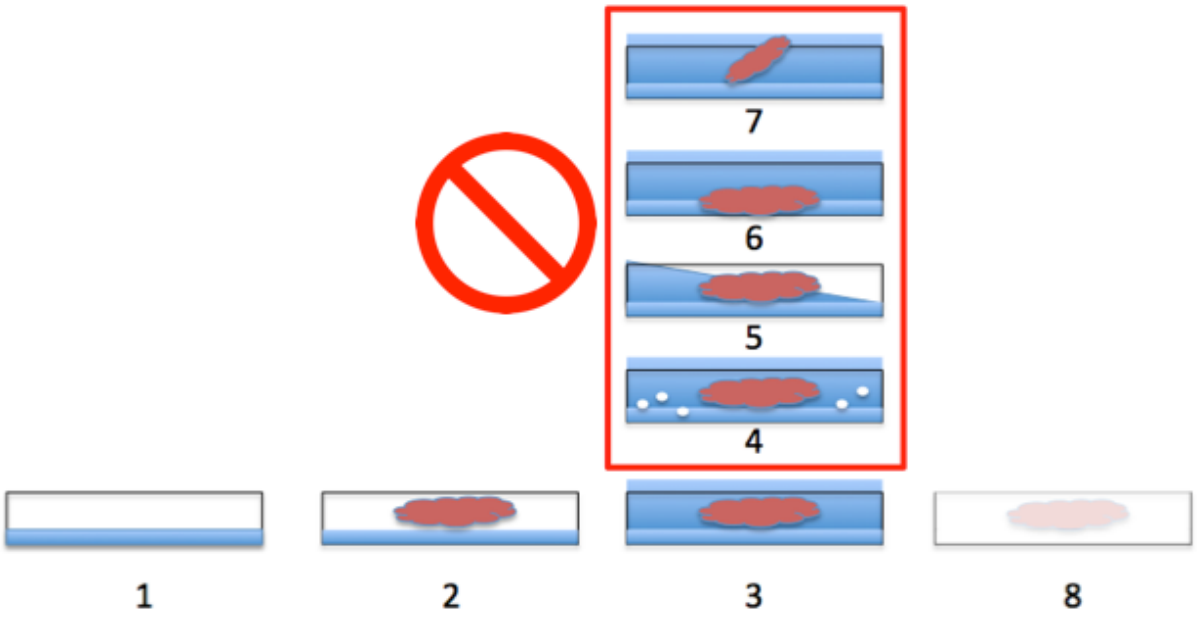
- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the cryocooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold.

- Record time when segments are placed in liquid nitrogen. No more than **30 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.




**Figures 1-8. Steps for embedding and freezing fresh tissue in OCT compound. See protocol. Figures 1, 2, 3, and 8- proper steps for embedding. Figures 4-7- improper methods for embedding- (4) air bubbles in OCT compound; (5) OCT not evenly covering tissue; (6) tissue sitting against floor of cryomold; (7) tissue not oriented flat in the mold.**

***Normal Tissue (Fallopian tube submitted for SEE-FIM sectioning)***

- If a fallopian tube grossly looks normal, collect up to 3 individual fimbria from that tube.
- Prepare each cryomold ("Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx) for tissue embedding and freezing. Label each cryomold with an appropriate ID and "N" to indicate normal tissue. Each cryomold shall have a unique ID. Fill each mold with OCT embedding compound (i.e. Tissue Tek #4583, Sakura Finetek) so that the bottom surface of the mold is covered with a thin (2-3 mm) layer of OCT (Fig 1). When dispersing the OCT into the mold, it is important to avoid creating air bubbles (Fig 4). Gently remove any air bubbles by pushing them to the side of the mold.

- Weigh and record the weight of the cryomold containing the thin layer of OCT.

- Record the time of excision.

- Divide the tissue which is procured for research purposes and not needed for clinical management into segments that are no larger than 1 cm x 1 cm x 0.5 cm. Depending upon the size of the tumor, 2-4 such tissue segments may be procured, embedded, and frozen.

- Gently place each tissue segment in the well of an "Intermediate" Tissue-Tek® Cryomold® (eg, Product No. 27183; http://www.tedpella.com/embed_html/27110.htm.aspx). The tissue should 'float' on top of the layer of OCT (Fig. 2). The tissue should not touch the bottom surface of the cryomold (Fig. 6). Place the tissue flat in the cryomold, with its largest two dimensions sitting parallel to the bottom of the mold. Avoid placing the tissue in the mold in any other orientation (Fig. 7). Each tissue segment should be placed in a different cryomold.

- Quickly weigh the cryomold with OCT and tissue, before adding any additional OCT. Subtract this weight from the initial weight of the mold + OCT, to calculate the weight of the tissue segment.

- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the cryocooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue (Fig 5). After 3-5 min. The OCT and tissue will be frozen. The OCT will turn from a viscous clear liquid to a white solid (Fig 8).

- Wrap the cryomold in pre-chilled aluminum foil, place in a pre-chilled tissue bag, and label each with the same ID as on the cryomold.

- Record time when segments are frozen. No more than **35 minutes** should have elapsed from the time of excision to freezing.

- Store at vapor phase LN2 temperature until shipping.

**Blood Collection Procedure**

- Peripheral venous blood **MUST** be collected prior to administration of anesthesia.

- Obtain 10 ml of peripheral whole blood collected by standard venous phlebotomy. The blood should be collected in the KEDTA (lavender top) vacutainer tube provided with the biospecimen procurement kit.

- Whole blood specimens should be processed and frozen as per CPTAC blood processing protocol within 1 hour of collection.