Retrospective Longitudinal Squamous Biopsy Cohort – OCT & FFPE Tissue Requirements and Processing Algorithms

RETROSPECTIVE: *Longitudinal LUSC bronchoscopy cohort*

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 a pre-invasive lesion and/or tumor along with access to a genomic control for each case that is enrolled. It is also anticipated that retrospective biospecimen tissue samples will primarily be prepared as Formalin Fixed Paraffin Embedded (FFPE) blocks that can be sectioned locally following the sectioning guidelines detailed below or as FFPE blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Alternatively, where possible, tissue may also be frozen, whereas tissue (pre-invasive/tumor lesion) samples are flash frozen (alone or with RNA preservative) that are then prepared in Optimal Temperature Cutting (OCT) media blocks that are sectioned locally following the sectioning guidelines detailed below or as blocks that will be sent to the Lung PCA Biorepository for sectioning and processing. Below we have provided detail for the retrospective longitudinal cohort with both OCT frozen and FPPE specimens that aims to outline the required clinical and biospecimen data to collect, along with the required biospecimens, tissue preparation and processing protocols and the details for shipping cases to our biorepositories.

- **Enrolling/Collecting Sites:** CU, UCL

- **Lung PCA Repository to ship cases:** No shipping of tissue required (all cases processed on site at either CU or UCL)

  - **Collection Timepoints:** *(estimated time between the collection of samples fresh or banked)*
    - T0 - Baseline bronchial dysplasia/CIS,
    - T1 - Bronchial dysplasia/CIS from the same lung location or invasive LUSC at 12months post baseline (at least 6M post BD, but later times also acceptable)
    - *Optional → T+ additional time points pre-baseline and post baseline (i.e. at separate site from T1) or post T1 can be considered when available. – authorization by repository required.*

  - **Required biospecimen to be collected:**
    - T0: Baseline
      - Bronchial Biopsy- FFPE and/or Frozen (if available) tissue blocks or sections
        - Frozen: (OCT/RNA protect) dysplastic lesions (*see sectioning guide A.1.1)*
        - FFPE: dysplastic lesions **(if providing frozen, FFPE is required for in situ assays)** *(see sectioning guide: A.1.2 (if providing frozen block see A.1.5))*
      - Genomic DNA
        - If tumor/pre-malignant lesion is frozen: (*see section A.2)*
          - 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*)
          - Alternatives: non-isolated blood samples, isolated DNA from FFPE normal lymph node (or less preferable, normal lung/ airway) or tissue scrolls (*see sections A.2 for sectioning guidelines*)

    - T1: Follow-up longitudinal (tumor from approximately the same lung location as T0/baseline biopsy)
      - Bronchial Biopsy or resected tumor block - FFPE and/or Frozen (if available) tissue blocks or sections*:*
        - Frozen: (OCT/RNA protect) Incident squamous cell carcinoma tumor (*see sectioning guide: A.1.1)*

- FFPE: Incident squamous cell carcinoma tumor **(if providing frozen, FFPE is required for in situ assays)** *(see sectioning guide: A.1.2)*

- **Required metadata to be collected:** *see the Lung* /data standards for details: (https://lci-test.whiterivercomputing.com/portal/data-standards)
  - Clinical:
    - **Baseline**: T0
    - **On Study Follow-Up**: T1
    - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 1 follow up or additional unscheduled visits*
  - Biospecimen:
    - **Specimen**: T0, T1 *(any T+ samples)*
    - **Site pathology**: T0, T1 *(any T+ samples)*

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections:

If not shipping whole tissue blocks use the following guidelines, where 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:

- Frozen/ FFPE Sections on slides for DNA/RNA isolation via LCM/macro isolation for bulk sequencing
- Frozen/ FFPE Sections for RNA isolation for bulk sequencing
- FFPE Sections for Bulk DNA/ RNA isolation from Frozen tissue and biopsy samples.
- FFPE Sections for multiplex/spatial assays and histologic evaluation (core Path)
- FFPE Cores for inclusion in TMAs for multiplex and spatial assays ***(optional for biopsy cohorts)***

