Cover Page

Title: Imaging and Understanding Bronchiolitis Obliterans Syndrome (BOS) in Lung Transplantation with Hyperpolarized ^{129}Xenon MR Lung Imaging

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Imaging and Understanding Bronchiolitis Obliterans Syndrome (BOS) in Lung Transplantation with Hyperpolarized ^{129}Xenon MR Lung Imaging

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1. ABSTRACT:

Lung transplantation has evolved into an effective therapeutic option for a large number of pediatric patients with end-stage pulmonary disease. Long-term survival after lung transplantation, however, is far worse than after the transplantation of other solid organs. Chronic allograft rejection in the form of obliterative bronchiolitis (BO) affects 50-60% of lung transplant recipients who survive 5 years after transplant. The time between transplantation and onset of bronchiolitis obliterans syndrome (BOS) can range from a few months to several years.

An established mouse model of BOS exists and gives insight to BOS mechanisms of specific epithelial injury and aberrant repair but mouse models do not translate perfectly to human pathophysiology. Additionally, we have developed MR-imaging biomarkers that will allow targeted biopsy and longitudinal monitoring without ionizing radiation.

This protocol aims to

- Develop new imaging methods that are sufficiently sensitive to allow early diagnosis of BOS.
- Improve patient treatment outcomes through earlier diagnosis of changes leading to BOS by obtaining image guided clinical biopsies of transplanted lung.
- Provide image guided research biopsies, where applicable, for use in future research of ex vivo biomarkers of BOS and in the development of treatments through future clinical trials.

We will achieve these aims through a prospective, non-randomized, longitudinal, observational study that will recruit about 5 subjects a year for 5 years. We will follow these post lung transplant patients at 6 months and 1 year with ^{129}Xe MRI (Hyperpolarized ^{129}Xenon Magnetic Resonance Imaging) and image guided bronchial biopsies to detect early BOS and to better understand BO disease progression. The research biopsies will provide samples for future research for rapid determination of cellular and molecular mechanisms that lead to BOS and to facilitate identification and validation of translatable pharmaceutical targets.

2. PURPOSE OF STUDY:

Lung transplantation has evolved into an effective therapeutic option for a large number of pediatric patients with end-stage pulmonary disease. Long-term survival after lung transplantation, however, is far worse than after the transplantation of other solid organs. Chronic allograft rejection in the form of obliterative bronchiolitis (BO) affects 50-60% of lung transplant recipients who survive 5 years after the transplant (1) and represents the most formidable challenge in this field. The time between transplantation and onset of bronchiolitis obliterans syndrome (BOS, the clinical surrogate of obliterative bronchiolitis) can range from a few months to several years, but this rejection is not well understood (2, 3). Current clinical practice calls for periodic post-transplant visits, where pulmonary function tests and airway biopsies determine a near-binary decision on the need for alteration of immunosuppression (4). The rational way forward to address the issue of long-term survival is a comprehensive, multidisciplinary and translational approach that combines image-guided biopsy, multi-modality longitudinal monitoring in lung-transplant recipients, and an established mouse model of BOS to understand this specific epithelial injury and aberrant repair. We intend to lay the framework here for such a combined approach that has a likelihood of improving clinical care in our own transplant recipients, while simultaneously determining molecular mechanisms of epithelial injury and repair related to BOS.

The histological hallmark of BOS is partial or complete occlusion of the lumina of terminal and respiratory bronchioles by fibrous tissue which leads to diminished airflow and attenuated alveolar function in a punctuate, heterogeneous pattern (Fig 1). The mechanisms that initiate and contribute to chronic rejection are not known, and there are no known biomarkers that predict the progression of BO lesions. Our lack of understanding of these mechanisms has made it difficult to successfully design immunosuppressive or other modalities that could treat or prevent the disease. BOS is likely initiated from a combination of epithelial injury and many allo-immune-dependent and independent factors.

We have recently established the first mouse model transplantation and BOS, which is a left-lung allograft transplant. After transplantation we can conditionally deplete all bronchiolar Clara cells of the graft, which is similar to an epithelial injury in humans that can be caused by influenza, smoking and other environmental stresses. While a normal lung (in a healthy, non-transplant patient/mouse) completely repairs within 1-2 weeks, the transplanted lung graft develops BO lesions and advances to chronic graft rejection. With the mouse model we are now able to specifically induce BO and follow disease progression by imaging, histopathology, and immune-phenotyping via flow cytometry and molecular analysis for protein and gene expression. This will allow rapid determination of cellular and molecular mechanisms that lead to BOS and facilitate identification and validation of translatable pharmaceutical targets. This thorough analysis of the mouse model will provide us with the basic understanding of BO disease progression but to translate into human models, we will work to validate with the biopsies from our patient cohort.

