Retrospective Cross-Sectional Squamous Cohort – FFPE only Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Cross-Sectional LUSC*

All cases identified for the retrospective cohorts 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. The cases selected for the Lung PCA are anticipated to have at the minimum biospecimens from an independent pre-invasive bronchial dysplasia and a pre-invasive lesion contiguous with invasive squamous cell carcinoma (SCC) along with access to a genomic control for each case that is enrolled. Tissue from the invasive SCC is also required. When frozen tissue with associated matched Formalin Fixed Paraffin Embedded (FFPE) tissue is available, either as OCT embedded or flash frozen (alone or with RNA preservative) tissues that are then prepared in Optimal Temperature Cutting (OCT) media blocks, these tissues will be sectioned and punched for tissue microarrays (TMAs) locally following the guidelines detailed 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 University of Colorado (CU) Core Repository are provided in the shipping guidelines.

- **Collecting Sites:** CU, RPCI, UCLA
- **Lung PCA Repository to ship cases:** CU- *see shipping guidelines for all samples going to CU.*

  - **Collection Timepoints:**
    - T0: Resection cases of LUSC where bronchial dysplasia is found in the tissue margin or remote airway from tumor AND in a tissue adjacent (contiguous with) the invasive SCC.

  - **Required biospecimen to be collected**
    - T0:
      - Resection- FFPE tissue blocks or sections *(see sectioning guide: A.1.2)*
        - FFPE Tumor with contiguous Bronchial Dysplasia (Lesion and Normal area) **AND**
        - FFPE Bronchial Dysplasia remote from tumor (Lesion area)
      - Genomic DNA
        - 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)*
          - Alternative: FFPE tissue Scrolls, Blood derived gDNA

  - **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
- Tissue cores in TMAs to be cut onto coverslips for Multiplex CODEX

Slide / Coverslip Product information:

- ***All Sections for H&E:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***All Sections for CODEX:*** Coverslips 22 x 22 only (EMS 72204-01)
- ***LUSC Sections for DNA & RNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

SECTIONING FFPE BLOCKS for slides/coverslips

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 (+) regular or frame (DNA/RNA sections) slides or coverslips without baking
6. All cut slides should be stored at -20 and shipped at 4°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 sections in and stored at -80°C until shipment or proceed to extraction
5. All cut samples should be stored at -20 and shipped at 4°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 below. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FFPE LUSC RESECTIONS FOR NUCLEIC ACIDS AND IN SITU ASSAYS FROM THE RETROSPECTIVE CROSS-SECTIONAL LUSC - PML COHORT (All samples)

NOTE: *Bronchial dysplasia adequacy classification is based on the number of 40X fields the dysplastic traverses. Below suggested sections are based on an average cell thickness of >/= 6-8 cells in dysplastic bronchial epithelium. If a lesion shows an average cell thickness that is definitely < 6 cells, please add 2-4 extra 10µM sections for DNA and RNA extraction if possible. For tissue collection from invasive SCC or normal tissue two categories are used with adequate showing a greatest dimension of .>/= 0.5 cm and borderline 0.1 – 0.5 cm and the collection algorithms listed at the bottom of this section are employed.*

Adequate dysplastic lesion – BD or SCC (Lesion spans >/= 7 40X fields): 17 fresh sections (~152 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-9** – 8 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)
- **Section 10** - 4µM Section for H&E (newly cut)
- **Section 11-16** - 6 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 17** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Intermediate dysplastic lesion – BD or SCC (Lesion spans 4-6 40X fields): 23 fresh sections (~212 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-13** – 12 x 10µM sections for DNA (DNA derived from microdissected epithelium)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Section 15-22** – 8 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 23** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline dysplastic lesion – BD or SCC (Lesion spans 2-3 40X fields): 31 fresh sections (~292 um)

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-17** – 16 x 10µM sections for DNA (DNA derived from microdissected epithelium)
- **Section 18** - 4µM Section for H&E (newly cut)
- **Section 19-30** – 12 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 31** - 4µM Section for H&E (newly cut)
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

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

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-4*** *– 3 x 10µM sections for DNA*
- ***Section 5*** *- 4µM Section for H&E (newly cut)*
- ***Section 6-8*** *– 3 x 10µM sections for RNA*
- ***Section 9*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

Borderline resected SCC or normal lung (0.1 - 0.5 cm in greatest dimension): 15 fresh sections (~132 um)

- ***Section 1****- 4µM Section for H&E (pre-existing acceptable)*
- ***Section 2-7*** *- 6x10µM sections for DNA*
- ***Section 8*** *- 4µM Section for H&E (newly cut)*
- ***Section 9-14*** *- 6x10µM sections for RNA*
- ***Section 15*** *- 4µM Section for H&E (newly cut)*
- **Core remainder of lesional tissue for placement in TMA on 22 x 22 coverslip**

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 Appendix D (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
    - If using an airway brushing
  - 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