LUNG TISSUE RESEARCH CONSORTIUM (LTRC)

PROTOCOL

Version 1.0

October 12, 2016

THE CONTENTS OF THIS PROTOCOL ARE CONFIDENTIAL AND ARE NOT TO BE CITED OR DISCUSSED EXCEPT WITH INDIVIDUALS TO WHOM IT HAS BEEN DISTRIBUTED ON BEHALF OF THE LTRC STEERING COMMITTEE

STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol and in accordance with all applicable regulatory requirements, including US Code of Federal Regulations (CFR) 21 Part 312 (where applicable) and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human participants. The study will be conducted in accordance with good clinical practices (GCP) in accordance with the International Conference on Harmonization's (ICH's) E6 guideline, all applicable participant privacy requirements, and the guiding principles of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB / IEC review and favorable opinion / approval to conduct the study and of any subsequent relevant amended documents
- Informed consent (and any amendments) to be obtained for each participant prior to participation in study procedures

Investigator reporting requirements (e.g., reporting of AEs/SAEs/protocol deviations to IRB / IEC):

- All individuals responsible for the design and conduct of this study have completed Human Participants Protection Training and are qualified to be conducting this research prior to the enrollment of any participants.

SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulations.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____ _____

Signature of Principal Investigator Date

_____

Name of Principal Investigator (printed or typed)

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List of Abbreviations

Table 1: List of Abbreviations

ABBREVIATION	FULL NAME OR TITLE
AIP	Acute Interstitial Pneumonia
ABG	Arterial Blood Gases
AE	Adverse Event
ALARA	As Low As Reasonably Possible
ATS	American Thoracic Society
BD	Bronchodilator
CC	Clinical Center
CL	Core Laboratory
CLIA	Clinical Laboratory Improvement Amendments
COPD	Chronic Obstructive Pulmonary Disease
eCRF	Electronic case report form
CT	Computed Tomography
1-DGel	1-Dimensional Gel
2-D Gel	2-Dimensional Gel
DCC	Data Coordinating Center
DLCO	Diffusing Capacity of Carbon Monoxide
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
ETC	External Tissue Contributor
FEV	Forced Expired Volume
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescent in Situ Hybridization
FRC	Functional Residual Capacity
FTP	File Transmission Protocol
FVC	Forced Vital Capacity
G_{aw}	Airway Conductance
HIPAA	Health Insurance Portability and Accountability Act of 1996
H&E	Hematoxylin & Eosin
HOPE	Hepes-Glutamic acid buffer mediated Organic
HRCT	High Resolution CT Scan
IAC	Image Acquisition Computer
IHC	Immunohistochemistry
IIP	Idiopathic Interstitial Pneumonias

ABBREVIATION	FULL NAME OR TITLE
ILD	Interstitial Lung Disease
IPF	Idiopathic Pulmonary Fibrosis
IRB	Institutional Review Board
ISH	In Situ Hybridization
LCM	Laser Capture Microdissection
LTRC	Lung Tissue Research Consortium
LVRS	Lung Volume Reduction Surgery
MOP	Manual of Procedures
MW	Maximum Voluntary Ventilation
NCHS	National Center for Health Statistics
NDI	National Death Index
NETT	National Emphysema Treatment Trial
NHLBI	National Heart, Lung, & Blood Institute
NSIP	Non-specific Interstitial Pneumonia
OCT	Optimal Cutting Temperature Compound
OSMB	Observational Study Monitoring Board
PACS	Picture Archive Communication System
PCR	Polymerase Chain Reaction
PFT	Pulmonary Function Test
PI	Principal Investigator
PVC	Premature Ventricular Complexes
PRC	Protocol Review Committee
QC	Quality Control
QT-PCR	Quantitative Polymerase Chain Reaction Solvent Protection Effect
R_{aw}	Airway Resistance
RCL	Radiology Core Laboratory
RV	Residual Volume
SELDI	Surface Enhanced Laser Desorption/mediated dUTP-fluorescence nick end labeling ionization
SLB	Surgical Lung Biopsy
TCL	Tissue Core Laboratory
TLC	Total Lung Capacity
TMA	Tissue Microarray
TUIMEL	Terminal deoxynucleotidyl transferase (TdT)
UIP	Usual Interstitial Pneumonia
VATS	Video Assisted Thoracoscopic Lung Biopsy

Protocol Synopsis

Table 2: Protocol Synopsis

PROTOCOL TITLE	LUNG TISSUE RESEARCH CONSORTIUM
Objective	• Primary: To collect lung tissue and blood samples from participants who undergo medically-indicated lung surgery. • Secondary: To annotate collected samples with an array of clinical data, limited exposure data, physiologic studies, and radiographic studies.
Study Design	Observational study of adults with and without lung disease.
Study Population	Adults age 21 and older.
Number of Participants	Up to 400.
Number of Centers	Four (4) clinical; 2 core laboratories.
Inclusion	Adults ages 21 and older undergoing lung surgery for suspected malignancy or metastases.
Exclusion	Diagnosis of cystic fibrosis or pulmonary hypertension. Any other condition that, in the judgment of the investigator, precludes participation. Failure to obtain written consent.
Study Duration	November 2016 through February 2019.
Participation Duration:	Typically not more than four weeks from time of consent, depending on participant's health status and schedule for lung surgery.
Safety	All adverse events and serious adverse events occurring within two calendar days following a protocol-mandated procedure will be monitored.

1. INTRODUCTION

1.1 Summary

The National Heart, Lung and Blood Institute (NHLBI) Lung Tissue Research Consortium (LTRC) was originally established with the primary purpose of collecting lung tissue and blood samples and a secondary purpose of collecting clinical data, limited exposure data, physiologic studies, and radiographic studies from participants with chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), other related idiopathic interstitial pneumonias (IIP) and interstitial pneumonias associated with connective tissue diseases who undergo medically-indicated lung resection. Under this contract period, the protocol will be modified slightly to enhance the collection of lung tissue and clinical data from control participants who require lung surgery. Control participants will be individuals with suspected lung cancer or metastatic disease to the lung, who have pulmonary function tests (PFT) showing normal lung function and normal pre-operative appearance of the lungs on CT imaging, except for lesions thought to represent discrete malignancies. For all participants, regardless of disease findings post-enrollment, non-neoplastic tissue will be collected remotely from a nodule or mass. All tissue and blood specimens and clinical data will be banked and stored for distribution to future investigators who have approved study proposals and are investigating the pathogenesis of lung diseases. It is the ultimate goal of this study that information derived from the LTRC will lead to novel interventional treatments for all disease categories under study.

There are four clinical centers participating in this consortium: 1) Mayo Clinic, Rochester; 2) University of Michigan; 3) University of Pittsburgh; and 4) Temple University. These centers will send de-identified participant tissue and clinical data to the Tissue Core Laboratory (National Jewish Health), Radiology Core Laboratory (Mayo Clinic), and the Data Coordinating Center (The Emmes Corporation). It is projected that approximately 400 participants and accompanying clinical data and specimens will be collected over a three year period. The organizational chart of the study is presented in the MOP.

1.2 Purpose of the Study Protocol

The LTRC protocol details the rationale, specifies the objectives, and describes the design and organization of the study. Maintaining a bank with specimens from a wide spectrum of participants with respect to the type and timing of progression of lung disease may lead to new insight into the cause of the disease and lead to new therapies to improve survival and quality of life. A large majority of participants enrolled under previous LTRC contract periods had diagnoses of COPD and IPF, with a much smaller number representing a control population. In order to balance the disease spectrum of LTRC participants, the current contract period will focus on participants undergoing surgery for suspected malignancy, since members of that group are more likely to yield control tissues than those undergoing surgery for other indications.

As the study progresses and the protocol is modified, the DCC will prepare and distribute (via the study website library) revised versions as necessary. Protocol modifications will be announced by the distribution of a numbered memo to all clinical centers, Radiology and Tissue Core Laboratories, NHLBI staff and the OSMB. The memo will also be posted to the LTRC website for future reference.

2. GOALS, BACKGROUND AND SIGNIFICANCE

2.1 Specific Goals

The primary goal of the LTRC is to identify participants meeting the following criteria:

- Individuals with suspected lung cancer or metastatic disease who are willing to provide informed consent for research use of their biospecimens and data.