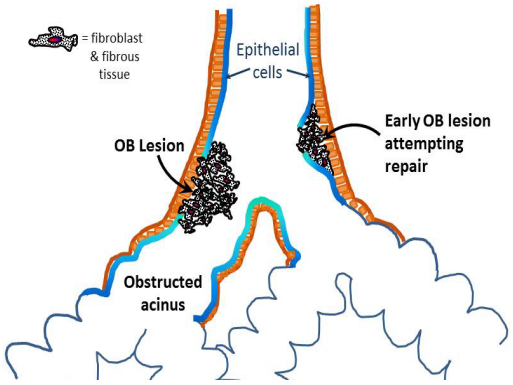

Figure 1: Representation of the initial progression of BOS at the last conducting and first respiratory airways (at the so-called "diffusion front"). Pathological findings indicate that fibrous tissue exists in both the terminal conducting airways and in the upper acinus in early BOS. It is believed that epithelial injury and its aberrant repair leads to myofibroblast infiltration, and fibrous tissue expands throughout the lower conducting and upper respiratory airways, causing restriction, obstruction, or both. ^{129}Xe MRI can detect these changes regionally, both by images of ventilation (obstruction) and restricted diffusion.

We have developed MR-imaging biomarkers that will allow targeted biopsy and longitudinal monitoring without ionizing radiation. Hyperpolarized ^{129}Xe Magnetic Resonance Imaging (HP ^{129}Xe MRI) can enable non-invasive, high-resolution imaging of lung structure and function. We have recently shown HP ^{129}Xe MRI to visualize pulmonary ventilation with high resolution, as well as the ability to show abnormalities of the alveolar microstructure (5-7). We have also demonstrated the fundamentally new capability to directly visualize the uptake of ^{129}Xe into the pulmonary capillary blood and tissues (8-10), which can provide an even more complete picture of pulmonary function by supplying regional gas-exchange information with or without exercise. New ^{129}Xe MRI techniques have potential for early BOS detection, with broad applicability across the spectrum of pulmonary disease. These techniques will also be used to easily follow regional progression in individual transplant recipients, in parallel with standard pulmonary function and blood tests, among others.

The purposes of this study are:

- Aim 1: To use HP ^{129}Xe and ^{1}H MRI to assess regional ventilation defects in post-transplant for BOS patients, during routine clinical visits near 6 months and 12 months and when BOS is suspected post-transplantation. This will help to guide the collection of relevant clinical biopsy samples for clinical decision-making purposes.
- Aim 2: To use imaging from Aim 1 to guide the selection of an additional 1-3 biopsy specimens, where applicable at the discretion of the clinician, per patient-visit during routine clinical biopsies post transplantation near 6 months, 12 months, and at any subsequent clinical visit when BOS is being screened for. Care will be taken to attempt that at least one specimen is in a high-likelihood BOS lesion (ventilation defect) and at least one specimen is from a putatively normal region. We will acquire specimens at time of transplant and through image guided biopsy from scheduled clinical bronchoscopy at 6 months, 12 months, and at any additional time points if BOS is suspected. Specimens collected for research purposes will be added to an existing tissue repository for future research.

3. BACKGROUND:

The twenty-third report by the registry of the International Society for Heart and Lung Transplantation reported survival rates of 49% and 25% 5 and 10 years after lung transplantation, respectively, which underscores the low long-term survival after lung transplantation (1). Chronic allograft rejection in the form of bronchiolitis obliterans represents the most formidable challenge in this field. The time between transplantation and onset of bronchiolitis obliterans syndrome can be as small as a few months; the probability is increased in patients with primary-graft-dysfunction scores (2). The histological hallmark of chronic lung allograft rejection is partial or complete occlusion of the lumina of terminal and respiratory bronchioles by inflammatory and fibrous tissue (Figure 1). It is generally thought that injury to airway epithelial cells and subepithelial structures with subsequent aberrant tissue repair ultimately leads to excessive fibroproliferation (3). Risk factors for the development of bronchiolitis obliterans following lung transplantation can be categorized into alloimmune-dependent (immune-mediated pathways such as rejection and autoimmune-like mechanisms), and alloimmune-independent factors. There is strong evidence for a relationship between the frequency and severity of acute rejection episodes and the subsequent development of chronic rejection (2). Alloimmune-independent risk factors include ischemia-reperfusion injury, bacterial and viral respiratory infections and gastroesophageal reflux (4). These alloimmune-independent risk factors are inflammatory stimuli that may damage the airway epithelium directly or alter the milieu in the lung allograft to increase antigenicity and thereby indirectly influence the alloimmune response. Therapeutic options for established bronchiolitis obliterans are limited as this condition is generally not reversible. Medical therapy such as augmentation of immunosuppression fails to prevent the progression of fibrotic airway obliteration in the majority of these patients and re-transplantation remains the only option.

Since BOS is often heterogeneously distributed in the lung, an imaging modality which is sensitive to ventilation defects due to decreased airway diameters in the terminal and respiratory bronchioles (Figure 1) will be particularly useful for localization of BOS lesions (11). Hyperpolarized gas MRI (^{3}He or ^{129}Xe) is currently the only technique that provides the ability to monitor real-time regional ventilation at high resolution (Figures 2 and 3) (12). Further, maps of restricted ^{129}Xe diffusion provide quantitation of alveolar duct size and geometry, which are presumably some of the first areas affected by BOS (11, 13-15). Methods developed by our research consortium have been shown recently to accurately measure the average major and minor diameters of acinar airways, allowing calculations (via imaging alone) of alveolar size and surface area, mean linear intercept, and total alveolar number (14).

