Prospective Biospecimen Collection Protocol
Colon Cancer
v1.7

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 1) a network of Tissue Source Sites (TSS) to obtain clinical specimens for proteomic and genomic analysis, 2) a Biospecimen Core Resource (BCR) to serve as a repository for tissue and associated, de-identified clinical data submitted to the program, and 3) a 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 colon adenocarcinoma (biopsy proven) 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 colon cancer.

The protocol builds on CPTAC experience with human tissues obtained from the TCGA programs 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 surgery for colon adenocarcinoma.

Patient Exclusion Criteria

- Prior history of other malignancies within the past 12 months except basal cell carcinoma of the skin.
- Other malignancies at the time of surgery.
- Any prior systemic chemotherapy, endocrine therapy or biological therapy for any cancer.
- Prior radiation therapy to the abdomen or pelvis for any cancer, including the current cancer.

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 tissue per protocol.
- 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 prior to tissue shipment. Slides will be returned.
- Blood per SOPs.
- Shipping Manifest completed and accompanying tissue shipment.
- CPTAC Tissue Submission Form (contains details regarding procurement such as ischemia times 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' 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.
- Less than 30 minutes total ischemia time, with a goal of 20 minutes from the time that the mesentery is completely divided (beginning of ischemia)

Tissue Procurement Procedure

- At least 30 minutes prior to specimen procurement fill the CryoCooler with enough LN2 to saturate the adsorbate pillow under the metal grate. The CPTAC collection kit box (or similar) should be placed on the metal grate to hold the specimens once collected. Be mindful to minimize warming of the cooler by always keeping the lid closed and locked when not actively inserting or removing specimens.

- 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 or "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 each cryomold containing the thin layer of OCT.

- Remove the particular section of the colon of interest with the goal of minimizing time of ischemia to the tissue. Record the time the mesentery is completely divided.

- Excise a >300 mg portion of the tumor mass and a separate >300 mg portion of adjacent normal colonic tissue. Place both specimens into separate specimen containers. To minimize total ischemia time for the tumor, **process the tumor material first**.

- 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.

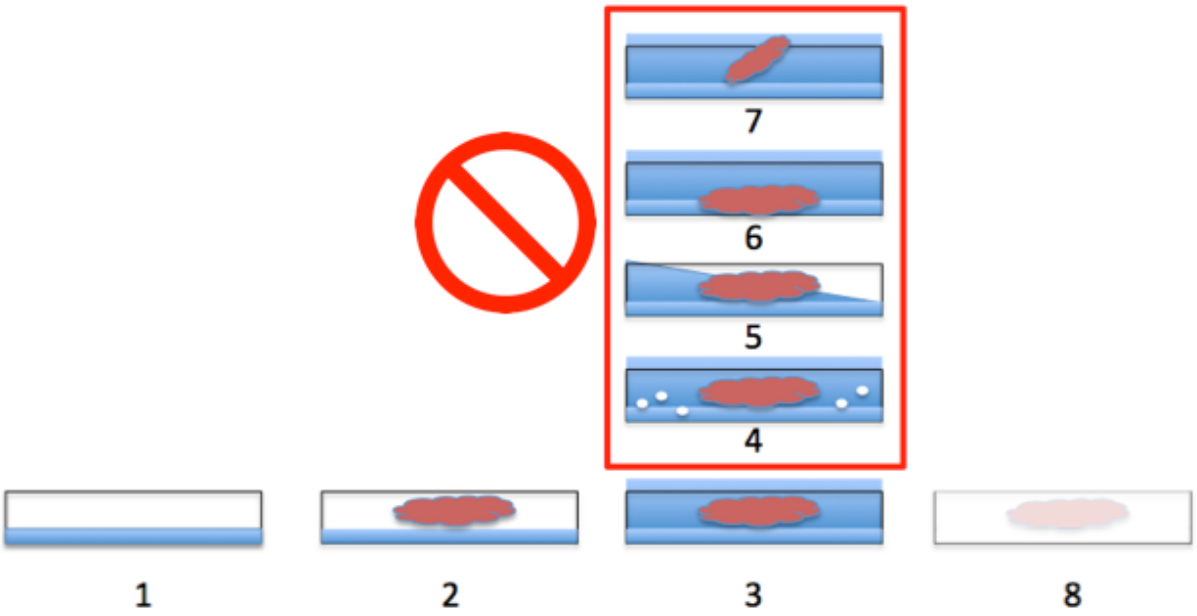
- Add additional OCT to completely cover the tissue (Fig. 3), avoiding additional bubbles (Fig. 4). Quickly transfer the cryomold to the CryoCooler. Lay the mold flat in the vapor phase; do not tilt the mold and ensure that OCT is evenly covering the entirety of the tissue (Fig 5). After 3-5 min. The 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. Be sure to close and lock the CryoCooler lid when not actively inserting or removing specimens.

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

- Store at vapor phase LN2 temperature until shipping.

- Repeat the above procedure for normal colon tissue segments, keeping in mind that ALL tissue (tumor and normal tissue) should be frozen within **30 min** of excision of the gross specimen.



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.

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. (CPTAC_Plasma_Collection_SOP_v2_0.pdf).