Secondarily, the LTRC investigators intend to collect clinical data, limited exposure data, physiologic studies, and radiographic studies from these participants. It is the goal of this study to collect tissue samples from approximately 400 participants in three years. This represents approximately three (3) participants per month for the three year period at each of the four clinical centers.

2.2 Background and Significance

Chronic lung diseases are a main cause of death and disability in the United States. COPD affects over 14 million individuals in the United States and represents the third leading cause of mortality. However, only one of six individuals who smoke develops COPD. This could imply either an individual susceptibility or an additional immunologic or infectious injury to lung cells (1, 2). Current treatment offers symptomatic relief, but does not prevent disease progression. Further understanding of disease pathogenesis, including the potential roles of lung parenchymal cell apoptosis, immunologic injury and inflammation, may lead to therapies that improve survival and quality of life (3).

Interstitial pneumonias, including IPF and those associated with connective tissue disease, are less common than COPD, but for many of these diseases there are poor outcomes. For example, IPF has a 50% survival rate 2-3 years following diagnosis and no treatment exists which prolongs survival. The prevalence of IPF is approximately 28 cases per 100,000 (4). The underlying histology of IPF is usual interstitial pneumonia (UIP), which can also occur in connective tissue diseases. The incidences of other interstitial pneumonias such as non-specific interstitial pneumonia (NSIP) or acute interstitial pneumonia (AIP) are less frequent but also occur as an expression of interstitial lung disease in the connective tissue diseases. Moreover there is significant crossover of these three interstitial pneumonias so that cases of IPF/UIP may also reveal fibrotic NSIP and be complicated by episodes of AIP. This implies common injuries but dissimilar histological responses. All of these processes are characterized by epithelial injury, uncontrolled fibroproliferation and the deposition of collagen, irrespective of the histology (5). It is clear that a further understanding of the genesis of the interstitial pneumonias is required before effective interventions can be developed.

2.3 Study Design

The donor group will include participants undergoing lung surgery for nodules and masses. These participants will provide tissues for LTRC investigations. Some of these participants may have mild or moderate COPD or ILD as determined by their post-consent PFTs and pathological examinations. Specimens will be collected regardless of post-consent pathology or lung function findings.

Participants will be evaluated as outlined in Section 4 to include demographics, clinical data, limited exposure data, radiographic scans of the lung, physiologic and exercise testing, and blood testing. The purpose of collecting these data is to stage disease severity and have clinical data correlations available for future investigators studying the tissue.

3. STUDY POPULATION

3.1 Overview

The LTRC will facilitate histopathological research of pulmonary diseases by collecting lung tissues from donors and preparing and distributing collections of tissue specimens to researchers within and outside the LTRC. Collections of specimens will be linked to individual clinical data appropriate to the particular disease.

3.2 Eligibility Criteria

All patients undergoing medically indicated lung surgery will be potential candidates for tissue procurement as part of the LTRC. Specifically, individuals with localized lesions representing suspected lung cancer or metastatic disease to the lungs will be eligible. After consent, participants will be entered on the site's screening log and enrolled into the study and data system. A participant may be enrolled more than once; the sampling unit is the surgical procedure. The DCC will be able to link multiple samples from the same participant as necessary for analyses.

3.2.1 Inclusion Criteria

- Adults ages 21 or older who are undergoing lung surgery for suspected malignancy or metastases.

3.2.2 Exclusion Criteria

- Diagnosis of cystic fibrosis or pulmonary hypertension.
- Any other condition that, in the judgment of the investigator, precludes participation.
- Failure to obtain written consent.

3.3 Recruitment Strategies

To obtain the widest representation of participants undergoing surgical procedures, a multidisciplinary approach will be encouraged. In an effort to optimize tissue procurement and clinical data for phenotyping of tissue donors, a diverse team will be needed to carry out LTRC study procedures at each clinical center, including:

- Principal and co-principal investigators
- Clinical coordinators (primary and liaison coordinators from thoracic surgery and lung transplant programs, if applicable)
- Pathologists and tissue processing technologists
- Thoracic surgeons and lung transplant physicians
- Radiologists, medical physicists, and CT technologists
- Referring physicians, if applicable

Each clinical center will be responsible for developing a cohesive group of researchers to identify potential tissue donors at their clinical center. All efforts should be made to recruit participants at the earliest time point in their clinical care as possible. This will optimize the collection of adequate data for clinical phenotyping. An active interaction between the LTRC Clinical Coordinator and

the Liaison Clinical Coordinator is encouraged. Similarly, active communication between the LTRC Principal Investigator and co-investigators and the thoracic surgical co-investigators will be crucial. The screening and consent processes will be the responsibility of the multidisciplinary team. These efforts will be carried out while preserving the participant's privacy and confidentiality. Limited data will be collected on a screening log to monitor the recruitment process for each participant and to check whether the participant has been previously enrolled.

The processes of recruitment, screening, and enrollment may vary slightly at each clinical center due to differences in staffing, infrastructure and interdepartmental relations. Each clinical center has outlined their workflow and procedures which should follow the general process flow outlined in the Manual of Procedures.

It is the expectation of the NHLBI that the LTRC clinical centers will screen approximately 10 potential participants per month and procure tissue from approximately 3 participants per month throughout the course of active recruitment. The primary focus will be on recruiting patients who are undergoing surgical lung resection for suspected lung malignancy (typically control donors and donors with mild COPD).

Monthly reports will compare age, gender, and race between participants who are recruited into the LTRC and those who are not recruited into the LTRC. The goal of these analyses will be to obtain the widest possible representation in each of these demographic groups, and to prevent bias in participant selection with respect to these groupings.

It is possible that the Steering Committee may decide at a future date to target recruitment of participants with a wider spectrum of pulmonary parenchymal disease processes.

3.4 Informed Consent

Written informed consent of the participant will be required before any LTRC procedure is performed. The participant will be given the consent form to read, after which the investigator or Study Coordinator will thoroughly explain the study and answer any questions. The LTRC consent form will include check boxes for the participant to decide if he/she consents to:

- A volumetric high-resolution CT scan obtained by LTRC-specific protocol parameters or a release of a CT scan obtained for clinical reasons within the six months prior to the surgical procedure (in a DICOM format)
- Genetic Testing

Participants will have the study goals of the LTRC fully explained to them. The amount of time required for the interviews and procedures and the risk of radiation exposures will also be explained. Participants will be informed that they can still participate in the main study if they choose to decline being contacted for the CT scan or Genetic Testing. Participants will be informed that refusal to participate in any part of the LTRC will not change their current or future care at the clinical center.

Each clinical center will be able to customize its own consent form based on the prototype developed by the Investigators. The DCC will review all sites' consent forms prior to submission to the Protocol Review Committee and local site Institutional Review Board (IRB). Investigators at each clinical center will work with their IRB to develop an effective sampling and monitoring strategy to ensure that the approved procedures are being followed.

3.5 HIPAA Compliance

Each LTRC clinical center will be responsible for its compliance with the current HIPAA requirements. This includes familiarity with what data are considered personal identifiers and should not be forwarded to NHLBI, the DCC or Core Laboratories. The DCC will design all LTRC study forms and databases to omit such variables, except the contact form, which will be kept at the clinical center. Additionally, each clinical center will fully explain their institution's HIPAA release form prior to obtaining the participant's signature. This form should include NHLBI, the DCC, and the Core Laboratories as institutions that may review the participant's data.

4. CLINICAL CENTER PROCEDURES

4.1 Overview of Study Procedures

Table 3: Visit and Procedure Schedule

Procedure	Requirement
Demographics	R
Informed Consent and Eligibility	R
Visit Documentation	R
Adverse Events	AN
Clinical Dx Report	R
Concomitant Therapy	R
Central Pathology Report	R
CT Scan Report	R
Environmental Questionnaire	R
Family Hx Questionnaire	R
St. George’s Respiratory Questionnaire	R
Laboratory Data	R
Local Pathology Report	R
Medical Hx Questionnaire	R
Occupational and Environmental Questionnaire	R
Pulmonary Function Tests*	R

Procedure	Requirement
SF-12 Health Survey	R
Smoking Hx	R
Symptom Questionnaire	R
Study Status	R
Six Minute Walk Test	R
Vital Status	R

R: Form is required

AN: Form to be completed only as needed.