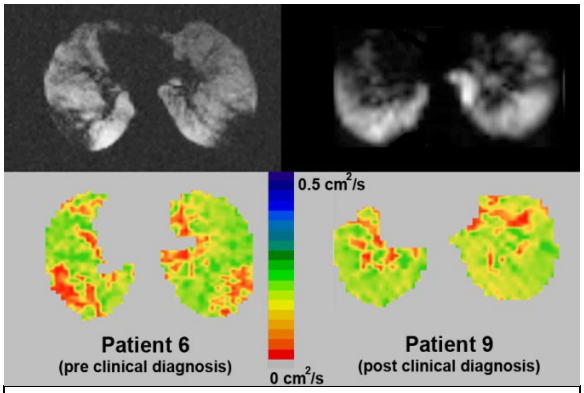

Figure 2: ^{3}He ventilation (top) and restricted diffusion (bottom, color) of two pediatric post-transplant patients. Patient 6 has focal ventilation defects (dark areas) and reduced diffusion (restricted airspaces, red on the color maps) 10 months *before* clinical BOS diagnosis, similar to Patient 9 after clinically-diagnosed "stable" BOS. These same techniques are available with ^{129}Xe gas.

MRI with hyperpolarized gases, however, requires specialized laser polarization equipment and customized MRI techniques and analyses. The PI and colleagues in Radiology are pioneers in the field of hyperpolarized-gas MRI in nuclear-spin polarization, technique development, and translation of imaging to clinical relevance; indeed over the past 3 years, the PI and colleagues have established ^{3}He and ^{129}Xe MRI capabilities at CCHMC with over 100 subjects (pediatric and adult) imaged to-date. We have developed myriad methods of obtaining restricted diffusion-weighted images that inform on acinar structure (13, 16-20). These techniques have been shown to be highly sensitive to early pulmonary disease (14).

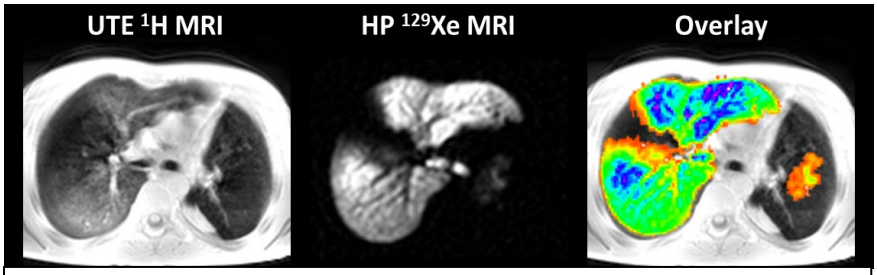

Figure 3: UTE ^{1}H and ^{129}Xe MR images in a 12 y.o. patient with BOS. There is a near absence of ventilation in the left lung and ventilation deficits in the right lung, which spatially correspond to regions of lower signal (trapped gas) in the UTE images, supporting these techniques as biomarkers to assess regional structure-function in BOS.

To date, studying immunological responses to lung transplantation has relied heavily on vascularized lung transplant models in large animals or rats (21-23). While these models have provided valuable insight into possible genetic targets that lead to the development of BOS lesions, most of these data have yet to be validated in human lung allograft recipients. The reasons for this disparity arise from the difficulty in finding early BOS lesions with conventional techniques in human lung allograft recipients and the fact that expression patterns of genes change over time. By identifying ventilation defects that precede the development of lesions in human allograft recipients, we will attempt to localize, capture and assess differential gene patterns that precede disease progression.

4. STUDY DESIGN:
This is a prospective, non-randomized, longitudinal, observational study to complete the following aims:

Aim 1: To use HP ^{129}Xe and ^{1}H MRI MRI to assess regional ventilation defects in post-transplant for BOS patients, during routine clinical visits near 6 months and 12 months and when BOS is suspected post-transplantation. This will help to guide the collection of relevant clinical biopsy samples for clinical decision-making purposes.

Aim 2: To use imaging from Aim 1 to guide the selection of an additional 3 biopsy specimens, where applicable at the discretion of the clinician, per patient-visit during routine clinical biopsies post transplantation near 6 months, 12 months, and at any subsequent visit when BOS is suspected. Care will be taken to ensure that at least one specimen is in a high-likelihood BOS lesion (ventilation defect) and at least one specimen is from a putatively normal region. We will acquire specimens at time of transplant and through image guided biopsy from scheduled clinical bronchoscopy at 6 months, 12 months, and at any additional time points if BOS is suspected. Specimens collected for research purposes will be added to an existing tissue repository for future research, maintained by the Heart Institute (and Dr Schecter).

Dr. Schecter and his team routinely acquire 5-10 biopsies at each post-transplant bronchoscopy. Specimens will be shared with the tissue repository as the volume of specimens allow (if the clinician finds that additional samples beyond what is needed for clinical decision-making may safely be obtained for research purposes). The primary use of the biopsy material will be for clinical decision making purposes. However, if for example, 6 biopsy samples are taken and sufficient for clinical use, the clinician may utilize judgement in the collection of up to 3 additional samples for research purposes and still be within the routine acquisition of up to 10 biopsy samples.

