Retrospective Cross-Sectional Adenocarcinoma Cohort – OCT & FFPE Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Cross-Sectional LUAD*

Retrospective cohorts include participants that are anticipated to be identified from banked biospecimens procured from patients that were consented for their tissue to be used in future studies including those that involve industry/pharma partners. For cases selected it is anticipated that each case will have at minimum, biospecimens from an independent, purely pre-invasive lesion and a pre-invasive (lepidic) component of a part-solid invasive adenocarcinoma along with invasive tumor and access to a genomic control for each case that is enrolled. It is also anticipated that some retrospective biospecimen tissue samples may include frozen tissue from the part-solid lesions with pre-invasive and invasive tumor components (in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an adjacent FFPE lepidic and invasive tumor biospecimen. Frozen normal lung tissue may also be available. Instructions for selecting and preparing these cases are provided below. These include details outlining the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols. Details for shipping cases to the M.D. Anderson Cancer Center (MDACC) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCL, UCLA, VUMC

- **Lung PCA Repository to ship cases:** MDACC *see shipping guidelines for all samples going to MDACC*

- **Collection Timepoints:**
    - Baseline-Resection cases of LUAD where AAH and/or AIS is found in association with the invasive tumor and at a site remote from the tumor

- **Required biospecimen to be collected**
    - **Baseline:**
        - Resection- Frozen tissue blocks or sections *(see associated sectioning guides A.1.1)*
            - Frozen Tumor with non-invasive lepidic component (Lesion and Normal area)
            - FFPE Tumor with non-invasive lepidic component (Lesion and Normal area) **AND**
            - FFPE Atypical Adenomatous Hyperplasia/Adenocarcinoma-in-situ remote from tumor **or**
            - Frozen remote (in invasive tumor block or additional separate block) if collected
        - Genomic DNA
            - If tumor/pre-malignant lesion is frozen: (*see section A.2.1)*
                - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat
                - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) or tissue scrolls (*see sections A.1 for sectioning guidelines*)
            - If tumor/pre-malignant lesion is formalin fixed, normal FFPE lymph node sample (or less preferable, normal lung) used for:
                - Preferred: Isolated DNA from FFPE (*see section A.2.2 FFPE)*
                - Alternative: FFPE tissue Scrolls (*see section A.1)*

- **Required metadata to be collected:** *see* the PCA portal/data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
    - Clinical:
        - **Baseline**
        - **Off- Schedule** data is *optional but may be populated any follow up or additional unscheduled visits*
    - Biospecimen:
        - **Specimen**
        - **Site Pathology**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

All sections must be fresh and cut just prior to shipping, to be delivered within 1-2 wks of being sectioned. ***Label all slides or coverslip boxes with only the PCA ID and section number (no MRNs or dates should be written)***

Sections needed for:

- Sections on slides for DNA isolation via LCM/macro isolation for bulk sequencing
- Sections on slides for RNA isolation via LCM/macro isolation for bulk sequencing
- Sections for Bulk RNA isolation from Frozen tissue and biopsy samples.
- Sections on slides for Multiplex IF

Slide / Coverslip Product information:

- ***All Sections for H&E and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUAD Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Positively charged glass slides (i.e. Fisher 12-550-109)

SECTIONING OCT BLOCKS for slides

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut sections using a cryostat in the order described below based on specimen abundance- note the section thicknesses and order
5. Sections should be prepared on positively charged (+) slides
6. All cut slides should be stored at -20 and shipped on dry ice.
7. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for genomic DNA isolation)*

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut 5-20 uM scrolls and store at -80oC until shipment or proceed to extraction, If tissue is prepared for single nuclei sequencing sections in to 1mL RNA protect and store in 1.8mL cryovial at -80^{o}C
5. H&E Sections should be prepared on positively charged (+) slides and stored at -20^{o}C until shipment
6. All tissue scrolls should be sectioned into a 1.5 mL tube with 1 mL of RLT buffer with BME and stored at -80^{o}C until shipment
7. All samples should shipped on dry ice as detailed in shipping guidelines.
8. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and assess adequate vs. borderline
3. Pre label all slides with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut sections described below based on specimen abundance* - note the section thicknesses and order
5. Sections should be prepared on positively charged (+) slides or coverslips without baking
6. All cut slides should be stored at -20 and shipped at 4^{o}C (with cold packs)
7. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for tissue scrolls *(for genomic DNA)*