* Spirometry is required but lung volume, diffusing capacity (DLCO), and arterial blood gas (ABG) components are optional

In most cases, a single LTRC study visit will be performed to clinically phenotype LTRC participants. This visit should not require more than a total of four to six hours to complete, but this visit may take place over a four-week period or until the participant is discharged from the hospital. In this circumstance, collection of LTRC phenotyping data may occur over a period of time. Collection of phenotyping data will include recording of relevant medical information and a limited exposure history, radiological evaluation, and pulmonary physiological and lung function testing as defined below. Blood specimens will be obtained both for defining the clinical phenotype of donors, as well as, for serum and plasma storage for later investigative purposes. Clinical questionnaires to determine the extent of symptoms, associated medical illnesses, smoking, limited environmental and occupational exposures, and quality of life will be administered. While the primary objective of the LTRC is to collect lung and blood tissues for the study, the presence of lung tissue without phenotyping data is of limited value, which makes the collection of phenotyping data a secondary objective. The LTRC investigators have set a goal of 90% completion of phenotyping data for the study.

4.2 Screening

Research staff will screen potential participants for eligibility requirements per local institutional policies.

4.3 Pre-surgery

After the participant has signed the IRB-approved consent form and after it has been determined that the participant satisfies all inclusion and meets no exclusion criteria, participants will be assigned a study ID, and the following evaluations/data will be recorded in the electronic case report form (eCRF):

- Participant demographics, including date of birth, gender, ethnicity, and race
- Pulmonary function tests (PFTs) (Spirometry is required; lung volume, DLCO, and ABG are optional)
- Prior and concomitant therapies

- Laboratory measurements
- CT scan reports (either as part of the study, or non-study scans occurring within six months prior to enrollment
- Six Minute Walk Test
- Adverse events
- Visit documentation
- Interviews/questionnaires (see sections 4.4.1 and 4.4.2)

Where able, the medical record will be used to collect information on the study case report forms (CRFs).

Clinical centers will strive to collect the information above prior to a participant's surgical procedure, as the tests are designed to assess the participant's phenotype prior to surgery. If needed, completion of specific questionnaires (Demographics, Medical History, Family History, Smoking History, Concomitant Therapy, Environmental, and Occupational Questionnaires) and the collection of historical information from the medical record or by participant interview may be done after.

4.4 Interviews and Questionnaires

4.4.1 Medical and Family History

A limited medical history will be obtained around the time of the participant's study visit. This will include data on the participant's medical history and family history in first degree relatives of chronic lung disease as documented by biopsy and rheumatologic conditions. We will determine the onset and progression of symptoms with respect to dyspnea on exertion, cough and sputum production. Queries concerning additional symptoms on coughing, dyspnea and phlegm will also be made.

4.4.2 Exposure History

Exposure history will include determination of smoking status and extent of exposure to tobacco smoke. Significant occupational exposure to asbestos, silicates and related hard rock dusts, underground coal mining, beryllium, cobalt and other hard metals, and other significant occupational exposures will be recorded. Environmental exposure histories to organic dusts and agents that induce hypersensitivity pneumonitis will be obtained. We will also obtain relevant history for exposure to drugs and radiation known to induce pulmonary fibrosis. These data will be obtained by a participant interview that will not exceed two hours in length.

4.5 Pulmonary Function Tests

4.5.1 Spirometry

Spirometry is a valid, reproducible means of monitoring for change in the severity of the respiratory component in lung disease. When properly performed with maximum effort and attention to the details of calibration of the instrument, spirometry maneuvers are among the most reproducible of biomedical measures. In a highly trained, experienced participant, a coefficient of variation of 2-3% is not unusual. Spirometry will be performed in a standardized manner in accordance with the American Thoracic Society (ATS) guidelines, as described in the MOP.

Spirometry is the timed-based measurement of the amount of air which can be forcefully exhaled from the lungs after a full inspiration. In the LTRC, this will refer to the measurement of the forced expiratory vital capacity (FVC) maneuver. In the forced expiratory vital capacity maneuver, a person inhales as big a breath as possible and then blows it out as fast and as long as possible until no more air can leave the lungs. The volume of air leaving the lungs is recorded continuously throughout the maneuver in order to allow calculation of important measures of lung function. Percent values will be calculated at the DCC using the Hankinson and Crapo reference equations (17).

4.5.2 Six-Minute Walk Test

The six-minute walk test is a timed walk involving a familiar activity and requiring minimal technical resources. It has been shown to be a reproducible objective indicator of functional performance. However, unlike cardiopulmonary exercise testing, it does not allow collection of basic physiologic data which may be useful in determining mechanisms of change or participant selection. It is important to emphasize that this is a test of maximum exercise performance; participants will be instructed and encouraged in a standard fashion to achieve maximal distance. Performance of this test will follow the recommendations of the American Thoracic Society (ATS) Statement for this test procedure (18).

4.5.3 Lung Volume Measurements

While spirometry measures the amount and rate of air flow exhaled from the lungs, it is not an accurate measure of the total lung capacity (TLC) which depends not only on the vital capacity (VC), the total air which is exhaled, but also on the residual volume (RV), which measures the amount of air remaining in the lungs at the end of a full expiration. Another measure, functional residual capacity (FRC), is a measure reflecting the air remaining in the lung at the end of a normal tidal breath. Usually, FRC also represents the volume at which the inward recoil of the lungs is balanced by the outward recoil of the chest wall. In restrictive lung diseases, such as IPF, the TLC, RV and FRC, are symmetrically decreased. In obstructive lung diseases such as COPD, these parameters are increased with the RV being disproportionately increased compared to the TLC. The LTRC will require a body plethysmography technique as detailed in the MOO, which allows measurement of trapped gas as well as ventilated lung units.

Decreased lung recoil and airway conductance account for varying degrees of functional derangement in emphysema, and this heterogeneity cannot be differentiated by maximal flow measures alone. Hence, the value of G_{aw} (the inverse of R_{aw}) lies in its ability to discriminate participants within this spectrum of pathophysiologic derangements who have a disproportionate severity of relatively fixed intrinsic small airways obstruction. Airway Resistance (R_{aw}) and Airway Conductance (G_{aw}) will be measured using a body plethysmography panting technique as detailed in the Manual of Operations.

4.5.4 Arterial Blood Gas

Like plethysmography, arterial blood gas analysis is used to measure other data elements that can be used to classify lung disease. Arterial blood is collected to determine the partial pressures of Pa02, PaC02 and the pH of the blood. These measurements assist the investigators in determining whether the participant is acidotic and/or is appropriately oxygenating the hemoglobin in the participant's red blood cells. Again these physical measures will allow the investigators to phenotype the participant. Details on the performance of arterial blood gas analysis will be presented in the MOP.

4.5.5 Diffusing Capacity of Carbon Monoxide (DLCO)

The DLCO test can be used to detect early stages of interstitial lung disease even when more standard tests such as spirometry and chest X-rays appear normal. In particular, the DLCO test will measure the lung's ability to perform gas exchange. This is done by measuring the amount of carbon monoxide that can be absorbed by the lungs for a specific time interval (usually ten seconds). The inability of the lungs to perform gas exchange is diagnostic for diseases such as emphysema and pulmonary edema or fibrosis. The specific procedures for performing the DLCO will be presented in the MOP.

4.6 Final Diagnosis

A final diagnosis of the participant's lung disease will be rendered by the clinical center PI. The clinical center PI will review all available local pathological and clinical participant information, diagnosis reports from the Tissue and Radiology Core Laboratories and consult with the relevant physicians at the LTRC clinical center, prior to making a final diagnosis. Once made, the final diagnosis will be submitted to the DCC. If an alternate diagnosis has been received from the TCL or RCL, the clinical center PI will communicate this new information to the participant's primary care physician to assist with the diagnosis and treatment of the participant. This will conclude the participant's direct participation in the LTRC. The participant may be contacted to clarify questions from the interview process at the end of this two-month period.