At a minimum the tissue repository will acquire research **specimens taken at time of transplant and** from scheduled **clinical bronchoscopy with image guided specimens at 6 months, 12 months, and at any additional time points if BOS is suspected.** Based on conservative numbers, we anticipate a total of around 15-18 biopsy research specimens per year/ per patient may be collected. Additionally, we will enroll an unknown number of subjects (approximately 15-20) who are post lung transplant within the past 10 years and may transfer to CCHMC Lung Transplant Center to be followed clinically. We will collect specimens at the first clinical bronchoscopy after they enroll in the study (study baseline) **and at any additional time points if BOS is being screened for.** We feel this allows a reasonable probability of several early BO lesions to be identified. Research biopsies will be processed for frozen sections and stained for histopathological assessment and validation of molecular targets identified in our related mouse model of transplantation. DNA will be processed and stored for current and future lung transplant studies.

5. DURATION:
We expect to be able to consent and scan 45 patients in the study over approximately 5 years. We will continue to monitor outcomes data for the enrolled subjects for 10 years post-transplant or until close of study.

6. SELECTION AND RECRUITMENT OF PARTICIPANTS

A. Number of Participants

The study group will include approximately 25 subjects who are scheduled for lung transplant.

An additional unspecified number of subjects (approximately 15-20) may be enrolled who are post lung transplant within the past 10 years and are being followed clinically through CCHMC lung transplant center.

B. Inclusion Criteria:

- Lung Transplantation within the last 10 years or being assessed for possible lung transplantation.
- Male or female participants will be considered for inclusion.
- 6 years of age and up.
- Participant must be able to hold their breath for up to 16 seconds.

C. Exclusion Criteria:

- Standard MRI exclusion criteria
- Bleeding disorders
- Participant is claustrophobic or otherwise unable to tolerate the imaging
- Pregnancy or positive pregnancy test
- Symptoms of respiratory infection within the past two weeks.
- Baseline oximetry at MRI visit of less than 95% on room air or less than 95% on a previously prescribed dosage of oxygen delivered by nasal cannula.

D. Recruitment:

Eligible patients will be identified at CCHMC through Dr. Marc Schecter, Director of Lung Transplantation at CCHMC or his team.

Recruitment of participants will include initial contact made by individuals (Dr. Schecter and team) who either are known to the potential participant or their legally authorized representative, or must have access to the potential participant and representatives by virtue of providing routine care and services, unless such contact has been previously authorized. Follow-up calls after the initial contact may be made by study personnel not directly involved in the patient's care. Any recruitment materials will be submitted for IRB approval before use. Eligibility based on inclusion/exclusion criteria will be reviewed before contacting potential study participants. Eligible candidates who are interested, willing to participate and give informed consent will be enrolled. After potential study subjects are identified, the study team will discuss with them the study and provide a copy of the consent form to interested subjects.

E. Vulnerable Populations:

For subjects under 18 years of age, additional efforts will be made to protect this vulnerable population. Assent will be obtained after age appropriate explanation of the study. The child is informed that participation in research (clinical trials) is voluntary and that they may decline or withdraw at any time for any reason independent of their parent/guardian. Assent will be reaffirmed before study procedure is performed. They will be informed that participation in the research (trial) will not affect their clinical care.

7. PLAN FOR OBTAINING CONSENT:
We are asking for a waiver of documented consent for screening that will include review of the MRI Safety Review Form, medical history information and inclusion/exclusion criteria with review of medical record and or patient provided information. We are requesting a waiver of documentation of consent for screening only because the questions that will be asked do not require documentation outside of research and the risk to subject confidentiality are minimal. We are requesting waiver of HIPAA authorization because this requirement would make it impossible to do the screening component of the study and add inconvenience to the participant and the family. Also, the risks to subject confidentiality for the HIPAA waiver are minimal.

For participants (parents) who express interest, the participants or if the participants is a child, his or her parent will be given the consent for the study, and it will be reviewed and time given for questions. When possible, the consent will be sent in advance of screening visit by mail or e-mail to allow additional time for review. All prospective subjects will have the study explained by a member of the research team in language that is understandable to them. The nature of the tests and procedures to be done will also be explained along with the potential hazards, possible adverse reactions, and financial costs. It will be clearly stated that participation is voluntary and that refusal to participate will involve no penalty or loss of benefits to which they are otherwise entitled. The participants and parent will sign the assent or consent after all questions are answered and a signed copy of the consent form will be given to the participants to keep. Consent will allow for multiple visits for capture of longitudinal data. No study related procedures will be done prior to consent being signed.

Non-English speaking participants will be consented using a short form consent process as per CCHMC SOP 41-1.8. The approved long consent form will serve as the summary of research.

Age of assent will be determined by the IRB, and those assenting will sign after all questions are answered. The study staff **will obtain assent for children when the child reaches age of assent** or at the next visit for those children previously enrolled in the study by parental permission only. Subjects who become adults during the research will be re-consented after they become 18 years of age.

