Prospective Longitudinal Squamous Biopsy Cohort – FFPE only Tissue Sectioning Requirements and Processing

PROSPECTIVE: ***Longitudinal LUSC bronchoscopy cohort***

Prospective cohorts include participants that are recruited from on-going or new screening cohorts and those seen for suspicion of lung cancer or from other pulmonary clinics that will be consented for use of tissue for this study and for future studies including those that involve industry/pharma support. For cases selected it is anticipated that each case will have at 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 prospective biospecimen tissue samples include fresh sorted/processed lesions for single cell assays, frozen tissue from the pre-invasive/tumor lesion (alone, in OCT [Optimal Cutting Temperature media] or with RNA preservative) and an FFPE biospecimen. However, in valuable cases if the frozen tissue does not represent both pre-invasive and invasive lesion for the site (as seen in the FFPE tissue), submission of the case with only FFPE derived sections can be considered. FFPE blocks are either shipped to the core repository for processing/sectioning or sectioned locally following the sectioning guidelines detailed below. Below we have provided detail for the prospective longitudinal cohort with FPPE specimens that outlines 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 Core Repository are provided in the shipping guidelines.

- **Enrolling/Collecting Sites:** CU, RPCI, UCL
- **Lung PCA Repository to ship cases:** CU- *see details in shipping guidelines for all samples going to CU.*

Longitudinal Sample Collection Overview: For the ***Prospective Longitudinal LUSC*** cohort, a minimum of 1 lung location will be longitudinally biopsied (up to 6-8 being followed at baseline) and the sampled at T1 (6-12 mos) and T2 (6-12 mos), where each location being followed will have at the minimum a formalin fixed and OCT frozen biopsy collected. For 1-3 selected sites followed overtime a minimum of 3 biopsies will be collected where 1) is collected in formalin, 2) is frozen in OCT, 3) is collected fresh for single cell sorting and where possible a fourth biopsy flash frozen in RNA protect (not in OCT) should be collected. The formalin and frozen samples will be shipped overnight on the day of collection to the LUSC core repository at the University of Colorado for processing where possible if formalin samples can't be sent they should be processed with in 24 hours at the site of collection and tissue blocks shared. The fresh tissue will be processed and single cell plates stored on site until the location to be further studied has been decided and plates will then be shipped to the BU team.

- **Collection Timepoints:** (*scheduled visits as part of the Lung PCA study)*
  - T(-1) (optional) Pre-screening sputum collection and potential pre-screening bronchoscopy
  - T0 - Baseline bronchial biopsies
  - T1 - Bronchial biopsies including follow-up fresh tissue from designated 2-3 sites of special interest
  - T2/incident LUSC - Bronchial biopsies or tumor block including follow-up fresh tissue from designated 2-3 sites of special interest
  - *Optional → T+ additional time points pre baseline, interim between T0-T1 or T1-T2 or post T2 can be considered when available – authorization by repository required.*
- **Required biospecimen to be collected: :** *(Additional details can be found in The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal along with the single cell processing protocol)*
  - **T0: Baseline**
    - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections
      - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations deemed to have highest grades of BD or that have shown persistent BD (see sections A.3 & C.3) **AND**
      - Frozen: OCT from all sites, and flash frozen in RNA protect from selected sites of highest grade dysplasia (to parallel those for fresh tissue when possible) **AND**
      - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)

        - Genomic DNA
            - If tumor/pre-malignant lesion is frozen: (*see section B.1)*
                - Preferred: Isolated DNA from blood sample including a whole blood/buffy coat *(see section A2.1)*
                - Alternatives: non-isolated blood samples, isolated DNA from frozen normal lymph node or (less preferable, normal lung/ airway) (*see sections A.1 for sectioning guidelines*)

    - **T1** (same lung location (s) at T0 1-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically nearest location)
        - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guidelines)*
            - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
            - Frozen: OCT/RNA protect from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection, when possible, **AND**
            - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)