4.7 National Death Index

At the conclusion of the LTRC, the vital status of participants will be updated based on a report from the National Center for Health Statistics (NCHS) National Death Index (NDI). Using participant information such as Social Security Number, name and birth date (all held at the clinical center), the clinical center Study coordinator will request an NDI report on the LTRC participants. Direct identifiers will be stripped from the report file and information about the follow-up date, whether the participant had died, the date of death, and cause of death will be linked to the participant's study ID, and the data will be forwarded to the DCC for inclusion in the LTRC database. The DCC will not request or receive the NDI reports as this process requires the use of identifying information.

4.8 Adverse Event Reporting

Adverse events (AEs) experienced by LTRC participants during a window that starts at the beginning of a protocol-mandated procedure and continues for two calendar days after the procedure is ended will be reported on LTRC forms. If a second procedure is started before the closure of the first window, the period of report shall end two calendar days after the beginning of the second procedure, etc. If a participant goes home before the two calendar day window is over, he/she will be encouraged to report any AEs to the LTRC investigators, and events will be collected from the medical record report of the event. All adverse events will be reported in each window. If the lung surgery begins while a participant is still in an AE reporting window for a protocol mandated procedure, all adverse events will be collected up to the start of the surgery; and only serious adverse events will be reported for a two calendar day period beginning at the start of the surgery. If it comes to the attention of clinical center staff that an adverse event is definitively related to an LTRC procedure, even if it is outside of a reporting window, clinical center staff will report the event to the DCC on the appropriate LTRC form.

If any event is "serious" in nature, the clinical center will complete the SAE section of the Adverse Event DCF with a complete narrative (removing the name of the individual), and complete the SAE eCRF in Advantage eClinical within three days of its occurrence. A serious event is defined as an event that

- Causes death
- Is life-threatening
- Results in hospitalization or prolongs a hospitalization
- Results in a serious or persistent disability
- Represents a serious hazard or could cause serious harm to the research participant.

All serious adverse events will be collected in the above stated periods and forwarded to the NHLBI and the OSMB Chairperson (within one week of notification), and the OSMB Chairperson will be queried as to further action that may be required to review the event. Reporting to the clinical site Institution Review Board (IRB) should be done per the institution SAE reporting policy.

5. TISSUE COLLECTION

5.1 Clinical Center Tissue Collection Procedures

The following materials will be collected at the LTRC clinical centers:

- Peripheral blood (up to 24.5 ml)
- Lung Tissue

5.1.1 Overview of Tissue Specimen Acquisition

Tissues will be collected for long-term storage at the TCL. Tumor samples should not be submitted to the LTRC. The LTRC will collect the 'non-tumorous' portions of lung tissue from surgical procedures performed for primary or metastatic lung tumors. These samples for the LTRC should be obtained from grossly uninvolved tissues, preferably maintaining an approximately 1.5 cm margin around the tumor. No portion of the tumor should be submitted. The local pathologist will be responsible for selecting the non-tumorous tissue. However, the technician should verify the absence of tumorous tissue which can usually be distinguished from 'normal' lung tissue by its hard, firm, usually white, appearance. The uninvolved lung tissue will appear tan to red (often with black specks) and will feel spongy. The LTRC will request only those tissues not used by the local pathologist for diagnosis.

There will be two clinical situations in which there is potential for tissue to be collected. These will include:

- Prospective Biopsy: The participant is enrolled in the LTRC and the participant's physician will take a biopsy of lesional tissue.
- Other Tissue Resection: The participant will undergo other tissue resection.

5.1.2 Acquisition of Fixed and Frozen Tissue Specimens

All tissues obtained prospectively by biopsy or other tissue resection and that are approved for research study should be prepared according to the instructions given in the LTRC Manual of Procedures (MOP).

If there is not sufficient tissue for at least one 100 mg aliquot per fixative, then follow the priority list - frozen, RNAlater and then formalin, with the understanding that all fixatives might not be collected. If there is not enough tissue to send a formalin aliquot, then the clinical center will send two blank formalin-fixed slides for each lobe from surgical pathology so that a diagnosis may be made at the TCL. Slides will be obtained from a block that does not contain tumor. These slides will not be returned to the clinical center.

It is very important that the period of time LTRC tissue remains ischemic is kept to a minimum. The tissue technician will work with the surgical/pathology staff to determine which tissue won't be needed for clinical purposes and will begin to process that tissue as soon as possible. The LTRC investigators have set as a goal that the ischemic period for LTRC tissue will be no longer than 15 minutes for VATS and biopsy specimens and 30 minutes for lobectomy tissue. Tissue and blood should be kept at the appropriate storage conditions and shipped to the TCL every other week.

5.1.3 Tissue Collection Kits Supplied by the TCL

The TCL will prospectively send out complete tissue and blood collection kits to each site. These kits will be either for blood or resection with individual components reflecting the type of procedure. The TCL will replace kits as they are used by the clinical centers. The individual specimen containers will be pre-labeled by the TCL with the tissue identifier (6-digit specimen number supplied by DCC), and type of fixative/preservation method. The TCL will make labels and containers for each kit. The clinical center should make sure that each container has the correct lobe marked at the time of collection. Each clinical center will be responsible for placing the appropriate specimens in each container, and labeling sample location (Right or Left and lobe). If there are unused containers for any case, they will be sent back to the TCL along with the rest of the specimen containers.

The TCL will supply the RNAlater fixative to each clinical center. The RNAlater will be stored at room temperature and does not have an expiration date. The clinical center will be responsible for providing 10% neutral buffered formalin and liquid nitrogen (or the equivalent) for flash freezing specimens.

5.1.4 TCL Diagnostic Slides

The TCL will make H & E and pentachrome stained diagnostic slides from a formalin fixed specimen for each lung lobe that is sampled from the participant.

5.1.5 Blood Specimens

Blood (20.5 ml) will be collected to perform specific laboratory tests designed to assist in phenotyping the participant. These tests are: White blood cell count, hemoglobin, hematocrit, platelet count, and alpha-1 antitrypsin.

The LTRC will also require a peripheral blood sample (up to 24.5 ml) to be divided into the following manner:

- One blue-top Qiagen 'PAXgene' DNA tube (8.5 ml)
- One red/grey top SST tube (8 ml)
- One green/gray top PST tube (8 ml)

Cells from these tubes will be used to generate DNA, serum and plasma, which will be separated and stored at the Tissue Core Laboratory.

Reference the LTRC MOP for details on processing, storage, and shipment of specimens.

5.2 Tissue Core Laboratory

5.2.1 Role of the Tissue Core Laboratory

The LTRC Tissue Core Laboratory (TCL) will coordinate the collection and distribution of specimens of lung tissue from open or thorascopic biopsy or lobectomy.

The role of the TCL is to:

- Collect and store pathological specimens sent to the TCL from the LTRC clinical centers; and
- Distribute the specimens based on Protocol Review Committee (PRC) recommendations and NHLBI approval

The TCL is the repository for all tissue specimens collected for the LTRC. TCL staff will be responsible for maintaining records on tissue sent to them by each clinical center.

Upon receipt of the tissue, the TCL will be responsible for the following:

- Completing fixation process
- Processing to paraffin or freezing
- Tissue diagnosis
- Storage (See MOP)
- Redistribution (See study website www.ltrcpublic.com)

For formalin fixation to paraffin, the samples are submitted to the National Jewish histology lab for automatic processing before being embedded at the Tissue Core Lab. H & E and pentachrome stains will be performed on representative paraffin-embedded tissue blocks for analysis and classification by the TCL pathologist:

- RNALater tissue is aliquoted and stored at -20°C for future distribution
- Flash (snap) frozen tissue is aliquoted and stored at -80°C for future distribution
- Blood in DNA tubes is stored at -80°C; Isolated DNA is stored at -20°C in 5 µg aliquots
- Serum and plasma samples are stored in 150 microliter aliquots at -80°C.

5.2.2 Tissue Diagnosis

The TCL pathologist will review and assess the tissue quality of the formalin specimen based on H & E and/or pentachrome staining. A histologic diagnosis will be rendered and passed to the DCC. The central pathology diagnosis will then be communicated back to the clinical center PI for possible medical management, and for the final 2-month diagnosis of the participant's lung disease.

The TCL will assess the RNA integrity of representative RNALater samples as appropriate.