8. STUDY PROCEDURES:
For the first year post-lung transplant, the clinical care plan includes post-operative visits at 2 weeks, 5-6 weeks, 3 months, 6 months, 9 months, and 1 year. These visits are considered as standard-of-care visits. Additionally as standard of care, surveillance trans-bronchial biopsies (TBB) are performed at 2 weeks, 6 weeks, 3 months, 6 months, 9months, 12 months, 18 months, and 24 months post-transplant. During the clinical consent process, patients/families are told that between 5-10 TBBs are taken. These biopsies are taken to assess clinical status and to provide information that may indicate signs of rejection after transplant. Clinical determination of rejection requires at least 4-6 TBBs. Since standard of care is to obtain up to 10 TBBs, additional samples above those required to determine possible lung rejection may be obtained/retained for research and still be within a routine amount of biopsy sampling.

Research imaging visits will be scheduled around the clinically scheduled visits at Baseline, 6 months post-transplant, and 12 months post-transplant. **An open number of additional research visits** will be scheduled in conjunction with any clinic visit if treating transplant physician suspects BOS and schedules a clinical bronchoscopy. Once BOS is diagnosed, research visits will continue to occur in conjunction with scheduled clinical bronchoscopies. It is not expected for

each clinical visit post diagnosis of BOS to include a research visit. Research visits will be decided by consultation and agreement between PI and treating transplant physician. All research visits will follow the same routine as below except Baseline Visit (Visit 1). For Visit 1, biopsies will be obtained during the transplant surgery, not during bronchoscopy. Remnant lung tissue from explanted lungs will also be collected at this time.

Time and Events for Aim 1 and 2:

Planned clinical visit schedule	Screening	**Research Visit 1 Pre-transplant[2]**	Transplant	2 weeks	5-6 weeks	3 months	**Research Visit 2** 6 months	9 months	**Research Visit 3 12 months[2]**	Visits up to 10 years post – transplant
Review of medical record and MRI safety sheet[6]	X	X		X	X	X	X	X	X	X[1]
Informed Consent		X								
Review of medical history and medication		X					X		X	X[1]
Height, weight, vital signs[5]		X					X		X	X[1]
Physical assessment		X		X	X	X	X	X	X	X[1]
Urine pregnancy test[4]		X[4]					X[4]		X[4]	X[1,4]
Spirometry (as tolerated and if none conducted within 6 months)		X					X		X	X[1]
Pulse oximetry		X					X		X	
Research MRI		X					X		X	X[1]
Collection of remnant explanted lung tissues			X							
Biopsies at Transplant Surgery			X							
Image guided bronchial biopsies at clinical bronchoscopy				X[2]	X[2]	X[2]	X	X[2]	X	X[1]
Safety assessment: Day of MRI, 1 day (+3), and 7 days (+3 to -1) after MRI[3]		X		X[3]	X[3]	X[3]	X	X[3]	X	X[1]

[1] Additional research imaging visits may be scheduled around any clinical visit, up to 10 years post-transplant where BOS is suspected.

[2] Image guided bronchial biopsies at clinical bronchoscopy as above and performed at 18 and 24 months; may also be performed if BOS is being screened for/suspected.

[3] Follow up can be conducted in person or by phone. Day 1 follow up should be performed prior to the clinical bronchoscope if feasible. Follow up will also be performed for additional visits when BOS is being screened for/suspected.

[4] Urine pregnancy test will be performed prior to any 129Xe MRI; urine pregnancy test will not be conducted on males, pre-pubescent girls or post-menopausal women or women who have undergone a hysterectomy.

[5] Vital signs include heart rate, respiratory rate and blood pressure. Clinical data may be used if collected on the same day.

[6] Medical record review may include demographics, diagnoses, lung tests, radiologic reports, imaging data, status of lung transplant

Imaging will occur before the clinically-scheduled bronchoscopy. The scan will take approximately 60 minutes. Anatomical lung MRI (^{1}H MRI) will be performed with coached spontaneous breathing with the intention of obtaining images at pulmonary FRC and FRC plus a tidal volume. This may include relaxed breaths and full inhalations with brief breath holds, as well as navigator-triggered free-breathing acquisitions.

^{129}Xe MR images will be acquired during a coached breath-hold maneuver (maximum duration 16 seconds) of hyperpolarized Xe gas. Pediatric subjects will be imaged with a Xe dose no greater than 1/6 of their predicted total lung capacity per ATS plethysmography-based estimates which uses subject demographics to predict TLC (24). The Xe dose for adult subjects will be no greater than 1 L. During the MRI, subjects will have routine monitoring pulse-oximetry with a licensed medical professional (e.g., MD, DO, NP, APN, RRT, or RN) present during the scan. In addition to an initial 1L or less Xe dose for calibrating the MR scanner, up to 4 doses of Xe gas may be administered during an imaging session. Image analysis will be performed after the MRI; a report on regional ventilation and corresponding ^{129}Xe diffusivity (both at the level of lung segments) will be prepared within following imaging and prior to the clinical bronchoscopy. All ventilation defects will be noted; and the PI will ensure that the clinical bronchoscopist and treating transplant physician receives the report before the clinical bronchoscopy.