    - **T2** (same lung location T0/T1 1-3 sites for extended collection of fresh and flash/RNA protect frozen tissue or tumor at site of previous collection or anatomically location)
        - Biopsy- Fresh sorted samples and Frozen and FFPE tissue blocks or sections *(see sectioning guide below and separate single cell sorting guideline)*
            - Fresh: biopsies single cell sorted into 96 well plates from 2-3 locations showing highest grades of BD or persistent BD – these will be pre-selected by the core repository and sites will be notified of which airway sites to collect for single cell sorting (see sections A.3) **AND**
            - Frozen: OCT from all sites, and flash frozen in RNA protect at sites with BD, from sites of fresh tissue collection, when possible, **AND**
            - Formalin/FFPE: From all sites, send tissue in formalin (if sending sections, i.e. from T(-1) specimen, see section A.1.2)
    - NOTE: For any sites at any time point, if invasive LUSC is present, collection of FFPE, OCT frozen, fresh tissue and flash frozen tissue in that order of priority should be undertaken with goal of collecting all

- **Optional Samples:**
    - *Please see The Lung Pre-Cancer Atlas: Prospective Longitudinal LUSC Biospecimen Manual also available on the Lung PCA portal for additional samples and processing information for this cohort.*

- **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, T2
        - *Optional:* **Off- Schedule** is for data for any irregular bronchoscopy visits with tissue collected for PCA *or T -1 or T + 2 follow up or additional unscheduled visits*
    - Biospecimen:
        - **Parent Specimen for each biopsy**: T0, T1, T2 *(any T+ samples)*
        - **Site pathology for each Parent Specimen**: T0, T1, T2 *(any T+ samples)*
        - **Derivative specimen for any samples processed (ie sectioned or analyte isolated)**

SECTION A.1 SECTIONING GUIDELINES

Preparation of tissue sections for FFPE only samples:

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 that may be needed based on tissue adequacy and availability:

- FFPE Sections for Bulk DNA/ RNA isolation FFPE biopsy samples.
- FFPE Sections on slides for DNA/RNA isolation via LCM/macro isolation for bulk sequencing
- FFPE Sections for multiplex staining and histologic evaluation (core Path)

Slide / Coverslip Product information:

- ***All Sections for H&E, RNA isolation and Multiplex IF:*** Positively charged glass slides (i.e. Fisher 12-550-109)
- ***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 slides or coverslips without baking
6. All cut slides should be stored at a minimum of -20 °C and shipped on dry ice
7. Cases can be sectioned and shipped in batches

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

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
8. All cut samples should be stored at a minimum of -20 °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 FFPE BRONCHIAL BIOPSY FOR NUCLEIC ACIDS AND IN SITU ASSAYS FROM THE PROSPECTIVE LONGITUDINAL LUSC - PML COHORT (All samples)

NOTE: *Biopsy lesion adequacy classification is based on the number of 40X fields the dysplastic epithelium 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. If the invasive SCC or normal tissue are collected from a resection specimen, 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 should be employed.*

Adequate dysplastic lesion IN FFPE – 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 (*DNA derived from micro-dissected epithelium*)
- **Section 14** - 4µM Section for H&E (newly cut)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 15-24** – 10 x 10µM sections collected into a 1.5mL tube for RNA (*RNA from whole sections*)
- **Section 25** - 4µM Section for H&E (newly cut)
- **Section 25-30** 4µM Section for spatial assays or **If amenable Core remainder of tissue for placement in TMA or section 5 sections for multiplex/spatial assays.**

Intermediate dysplastic lesion IN FFPE – BD or SCC (Lesion spans 4-6 40X fields): 33 fresh sections (~312 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)
- **Use pre-cut scrolls 12 x 10 µM – for RNA (RNA derived from whole sections) OR**
- **Section 19-32** - 14 x 10µM sections for RNA (RNA derived from whole sections)
- **Section 33** - 4µM Section for H&E (newly cut; NOT necessary if pre-cut scrolls used for RNA)
- **Section 33-38** 4µM Section for spatial assays or **If amenable Core remainder of tissue for placement in TMA or section 5 sections for multiplex/spatial assays.**

Borderline dysplastic lesion IN FFPE – BD or SCC (Lesion spans 2-3 40X fields):

- **Section 1**- 4µM Section for H&E (pre-existing acceptable/preferred)
- **Section 2-25** – 24 x 10µM sections for DNA/RNA
- **Section 26** - 4µM Section for H&E (newly cut)
- **Section 27-31** 4µM Section for spatial assays or **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