The quality review process begins with visual inspection upon TCL receipt of the shipped tissue. Sample size, adequacy of fixative/preservative immersion, and shipping variables (container

condition, adequate dry ice, etc.) are noted. A hematoxylin and eosin stained slide and a pentachrome (Movat) stained slide from each formalin-fixed lobe is reviewed by the TCL pathologist to assess tissue quality and to provide a histopathologic diagnosis.

RNA quality and viability for use are primary quality variables. Based on LTRC II steering committee recommendations, 20% of individual subjects with RNAlater tissue are randomly selected for RNA quality testing. As RNA quality testing requires the sacrifice of some tissue, subjects with < six available tissue aliquots are grouped into a separate sampling stratum in order to minimize the number of tested subjects with only one available aliquot. Within this stratum, for every 100 subjects, 10 subjects are randomly selected for testing. RNA quality is measured by running a sample of total RNA on an Agilent Bioanalyzer chip. A RIN number greater than or equal to “7” is considered acceptable quality and recorded as “Plus” within the database, and the subject's RNAlater and Frozen tissue are considered acceptable for experiments requiring intact RNA and are recorded as "Use for RNA". A RIN number less than “7” is considered unacceptable quality and recorded as “Minus” within the database. RNAlater and frozen tissue from these subjects are noted as "Not for RNA." Additionally, we monitor user responses for determination of potential problems.

6. RADIOLOGY

6.1 Goal

The goal for the LTRC radiology component is to obtain CT image data on greater than 90% of the recruited LTRC participants. Below we have listed, in order of preference, the CT image of the chest that should be obtained for each participant in the LTRC.

- LTRC Protocol: This is a volumetric high-resolution CT scan protocol of the chest that will be obtained specifically for research purposes, with acquisitions of images including supine inspiratory, supine expiratory and prone inspiratory imaging with specific acquisition parameters and multiple reconstructions to optimize diagnostic utility and consistency in quantitative analysis. The prone inspiratory acquisition is not required for subjects who are unable to comply with positioning due to physical limitations. This scan should be obtained after enrollment but before surgery.
- For participants who cannot or wish not to have an LTRC scan but who have recently undergone a CT of the chest for clinical reasons (less than six months) or who will have a clinical scan before lung tissue is obtained, these images will be accepted by the RCL for evaluation. These images must be electronic DICOM images from the clinical center or from another institution. Volumetric high-resolution imaging (slice thickness less than 2mm) with non-contrast techniques is preferred. The CT portion of a PET/CT, imaging performed with intravenous contrast, image acquisition/reconstruction that is not volumetric (i.e., HRCT with gap) or slice thickness greater than 2mm is not preferred. Participants who do not have historical imaging with a preferred technique should be strongly encouraged to obtain the LTRC Protocol CT prior to surgery.

Clinical center staff need only carry out one of the above procedures to obtain CT data for the LTRC. The CT scan can be used for medical management, or another CT scan can be collected. This section lists, in order of priority, the type of CT scan the LTRC investigators would like to collect from each participant. Below, a brief description of each of the different types of CT scans has been presented.

6.2 LTRC CT Scan

The LTRC CT Scan is the preferred CT scan protocol to be collected for phenotyping the participant in the LTRC. The LTRC CT Scan is designed specifically to allow quantitative analysis of the lung as part of the LTRC project, and requires specific acquisition and reconstruction parameters that enable data to be as comparable as possible between the different participants. A subset of the CT scanners available at the clinical centers will be certified for use in obtaining this specific LTRC CT scan trial through an ongoing QA assessment and Protocol validation process. The precise parameters used for obtaining this LTRC CT dataset will be optimized for each specific CT scanner at a clinical center. Specifically, the full LTRC CT Scan Protocol will consist of helically acquired datasets on a multidetector CT scanner (with no less than 16 detectors) as well as scout/localization images as needed. Thin-slice images (1.0 mm or less) will be obtained and reconstructed at 50% overlap in both a high-resolution kernel (with specific accommodation for this kernel's density accuracy) and a kernel with lower noise and spatial frequency. These scans will be obtained in the supine position at full inspiration, supine and suspended full expiration and prone full inspiration. Scans will be optimized to allow for single breath acquisition in less than 10 seconds. Radiation dose will be adjusted for the participant's size. The ALARA (as low as reasonably allowable) principle will be utilized in these studies with accommodation for the adequate signal/noise ratio to realize the experimental quantitative analysis goals of the LTRC. The effective dose for the inspiratory phase LTRC CT Scan will be approximately 3-5 millisieverts (mSv), and a lower dose expiratory phase and prone series will each be approximately 2-3 mSv.

The images obtained from these scans will be sent to the Radiology Core Laboratory for analysis and storage as described below. Several scan protocols, based on different CT scanner brands and models, with appropriate weight-based dose adjustments and specific breathing instructions for participants and technologist information sheets are included in the LTRC Manual of Operations.

6.3 Historical CT Scan

A less desirable alternative to the LTRC CT Scan for participants enrolled in the LTRC that still fulfills the imaging requirements is a CT scan of the chest previously obtained for clinical reasons. These historical exams must have been obtained less than six months from the time of evaluation at the clinical center and must also be in digital DICOM format for transfer to the RCL. Volumetric high-resolution imaging (slice thickness less than 2mm) with a field of view sufficient to visualize the entire lung volume with non-contrast technique is preferred. The CT portion of a PET/CT, imaging performed with intravenous contrast, image acquisition/reconstruction that is not volumetric (i.e., HRCT with gap) or slice thickness greater than 2mm is not preferred. Subjects who do not have historical imaging with a preferred technique should be strongly encouraged to obtain the LTRC Protocol CT prior to surgery. Radiographs (chest x-ray) and other imaging modalities (MRI, V/Q scans, Ultrasound) are not acceptable. The participant must consent to use of a clinical scan for research purposes, its storage at the Mayo Clinic, or another repository designated by the NHLBI, and the sharing of results and de-identified image data with future researchers as part of the LTRC data repository. If more than one historical exam is available, the most recent examination with optimal acquisition and reconstruction parameters (non-contrast-enhanced, volumetric, high-resolution CT images with (thickness of 2mm or less) will be sent to the RCL for analysis and storage as described in the sections below. In order of preference, non-contrast exam, volumetric high-resolution technique and age of exam are to be considered. For example, if a 1 week old enhanced pulmonary embolism protocol CT, a 1 month old PET/CT and a 3 month old non-contrast volumetric high-resolution CT are available, the 3-

month old non-contrast high-resolution CT would be preferred). The LTRC Protocol CT acquired prospectively would be preferred to any of the historical exams, if that can be acquired prior to surgery.

6.4 Radiologic Evaluations

CT data acquisition and data management at each clinical center will be the responsibility of the clinical center. Per best practices, it is expected that the clinical center will be able to acquire and store participant data securely using standard clinical radiology management systems (i.e., a local PACS). There are two options for image data transfer to the RCL. The preferred mechanism is to use the LTRC Data Transfer Tool which will de-identify the image data at the clinical site and then submit the de-identified data electronically to the RCL. If it is not possible to use the LTRC Data Transfer Tool, the clinical site may use their own tools to de-identify the data, record it to physical media (CD/DVD/USB), and mail the de-identified data to the RCL. The RCL will not take responsibility for data on physical media that is improperly de-identified. If the RCL received non-de-identified data on physical media, the media will be destroyed, and the site will be requested to resubmit the data with proper de-identification. The RCL will not accept other forms of electronic image data transfer from the clinical sites (i.e., third-party data sharing sites) unless previously approved by RCL staff.

6.4.1 Image Acquisition and Initial Clinical Center Storage Procedures

Each clinical center will be required to store the original image data acquired by the LTRC protocols either to a local archive, such as a local PACS archive, or to digital archive media approved by the CT scanner manufacturer for the duration of the LTRC study. This local archival is utilized to minimize the risk of data loss. Prior to image transfer to the RCL, the clinical site study coordinator should request physical media of the LTRC scan (or the appropriate historic scan). If the clinical site is using the LTRC Data Transfer Tool, the image data does not need to be de-identified (see below). If the clinical site is submitting data via physical media, the study coordinator is responsible for ensuring the data is properly de-identified prior to submission.

6.4.2 Electronic Image Data Transfer

Electronic transfer of the data using the LTRC Data Transfer Tool is the preferred method of data transfer. The tool is selected from the LTRC data transfer website: https://rportal.mayo.edu/LTRC/. Upon clicking on the website link, the tool will be downloaded and can be run by the clinical site study coordinator. It is recommended that the clinical site always run the tool from the website to ensure the latest version of the tool is being used.