The lung lavages and biopsy specimens are performed as a part of routine clinical care. Where research biopsy samples are deemed safe and appropriate by the clinical staff, the target areas for the 1-3 research biopsy specimens will be any two lung segments with the worst ^{129}Xe MRI ventilation defects (as determined by image as reduced diffusivity) and a segment with uniform ^{129}Xe ventilation and normal diffusivity. Since our regional specificity for biopsies will be at the level of lung segments, we anticipate no significant problems obtaining the image-targeted biopsy samples. **Final decision on obtaining research biopsies will be made by the clinical bronchoscopist and treating transplant physician at the time of the bronchoscopy based on patient safety and their clinical judgment.** If image guided biopsies cannot be obtained on a research visit for safety reasons, the subject will be continued through the study with image guided biopsies at future visits as clinical condition allows.

The Pulmonary Division and Heart Institute will be responsible for ensuring that specimens are collected, registered and stored properly.

Standardized Hyperpolarized ^{129}Xe MRI Procedure
Subject will be supine in imaging chamber with ear protection.

Prior to the MR scan, subjects will practice the inhalation and breath-hold maneuver with the Tedlar or polyethylene (PE) bag using room air.

Subject will be supine in imaging chamber with ear protection and oximetry monitoring. There will be one bag with a breathing tube and mouthpiece near the subject's head. This bag will contain hyperpolarized ^{129}Xe gas mixture (Xe gas may be dilute with nitrogen).

Total time in the MR scanner will be about 60 minutes.

- Once the subject has been placed and is acclimated to the scanner, baseline vitals including SpO_2 and heart rate will be collected. These vitals will be used for safety monitoring throughout the scans involving xenon gas administration, and monitoring for safety parameters.

- The MR portion of the procedure will include anatomic proton (^{1}H) scans (no gas inhalation).
- After anatomic MR scans, a calibration scan will be performed with a small amount of xenon. The participant will be instructed to exhale a tidal volume (down to functional residual capacity, FRC) and then inhale ^{129}Xe and hold breath for up to 16 seconds. The subject will be instructed to exhale the breath at the end of the scan sequence and take deep breaths of room air, talk, etc.
- For the Xenon MRI scan, the subject will be instructed to exhale a tidal volume (down to functional residual capacity, FRC) and then inhale the ^{129}Xe and hold breath for up to 16 seconds. Subject will be instructed to exhale directly into room air after imaging and to take deep breaths of room air, talk, etc.

SpO_2 will be monitored before, during and through 2 minutes after ^{129}Xe maneuver. Lowest SpO_2 and time to recovery to pre-maneuver and/or baseline SpO_2 will be recorded. If the subject's blood oxygen saturation and heart rate do not return to within 5% of the established baseline SpO_2 and the heart rate to within 20 bpm of baseline within 2 minutes, the medical professional will continue to encourage the subject to breathe/talk and try to get SpO2 and heart rate back to acceptable levels on their own. If the subject's blood oxygen saturation drops greater than 10% from their reclining baseline value or their heart rate rises by 40 bpm, oxygen may be administered to assist the subject in returning to the acceptable SpO2 and heart rate levels. If the subject returns to acceptable SpO_2 and HR values, they may be given the decision to continue to the next inhalation. Prior to additional doses, if applicable, the subject will breathe normal room air for at least 2 minutes.

Afterwards, the patient will exhale fully and breathe normal air before repeating this procedure. Subjects may be given more than one dose but each dose will be separated by at least 2 minutes of breathing room air. In addition to small MR-scanner calibration dose, a maximum of 4 ^{129}Xe doses will be given.

About ^{129}Xe MRI

The PI and colleagues in Radiology have been pioneers in the field of hyperpolarized-gas MRI—in nuclear-spin polarization, technique development, and translation of imaging to clinical relevance and developing myriad methods of obtaining restricted diffusion-weighted images that inform on acinar structure (13, 16-20). These techniques have been shown to be highly sensitive to early pulmonary disease (14)

MR imaging will be directed by Dr. Woods, with medical oversight for review of adverse events and clinical interpretations of unexpected MR findings from a physician within the Pulmonary Division. Dr. Woods has extensive experience acquiring and analyzing hyperpolarized-gas images. Images of gaseous ^{129}Xe will be acquired during separate breath holds: typically using high-resolution FLASH (for ventilation) or multi-b-value diffusion-weighted MR (for measurement of acinar-airspace narrowing), both via fast-gradient echoes. Both sequences are in place and have been utilized in studies conducted at CCHMC (5, 6). Ventilation and dissolved ^{129}Xe uptake may also be evaluated using other MRI sequences such as SPIRAL or UTE. Spectroscopy scans may also be used. However, in no instance will the breath-hold period exceed 16 seconds.

Image analysis will be performed after MRI including a report on regional ventilation, corresponding ^{129}Xe diffusivity, or ^{129}Xe exchange as appropriate for the type of imaging performed.

Image analysis will be performed after MRI including a report on regional ventilation and corresponding ^{129}Xe diffusivity (both at the level of lung segments).