1. Using a fresh blade for each specimen/block cut and discard the first 2- 5 µM sections from a block
2. Cut a 4µM section and confirm each histology by H&E and to assess adequate vs. borderline
3. Pre label all slides and 1.5 mL tubes with PCA ID and section number ***NO MRNs or DATES***
4. Proceed to cut 5-20 uM scrolls and store at -80^{o}C until shipment or proceed to extraction
5. All cut samples should be stored at -20 and shipped at 4^{o}C (with cold packs)
6. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN LUAD RESECTIONS FOR THE RETROSPECTIVE CROSS-SECTIONAL LUAD-PML COHORTS (all tissue – usually just part-solid nodule)

NOTE: *When Frozen tissue is available, DNA and RNA sections will be derived from the OCT embedded frozen tissue and sections for multiplex IF will be derived from the FFPE tissue of the same lesion. Adequacy of premalignant and invasive adenocarcinoma associated lesions is determined by the greatest dimension of the lesion where those in which this measurement is >/= 0.5 cm are classified as adequate and those where this measurement is 0.1 – 0.5 cm are classified as borderline. When non-invasive lepidic premalignant tissue contiguous with invasive adenocarcinoma in part-solid nodules is discontinuous, the sum of the greatest dimensions of the areas of lepidic tissue is used to determine the appropriate adequacy classification.*

Adequate lesion or normal lung (>/= 0.5 cm in greatest dimension): 9 fresh sections (~72 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-4** – 3 x 10µM sections for DNA
- **Section 5** - 4µM Section for H&E (newly cut)
- **Section 6-8** - 3x10µM sections for RNA
- **Section 9** - 4µM Section for H&E (newly cut)

Adequate lesion FFPE tissue (>/= 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

Borderline lesion (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-7** – 6 x 10µM sections for DNA
- **Section 8** - 4µM Section for H&E (newly cut)
- **Section 9-14** – 6 x 10µM sections for RNA
- **Section 15** - 4µM Section for H&E (newly cut)

Borderline lesion FFPE tissue (0.1 - 0.5 cm in greatest dimension): 7 fresh sections (~28 um)

- **Section 1** - 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-6** – 5 x 4µM sections for in situ multiplex IF
- **Section 7** - 4µM Section for H&E (newly cut)

SECTION A.2 GENOMIC DNA ISOLATION & GENOMIC SAMPLE PREPARATION

A.2.1 PREPARATION OF BLOOD AND FROZEN SAMPLES FOR GENOMIC DNA ISOLATION

- **Blood samples:**
  - Acceptable blood samples: Whole Blood, Buffy Coat, PAX gene, STRECK
    - For buffy coat and other blood derivative specimen processing protocols, *see protocol (Synapse Link: https://www.synapse.org/#!Synapse:syn18352221/wiki/588708) The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual (Blood Collection Processing Section: Serum Processing & Plasma and Buffy Coat Processing)*
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Mini and Blood Mini Protocol*)
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal
- **Frozen tissue**
  - Acceptable Frozen tissue samples: normal LN, normal lung/airway (including bronchial and nasal brushings)
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DNA Micro Protocol*)
    - If using normal tissue see Section A.1 ***SECTIONING OCT BLOCKS for tissue scrolls for RNA and genomic DNA isolation*** for details
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal

A.2.2 Preparation of Lymph Node or FFPE Samples:

- **FFPE tissue**
  - Acceptable FFPE tissue samples: normal LN, normal lung
  - Basic prep: (*for detailed protocol see: Qiagen - QIAamp DSP DNA FFPE Tissue Kit Protocol*)
    - If using normal tissue see Section A.1 **SECTIONING FFPE BLOCKS for tissue scrolls for genomic DNA**
  - Minimum DNA amount (in 30 uL): 250 ng, 500-1000 ng is optimal