The tool only works on Microsoft Windows computers. It is expected that the computer system will have sufficient resources (computational power and memory) to support the de-identification and data transfer process. Additionally, it is expected that the system will have a physical media drive (i.e., a DVD/CD drive) in order to load the data into the tool. The tool will not require installation and will not install any software on the local system. Under the guidance of the clinical site study coordinator, the tool will index the LTRC Image data. All data is expected to be in the DICOM format. The study coordinator will be required to answer a limited set of questions regarding the image data. Proper operation of the tool is detailed in the instructions provided with the tool.

Prior to electronic submission, the data is de-identified using the DICOM recommended Basic Attribute Confidentiality Profile defined in the DICOM Part 15 guidelines (16). This de-identification process will protect patient information (through removal or re-assignment of personal health

information with study identifiers) prior to data transmission. Data are submitted to the RCL using the Secure FTP protocol. Accordingly, the study coordinator's computer system must be connected to the internet, and it must be able to connect to the RCL through that internet connection.

During the file de-identification and transfer process, the study coordinator should not exit the software or log out of the machine as this will disrupt the transfer. Instead, the coordinator should track progress according to the progress meter within the tool. Once the transfer is complete, the coordinator can exit the software application.

6.4.3 Image Data Transfer via Physical Media

If the site is unable to use the electronic transfer tool, the site can choose to provide physical media to the RCL. Read-only DVDs are preferred; however, a site may also choose to provide images on USB pen drives or portable hard drives. When submitting images via physical media, it is the responsibility of the clinical site to ensure the data has been properly de-identified. It is recommended to use the DICOM Part 15 guidelines (16) in this process. If non-de-identified data is received by the RCL, the RCL will destroy the media.

In order to submit data as physical media, the clinical site study coordinator should print and complete the PDF form found on the RCL data transfer website (https://rportal.mayo.edu/LTRC/). A copy of the completed form should be retained by the clinical site while the original should be submitted along with the data. The mailing address of the RCL is included in the form along with additional instructions. Any image data should be mailed using a certified mail carrier service.

6.4.4 Receipt of Image Data and Quality Assurance

When the RCL receives LTRC image data, the data will undergo an initial review. The review process does not include an assessment of the quality of the image data; it is only used to ensure the integrity and completeness of the data. The data will be reviewed to ensure that the entirety of the imaging study has been submitted and that the form information is complete. Additionally, if physical media is provided, it will be scanned for malicious code and checked for de-identification. If problems are found with the physical media, the media will be destroyed.

Following the initial review of the data, the clinical site will receive an email confirming the receipt and acceptance (or rejection) of the dataset. Information will be provided if problems are detected. At that time, the data will be incorporated into the processing pipeline.

6.5 Processing of CT Scans

6.5.1 Image Quality Scrutiny, Semi-Quantitative Reporting and Quantitative

6.5.1.1 Analysis Interpretation

Due to the nature of the disease processes to be studied by the LTRC, including emphysema and infiltrative lung diseases, no single measure or objective criteria can be utilized to quantify the type and extent of pulmonary disease. The Principal Investigator, co-investigators and RCL staff will provide expert subspecialty evaluation, image quality assessment and semi-quantitative analysis of the images of the chest for all studies provided to the RCL.

All interpretations by the local clinical center radiologist will be placed in the participant's medical record. The RCL report will include unexpected pulmonary findings pertinent to clinical care and prudent efforts will be made to report clinical important unexpected findings to the clinical center LTRC investigators via the reports submitted to the DCC.

6.5.1.2 Expert Semi-Quantitative Analysis

As the primary component of the RCL reporting by the radiologists, a semi-quantitative assessment of the type and extent of pulmonary pathology will be provided. Specifically, a matrix of specific radiographic findings and their anatomic localization will be provided by the interpreting radiologist, based on accepted terminology. This characterization and structured reporting of specific abnormalities is designed to take advantage of the skills of the RCL subspecialty thoracic radiologist investigators and provide a database of semi-quantitative data that can be utilized by the DCC and LTRC Investigators to correlate with clinical and pathologic findings.

The semi-quantitative and qualitative evaluation of pulmonary disease is inherently subjective and, to some degree, depends on the skill, training and experience of the radiologist. Inconsistently applied criteria or systematic bias can significantly reduce the power of the study. Because of this dependence, standardized terminology, accepted descriptive criteria, careful training and testing of reader performance is required. The goal is to assess the various pulmonary pathologies within the LTRC studies reproducibly and produce excellent inter- and intra-reader consistency.

6.5.1.3 Image QA/QC and Subjective Assessment

The RCL will maintain a QA validation procedure to validate the techniques used to acquire images at the clinical centers. This program will provide ongoing assurance of image quality. Each radiologist report will include an assessment of image quality and address any specific participant-related or unexpected image quality abnormalities, anomalies or artifacts.

These assessments will be logged and reviewed on a regular basis to assess for significant trends or correctable issues which would be addressed through contact with the clinical center CT technologist liaison. Studies deemed to have significant QA/QC issues or be non-diagnostic by the radiologist shall be reviewed at regular RCL administrative group meetings, and, if deemed necessary, steps to correct or minimize systematic problems will be reported to the Steering Committee for further analysis. The RCL will propose to the Steering Committee any needed modifications in the methods of image acquisition and transmission.

6.5.1.4 Quantitative Analysis Procedures, Goals and Hypotheses

The Biomedical Imaging Resource at the Mayo Foundation will be involved in the design and implementation of computer-based techniques for quantitative 3D analysis for the CT datasets obtained using the LTRC specific volumetric high-resolution CT acquisition parameters. Datasets which are not obtained in accordance with the specific instructions and parameters outlined for the LTRC protocol in this manual (such as historical CT scans) will be processed, if possible, as by the RCL for quantitative information. A single series from each LTRC participant will be processed. For the LTRC Protocol and any historical imaging, specifically the inspiratory supine volumetric HRCT from the LTRC protocol and any available from historical exams, the volumetric HRCT performed without contrast will be processed. For historical exams, no studies performed with intravenous contrast, with slice thickness greater then 2mm, with strong edge-enhancing reconstruction kernel (i.e., GE Lung and Siemens b70 or 80) or reconstructed as a non-volumetric dataset (i.e., thin slices with gap) will be processed. For the LTRC Protocol, additional

measurement of airways and the manual segmentation of the lobes of the lungs will be performed. These airway measure and lobe segmentations will not be performed on CT scans that are not performed by the specific LTRC volumetric HRCT protocol. The quantitative 3D analysis will include techniques utilizing a combination of multiple algorithms, including as automated and semi-automated segmentation driven by direct visualization, and displayed through a standardized interface.

Mayo Biomedical Imaging Resource will leverage the AVW imaging library (a collection of over 600 functions that have been utilized to build advanced image-based application solutions, including the powerful Analyze software package) and a customized version of the CALIPER (Computer-Assisted Lung Informatics for Pathology Evaluation and Rating) application for processing LTRC CT scans. CALIPER is an application designed for the semi-automated segmentation, visualization and quantification of chest CT data. The CALIPER analysis scheme will include whole-lung segmentation and application of algorithms which measure representative features of the pulmonary pathology present in LTRC participants. The CALIPER interface will guide an analyst technician through the process of anatomic segmentation and feature quantification. Regional decomposition of image analysis will be accomplished by performing 2D image measurements over the entire lung relative to starting and ending section locations, and by using mathematical morphology to define peripheral and central lung regions for both 2D and 3D measurements.

This analysis application will directly access participant data in a DICOM database, and will record analysis results along with all operator-selected segmentation and analysis parameters. The results, including anatomic segmentation maps, lung volumes, tracheobronchial tree measures and quantitative measures will be made available to the DCC and other investigators, as approved by the NIH/NHLBI and LTRC Steering Committee.

6.6 Storing CT Scans

The submitted CT data from the clinical centers would be stored in the LTRC DICOM repository and loaded onto a RCL LTRC workstation for analysis by CALIPER and review by a radiologist. The Clinical Coordinator will verify the receipt of images at the RCL via the DCC's Advantage eClinical image management and verification system. The study coordinator or a CT technologist will verify study information, series and image counts and enter this information into the CT Scan Report eCRF.