Process for hyperpolarization of ^{129}Xe

Hyperpolarized ^{129}Xe will be produced, by trained staff, using research polarizers housed at Cincinnati Children's Hospital Medical Center. Each dose will be produced with its own unique batch record. The dose of xenon for imaging will consist of no more than 1 liter of either natural-abundance xenon (26% ^{129}Xe isotope), or isotopically-enriched xenon (>26% ^{129}Xe), with medical grade UHP N_2 added as needed to adjust the total dose volume. Xenon will be diluted *in situ* within the dose bag prior to inhalation. This will be accomplished by delivering the xenon and the diluent gas directly to the bag through a gas-handling manifold made of a suitable, non-relaxing material such as aluminum or inert polymer. The exact xenon percentage in the mixture will depend on the technical details of the studies. In high resolution ventilation imaging, it may be necessary to have subjects inhale 100% xenon for the individual short breath hold. In spectroscopy, where the signal intensity demands are more modest, a more dilute mixture may be delivered to reduce the risks of paresthetic effects. In general, we will use the minimum xenon percentage need to achieve the study goals, and we anticipate that the xenon percentages will range from 100% to 10%. The decision to release an HP ^{129}Xe dose for administration will be made based on its measured polarization, and which is obtained by a calibrated measurement station in conjunction with the polarizer.

Hyperpolarized ^{129}Xe will be dispensed in preparation for each MRI based on the dose (xenon volume) required for that subject and study, and each dose will consist of up to 1L of xenon for adults or a fraction of that for children, based on predicted Total Lung Capacity calculations. Only one dose of gas will be transported to the MRI chamber at a time. This is to ensure that a magnetic environment is maintained and the polarization of the dose is maintained. Additionally, this spatial control of the dose serves to mitigate multiple doses being administered without adequate monitoring and room air in between. The total gas dose volume (xenon and any diluent gas) will be delivered in a single breath, and the timing of inhalation will be controlled by the administrator of the gas. This will ensure that the subject will not inhale more than one breath from the gas bag and will not inhale additional gas until monitoring has taken place. If an additional dose is required to continue the study, it will be placed and delivered by the administrator per the protocol assessment requirements. After the dose is exhaled into the room, ambient air will immediately dilute the xenon concentration to levels well below the paresthetic threshold.

At present, ^{129}Xe has been classified by the FDA as a drug but has not been given FDA approval despite the long record of safety of xenon in medical use.

About the MRI: Coil safety data: Custom MRI coils built in-house by the CCHMC Imaging Research Center (IRC) Engineering Lab will be used for MR imaging.

Specific Absorption Rate (SAR): The radio frequency (RF) field is an oscillating electromagnetic field, pulses of which are used to generate the MR signal. The body absorbs a portion of the transmitted RF energy that may result in tissue heating. SAR is a measure of the amount of RF power absorbed per unit of mass of tissue. It serves as an indicator of heating potential and is the dosimetric means used to report the estimated amount of heat dose received by the patient. This value is expressed as watts of power per kilogram of the patient's body weight (W/kg) and is routinely estimated by Philips MRI operating system software. The SAR algorithms for the MR systems calculate SAR values for each MR acquisition and prevent the system from operating above FDA mandated guidelines.

9. DATA ANALYSIS METHODS:

Biopsy specimens: 50% of total surviving patients are diagnosed with BOS 5 years post-transplant. Assuming 6 lung transplants per year during the enrollment period of this study, we expect 5-6 patients to be in our study cohort during the first year. With 1-3 biopsy specimens per patient per time point, we anticipate a total of useable 15-18 biopsy specimens at the conclusion of the study, which allows a reasonable probability of several early OB lesions to be identified, providing good preliminary data for our future R01 proposal in 2 years. Biopsies will be processed for frozen sections and stained for histopathological assessment and validation of molecular targets identified in our related mouse model of transplantation.

One analytic focus of this project is to evaluate the accuracy of using MRI imaging to detect the presence of BOS under the assumption that the biopsy yields the gold standard determination. Accuracy measures including sensitivity (sen), specificity (spc), positive predictive value (PPV), and negative predictive value (NPV) will be estimated at the lung segment level based on the sample collected at each research visit time post lung transplantation as well as the total sample collected during the study. Appropriate correction for the correlation within a patient will be applied when estimating the variance of the accuracy measures. In addition, for those patients with at least one BOS lesion determined by biopsy during the study period (i.e. BOS patients), the number of apparent MRI-false positives will be summarized at each time point to show if MRI can detect BOS earlier than biopsy.

Sample size consideration: The goal of this study is to collect biopsy samples from three locations (2 at ^{129}Xe MRI-detected segments with ventilation defect and 1 at a segment with normal ventilation) in the lung at each of three time points in about 15 patients for a total of 135 samples. The sample size justification is based on the biopsy samples collected at 12 months post lung transplantation and the precision with which we can estimate the expected positive predictive value (the proportion of true BOS determined by biopsy among those detected by MRI based on ventilation defect). Assuming 5 lung transplant patients are enrolled per year in the study, we will have 15 patients enrolled during the three years enrollment. Considering the reported 83% 1-year survival rate for pediatric lung transplant patients, we will have 12 patients surviving with 36 (=12x3) biopsy samples collected at 12 months (24 MRI detected possible BOS lesions, 12 normal). With 24 samples with MRI detected possible BOS lesions, the 95% confidence interval of the PPV will extend 19% from the observed value for an expected PPV of 65%. In the case of low enrollment, for example only 2 patients enrolled per year, we will have 6 patients within three years enrollment. With the same assumption of 83% 1-year survival rate, 5 patients will remain in the study with 15 MRI guided biopsy samples (10 MRI detected BOS lesions, 5 normal) collected at 12 months post-transplant. With 10 samples with MRI detected possible BOS lesions, the 95% confidence interval of the PPV will extend 30% from the observed value for an expected PPV of 65%.