Slide / Coverslip Product information:

- ***All FFPE Sections for H&E. RNA & Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***LUSC Sections for DNA Isolation from lesions in tumor blocks:*** Frame slides– (Applied Biosyst. LCM0521)

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
1. All cut slides should be stored at a minimum of -20 °C and shipped on dry ice
6. Cases can be sectioned and shipped in batches

SECTIONING OCT BLOCKS for tissue scrolls *(for RNA and 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. H&E Sections should be prepared on positively charged (+) slides and stored at -20°C until shipmen
4. Pre label all slides and 1.5 mL tubes with PCA ID and section number *NO MRNs or DATES*
5. H&E Sections for biopsy samples should be prepared on positively charged (+) slides and stored at -20°C until shipment
6. Proceed to cut 5-20 uM sections for DNA and for RNA of biopsy samples refer to details below* - note the section thicknesses and order and store at -80°C until shipment or proceed to extraction
7. All tissue scrolls for RNA isolation should be sectioned in to a 1.5 mL tube with 1 mL of RLT buffer with BME and stored at -80°C until shipment.
8. All samples should be shipped on dry ice as detailed below.
9. Cases can be sectioned and shipped in batches

SECTIONING FFPE BLOCKS for slides

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

SECTIONING FFPE BLOCKS for tissue scrolls *(for RNA or 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 for DNA and for RNA refer to details below and store at -80^{o}C until shipment or proceed to extraction
9. All cut samples should be stored at a minimum of -20 ^{o}C and shipped on dry ice
5. Cases can be sectioned and shipped in batches

*Specimen abundance is measured by techniques described below in sections A.1.1 – A.1.5. Follow these guidelines to determine how many slides or sections to cut for each lesion.

SECTION A.1.1 FROZEN BRONCHIAL BIOPSY FOR NUCLEIC ACIDS AND FFPE BRONCHIAL BIOPSY FOR IN SITU ASSAYS FROM THE RETROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *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. Can be applied for the Prospective LUSC cohort if processing locally*.

Adequate dysplastic lesion IN OCT – BD or SCC (Lesion spans >/= 7 40X fields): 25 fresh sections (~232 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-13 – 12 x 10µM sections for DNA (or DNA/RNA) (*from micro-dissected epithelium*)**
- **Section 14 - 4µM Section for H&E (newly cut)**
- **Section 15-24 – 10 x 20µM sections collected into a 1.5mL tube for RNA (*RNA from whole sections if possible*)**
- **Section 25 - 4µM Section for H&E (newly cut)**

Adequate dysplastic lesion IN FFPE – BD or SCC (Lesion spans >/= 7 40X fields):

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**

FOR MORE LIMITED SPECIMENS, COLLECTION OF DNA FROM OCT FOLLOWED BY COLLECTION OF IN SITU/MULTIPLEX *AND* RNA FROM FFPE CAN BE CONSIDERED AS OUTLINED BELOW

Intermediate dysplastic lesion IN OCT – BD or SCC (Lesion spans 4-6 40X fields): 18 fresh sections (~168 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-17 – 16 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)**
- **Section 18 - 4µM Section for H&E (newly cut)**

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 16 fresh sections (~148 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-15 – 14 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 16 - 4µM Section for H&E (newly cut)**
- **If amenable Core remainder of tissue for placement in TMA or section 5 sections for multiplex/spatial assays.**

Borderline dysplastic lesion IN OCT – BD or SCC (Lesion spans 2-3 40X fields): 26 fresh sections (~248 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 2-25 – 24 x 10µM sections for DNA (DNA derived from micro-dissected epithelium)**
- **Section 26 - 4µM Section for H&E (newly cut)**

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields): 22 fresh sections (~208 um)

- **Section 1- 4µM Section for H&E (pre-existing acceptable/preferred)**
- **Section 9-21 – 20 x 10µM sections for RNA (RNA derived from whole sections)**
- **Section 22 - 4µM Section for H&E (newly cut)**
- **If amenable Core remainder of tissue for placement in TMA or section 5 sections for multiplex/spatial assays.**

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