Validated studies received by the RCL will be archived in a dedicated LTRC research pool within the Mayo Institutional Digital Image Archive (MIDIA). This institutional archive resource will allow for storage of massive quantities of LTRC DICOM data in a high-availability dual-datacenter environment. As part of the storage process to MIDIA, a final archive verification report will be automatically generated, which will be compared to the tracking information in the-LTRC DCC database by the archiving technologist or study coordinator for final data integrity assurance. The standard procedures utilized at the Mayo Clinic for storage verification of research related and clinical images to the MIDIA archive will be followed for LTRC Data.

6.6.1 Radiology Center Image Data flow and Tracking

The RCL Clinical Coordinators will utilize a local RCL workstation to send the clinical center images to the LTRC MIDIA archive, which in turn automatically forwards the newly acquired DICOM images to the LTRC analysis database. These images can then be loaded by a 3D Analyst to a LTRC analysis workstation. At the time of 3D analyst loading of an exam, validation of the study type and the technique utilized for any LTRC HRCTs would determine whether the

study sent from the clinical center can be processed for quantitative analysis and segmentation. The 3D analyst will also perform an additional final verification of the study content by reviewing the number or series, number of images, and DICOM header elements relevant to CT technique for each exam. If the CT scan parameters match those of the LTRC specific protocol, these images will undergo anatomic lobe segmentation, and tracheobronchial tree measurement as well as processing for parenchymal quantitative information. Exams that are not performed by the LTRC specific protocol will be loaded for automated lung volume measurements and parenchymal classification if the non-contrast volumetric HRCT series images are of sufficient quality and a volumetric HRCT series are available. Other series and non-LTRC protocol exams transferred to the RCL will be loaded for viewing but will not undergo quantitative processing. The CT 3D Analyst will record the results from the quantitative analysis and anatomic segmentation results into the RCL LTRC database and will automatically have representative JPEG images of the upper, middle and lower lungs generated for transfer to the DCC.

After completion of the quantitative analysis by the CT 3D analyst, the LTRC radiologist will view all images for the LTRC participants, note the quality of each series, review and validate the segmentation of anatomic structures and the quantitative processing results, if available. The LTRC radiologist will also record semi-quantitative results and other interpretation data into the LTRC RCL CT Scan Report eCRF. If the clinical center media is not re-writable (such as a CD or DVD), it will be stored for the duration of the LTRC protocol as an additional backup. If the transfer media can be written to multiple times, it will be reused. Specifically, after the LTRC data archival has been verified and no discrepancies remain, the transfer media will be erased in a secure manner and returned to the clinical center.

6.7 Selection and Shipment of CT Scans for Sub-studies

The method for individual case selection and access to CT scans for sub-studies is detailed in the Manual of Procedures. Briefly, clinical data will be sent to an investigator using the de-identified CT study number (i.e., no clinical center designation and no sequence number within the clinical center). If specimens and/or CT images are requested, the corresponding specimen and/or CT numbers will be included in the data file from the DCC to allow the investigator to link their interpretations from RCL materials to the clinical data.

The CT Images will be prepared for distribution to investigators in standard DICOM format on either CD media, DVD media or other physical storage media such as flash storage cards or external hard drives as necessary to store the capacity of data transferred. Data sets and tissue specimens will be prepared in accordance with the NHLBI Limited Access Data Clause. Investigators will agree not to transfer data to other investigators nor use the data for other purposes unless it is approved by submitting a new proposal or an addendum to an existing proposal. At the end of the LTRC project, the complete CT Image Data with full documentation will be provided for use by investigators.

6.8 Quality Control

A detailed quality control program for calibrating and collecting images will be carried out as part of the LTRC. The Radiology Manual of Procedures is included as an appendix in the LTRC Manual of Procedures and provides a detailed description of the methodology for collection of specimens, certification and calibration of machines.

7. DATA COLLECTION AND MANAGEMENT

The investigator is obligated to conduct this study in accordance with U.S. Federal Regulation 21 CFR 312.60-69 as specified on the signed form FDA 1572, applicable federal laws, and the ICH: GCP: Consolidation Guideline. The investigator is responsible for informing the IRB/REB of any safety issues related to the study and the study drug, including reports of SAEs, if required, and all expedited safety reports.

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the DCC to the sites to record and maintain data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents, or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

7.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical study staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study. The DCC will be responsible for data management, quality review, analysis, and reporting of the study data.

7.2 Data Capture Methods

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

7.3 Timing/Reports

The OSMB will convene and make recommendations on study continuation based on the safety data collected annually.

7.4 Study Records Retention

Records and documents pertaining to the conduct of this study, including data collection forms, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator per federal or provincial regulations. No study records will be destroyed without prior authorization from the NHLBI.

8. SITE MONITORING

Site monitoring will be conducted to ensure that human participant protection, study procedures, laboratory procedures, and data collection processes are of high quality and meet, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and LTRC DCC as well as the National Heart, Lung, and Blood Institute's (NHLBI) policies. The LTRC DCC and/or NHLBI will conduct site-monitoring visits as detailed in the site monitoring plan or in the MOP.

Site visits will be made at standard intervals as defined by the site monitoring plan and may be made more frequently as directed by the NHLBI. Monitoring visits will include, but are not limited to, review of regulatory files, data collection forms (DCFs), informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

Currently site visits to each LTRC clinical center will be conducted every year during the recruitment and tissue collection process. Plans for site visits will be provided to the LTRC Steering Committee, NHLBI Project Officer, and LTRC Study Chair on a schedule agreed to with the NHLBI Project Office. The site visit teams will consist of a core group of professionals working on LTRC at the DCC as well as a representative from NHLBI if needed.

A report summarizing the findings of the site (audit) visit will be sent to the Steering Committee, the clinical center Principal Investigator, the NHLBI Project Officer and members of the OSMB.

The components of the site visit will include the following activities:

- Participant recruitment - The clinical center's methods for identifying and recruiting eligible participants will be reviewed. An item on the site visit agenda will cover the methods clinical center staffs use while the participant is being screened, recruited, interviewed, and tested to preserve the risk to disclosing the participation of the participant in the LTRC.
- Clinical Center Operations - The methods of scheduling and record keeping will be reviewed and study files examined.
- Review of Medical Records and Study Forms - Selected study forms for a sample of enrolled participants will be reviewed. The data on the forms for the participants will be reviewed. The data on the forms for the participants will be compared against listings of data on the database at the DCC and against records from the clinical center. Consent forms will be reviewed for participants.
- Retraining, Reinforcement of Standardization of Data Collection Methods - These steps will be ongoing and individualized to suit the needs identified for each clinical center.
- Data Management Activities
- Procedures for data entry will be reviewed as well as the procedures for filing records.
- Data quality review

9. CORE LABORATORY DATA MANAGEMENT

9.1 Specimen Tracking

The performance of each of the Core Laboratories (CL) participating in a study will be summarized in monthly reports. This report will include the number of specimens received and processed, the number of specimens not prepared or not labeled properly, studies of the monthly variation

associated with reported results (Shewart plots) and test-retest reliability studies of CL evaluations.

The DCC staff will compare the performance of each LTRC CL to its own past performance and to agreed-upon study standards. Quality control charts (Shewart Plots) will be used to examine the means and frequencies of the assays and evaluations performed over time. An investigation will be undertaken to determine whether any shift represents a change in the population being studied or shift in the methods for performing specified assays.

10. STUDY ADMINISTRATION

10.1 Organization Overview

The LTRC will be conducted by the collaborating investigators of a Data Coordinating Center (DCC), four clinical centers (CC), two Core Laboratories (a Tissue Core Laboratory (TCL) and Radiology Core Laboratory (RCL)) and the NHLBI Program Office. An organizational chart of the LTRC is presented in the MOP.

The Protocol Review Committee (PRC) will be responsible for evaluating the basic science and feasibility of the LTRC Protocol. They will review the protocol prior to participant recruitment and will review any subsequent modifications to the Protocol. They will review all requests for LTRC specimens and forward their recommendations to the NHLBI.

The Observational Study Monitoring Board (OSMB) will be responsible for reporting to the NHLBI on the overall progress of the study, the validity of LTRC evaluations, and on the safety profile of the approved study procedures.

The Steering Committee (SC) will be composed of the NHLBI Project Officer and the Principal Investigators of the CCs, the DCC, the RCL and TCL. This committee will be the focus for discussions and decisions on study design and performance. The DCC will abide by decisions of the SC and the NHLBI throughout the course of this study. This committee forms the basis of DCC distribution lists to ensure that all study staff receive the necessary materials to be trained in study procedures and information on the implementation of study procedures.

The DCC will compile an address directory that identifies the name, address, phone and fax numbers, and email address of all clinical centers, Core Laboratory, DCC, NHLBI staff and all committee members. This address directory will be emailed to all LTRC study investigators and staff, and posted to the LTRC Study Web page. This directory will be updated as needed to reflect staffing changes in the LTRC.

Once the study is underway, the DCC support staff will take primary responsibility for presenting information on the progress of the study with respect to collection, analyses, completeness and quality of required data. DCC support staff will be responsible for setting up meetings and conference calls of the LTRC including the identification of the meeting site, meeting rooms, distribution of materials, and recording of minutes. All study materials and minutes not classified as confidential will be uploaded to the LTRC Web page.

10.1.1 National Heart, Lung & Blood Institute

This study is being funded by the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH). The NHLBI Project Officer is responsible for overseeing the study design, implementation, data quality and information dissemination. The Contract Officer is responsible for monitoring the receipt of all deliverables as required by the NHLBI and dispersal of payment to the contractors.

10.1.2 Study Chair

The NHLBI has appointed a study chair (Dr. Robert Wise, Johns Hopkins University Allergy and Asthma Center) to assist the NHLBI Project Officer and oversee the operation of the LTRC. Dr. Wise is not a staff member at any LTRC participating institution.

10.1.3 Clinical Centers

The Principal Investigators of the clinical centers (CC) have agreed to abide by the Protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the LTRC including: Recruitment and characterization of the participants as specified in the Protocol, accurate data collection and the transmission of information and specimens to the DCC and Core Laboratories.

10.1.4 Data Coordinating Center

The Data Coordinating Center (DCC) is responsible for the logistics of study coordination, data management, study monitoring, quality control measures and descriptive data analysis. The DCC will be responsible for all documents and the LTRC Web pages. As study proposals are received, the DCC will be responsible for facilitating the review, querying the LTRC database for appropriate samples, supplying shipping lists to the Core Laboratories, and providing clinical and phenotypic data to the investigators.

10.1.5 Tissue Core Laboratory

The Tissue Core Laboratory (TCL) will be responsible for developing the standardized procedures to be used in obtaining the lung tissue specimens. This includes supplying tissue collection kits and appropriate shipping instructions, receipt of all specimens, division and cataloging of specimens, and long-term storage of specimens. Once a study is approved, the TCL will be responsible for shipping selected specimens to the appropriate investigators as directed by the DCC.

10.1.6 Radiology Core Laboratory

The Radiology Core Laboratory (RCL) will be responsible for developing the standardized procedures for obtaining the CT scans. This will include methods for calibration of equipment at the clinical centers and the cross calibration of densitometers between the Clinical Centers with the use of various phantoms. Once a study is approved, the RCL will be responsible for shipping selected scans to the appropriate investigators as directed by the DCC.

10.2 Committees

10.2.1 Steering Committee

The Steering Committee (SC) consists of ten members: The NHLBI Project Officer, the Study Chair, the Principal Investigators of each of the four clinical centers, the Principal Investigator and Co-Principal Investigator of the Data Coordinating Center and the Principal Investigator of the Tissue and Radiographic Radiology Core Laboratories. The Steering Committee has the responsibility for developing the Protocol, study implementation, recruitment and Protocol adherence.

10.2.2 Observational Study Monitoring Board

The Observational Study Monitoring Board (OSMB) will be charged with reviewing the Protocol and consent forms with respect to ethical and safety standards and advising the NHLBI. During the study, the OSMB will meet periodically to review safety issues and to monitor contractor performance in execution of the Protocol. Prior to each meeting, the OSMB will be provided with the LTRC monthly report. At the beginning of each meeting, the NHLBI Project Officer, Study Chairman and the DCC Principal Investigator will report on study progress and answer any questions.

10.2.3 Protocol Review Committee

The Protocol Review Committee (PRC) will be appointed by the NHLBI Project Office and charged with the duty of reviewing the LTRC Protocol prior to recruitment. This committee will offer an independent, unbiased review of the LTRC Protocol to ensure scientific soundness. At least annually, the PRC would review proposed modifications to the LTRC Protocol.

The PRC is responsible for reviewing proposals, from both LTRC and external investigators, for the use of these specimens. The PRC will review the proposals for feasibility and scientific merit and forward their comments and recommendations to the NHLBI. The DCC will assist the PRC by providing statistical support and current specimen inventories. Upon approving a request based on the PRC recommendation, the NHLBI will instruct the DCC to release the appropriate specimens for shipment.

11. POLICY MATTERS

11.1 Training and Certification

11.1.1 LTRC Training Sessions

Prior to participant recruitment, all LTRC clinical center staffs are expected to attend a central training session. At this time, the LTRC Manual of Procedures (MOP) will be reviewed in detail. All training session materials will be posted on the study website for future reference. Clinical center staff joining the LTRC after this training session should review these materials and receive training from previously certified clinical staff. As needed, additional training will be provided by the DCC at Steering Committee meetings and site visits. Reference the LTRC MOP for more details regarding training requirements and procedures.

11.2 Distribution of Tissues and Data

The LTRC will generate considerable new data about participants with lung disease. Investigators will submit concept sheets that are reviewed by the Protocol Review Committee (PRC) with DCC input. If the PRC recommends approval of the concept sheet, the NHLBI Project Office will make a final decision and authorize the release of specimens and/or data to the investigator. The investigators will be expected to perform their analyses in a rapid and efficient manner. Approved concept sheets and published manuscripts will be posted on the LTRC Public Web page.

Investigators at all LTRC clinical centers, the DCC, and the NHLBI Project Office have equal status with regard to developing concept sheets and collaborating in the development and publication of research papers based on study material. Study coordinators and other staff at these centers are encouraged to submit studies.

11.3 Reports on Methodology

Manuscripts concerning the overall design, protocol, procedures, or organizational structure of the study may be published prior to the end of the LTRC. Such preliminary publications will be developed and reviewed according to the same guidelines used for other reports of findings.

Many public presentations about the LTRC that do not involve protocol data, or ancillary study data (e.g., grand rounds talks concerning the study's general design and objectives) do not require formal preliminary review and approval by the Steering Committee. However, if there is any doubt, investigators are asked to first consult with the Steering Committee indicating their intention to present the material, in order to avoid the premature release of study data or the inappropriate publication of confidential information.

11.4 Conflict of Interest Policy

11.4.1 General Principles

The LTRC investigators have agreed to a policy on conflict of interest which has few specific restrictions, but a broad indication for disclosure of potential conflicts of interest. The LTRC investigators wish to endorse the spirit and content of the 21st Bethesda Conference: Ethics in Cardiovascular Medicine (Frommer, et al, 1990) dealing with these issues, and seek to make this policy consistent with the record of that conference.

To address actual or perceived conflict of interest in the LTRC, the participating investigators voluntarily agree to abide by the guidelines described in the policy statement developed for the LTRC. See the Policy for Acquiring Specimens and Publishing Results for a copy of the Conflict of Interest Statement and additional details on these matters.

11.4.2 Individuals to be Governed by These Guidelines

Members of the LTRC Study Group who will be governed by these guidelines include the Study Chairman, the Principal Investigator at each clinical center, key personnel in the Data Coordinating Center, and the Principal Investigators of the Core Laboratories. Co-Investigators and other staff who have major responsibility for enrollment, recruitment, follow-up or collection of data for the LTRC at clinical centers or Core Laboratories will also be governed by these guidelines. The Principal Investigator for each LTRC Center will submit a list of individuals who will be governed by these guidelines at the beginning of the study and revise, as necessary, annually. The Principal Investigator of each participating unit will review the guidelines with all appropriate staff prior to the start of participant recruitment and at least annually thereafter.

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