10. FACILITIES and PERFORMANCE SITES:

Study Location: Cincinnati Children's Hospital Medical Center (CCHMC) Imaging Research Center (IRC)
CCHMC Department of Radiology
CCHMC Surgical Suites
CCHMC Heart Institute Bio-Repository, R Basement

11. POTENTIAL BENEFITS:
We do not anticipate any direct benefit to any participant. However, it is possible that we may discover unanticipated findings that are clinically relevant. The treatment of these unanticipated findings could result in improved outcomes. Future lung transplant patients and the scientific community may benefit from the knowledge obtained from this study.

12. POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS:
Other than lack of oxygen during the brief period (10-16 seconds) the participant is breathing the ^{129}Xe MRI, we know of no serious risks related to ^{129}Xe. To mitigate the risks associated with the ^{129}Xe, administration of the gas will be done with a licensed medical professional (MD, DO, NP, APN, PA, RRT, RN) present and the subject will be monitored throughout the visit until all scans involving study drug are complete and the subject's vital signs and SPO_2 have returned to adequate values per protocol. Additionally, the administrator of the gas will control the delivery of the single dose of gas and the timing of inhalation. Only one dose will be available in the MRI suite at any time as the gas is stored in the polarizer to maintain adequate polarization for optimal imaging, thus the volume of gas available to the subject will be controlled. Furthermore, using minimal ^{129}Xe percentages per experimental goals will mitigate additional risk.

Risks

^{129}Xe :

Common:

- Mild paresthesia which includes
  - Light headedness
  - Dizziness
  - Tingling
- Euphoria

We expect the duration of Xe effects to be short in duration; typically about 3 minutes after exhalation is expected

Breath-Hold:

Common:

- Decline in SPO_2 due to the administering of an anoxic gas mixture, with usual return to baseline values within the next minute after exhalation

Uncommon

- Heart rate change

MRI:

Common:

- Discomfort from being confined in the small space of the MRI. Comfort measures will be used as appropriate. They may include watching movies or swaddling.
- Loud noise. Ear plugs and head phones will be used to protect hearing.

Uncommon:

- Panic (claustrophobia). If this happens, we will stop the test. Subject will be able to stop the test at any time.

Rare:

- Injury from ferrous metal inside the magnet room. All subjects are screened to prevent any injury from metal and the MRI magnet.

Pulse Oximetry:

Common:

- Temporary pressure marks from monitor probe

Transbronchial Biopsy:

All bronchoscopies are being done for clinical purposes. We feel the incremental risk from 1-3 additional biopsies is small. The final decision on patient safety for obtaining the biopsies will be decided by the clinical judgment of the bronchoscopist and treating transplant physician on the day of the bronchoscopy.

Common

- Minor bleeding within the airways and lungs that will be controlled with local bronchoscopic measures,
- Mild hemoptysis during the 24 hours after the procedure; temporary hoarseness and sore throat.

Rare:

- There is a 1% risk of a pneumothorax (collapsed lung) with chest tube placement necessary to re-expand the lung in many of those instances.
- Hypoxemia (decreased oxygenation), infection, and respiratory and/or cardiovascular instability leading to death

Pregnancy:

There is no known risk from MRI or xenon to pregnant women or fetuses but for additional safety, we will exclude pregnant women from this research study. Clinical testing as part of pre-surgical preparation may be used to confirm non-pregnancy at visit 1 if available. Urine pregnancy tests will be performed prior to MRI scans and negative results confirmed prior to MRIs.

13. RISK/BENEFIT ANALYSIS:

Although this study involves slightly greater than minimal risk there may be a direct benefit to the study subject. The treating physician will receive through high resolution MR images that may help in the early diagnosis of BOS. Additionally, this study may help in the development of improved outcomes for lung transplant patients from advancing our understanding of the biochemical mechanisms that occur after lung transplantation and their imaging correlates.

14. DATA SAFETY & MONITORING:

No Data Safety Monitoring Board (DSMB) will be created for this study. The Data Safety Monitoring Plan (DSMP) will be through oversight of the PI in reporting all adverse events. Medical management and decision for unexpectedness, relatedness and severity of adverse events due to bronchial biopsies will be determined by the treating transplant physician.

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with

International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by a monitor independent of the study team.

- Initial monitoring visits will focus on review of the consenting process, eligibility criteria, and investigator/staff training and qualifications. On-going visits will occur at least annually; additional monitoring may occur at the discretion of the sponsor. On-going visits will include targeted verification of safety and efficacy endpoint data of a percentage of enrolled subjects. The percentage will be determined based on enrollment. Additional monitoring activities will take place when needed to supplement the scheduled monitoring visits and ensure follow-up on any outstanding issues noted at scheduled visits. If systemic issues are identified during monitoring visits (i.e., consent process, ineligible participants, regulatory violations, missing data, etc.) re-training of the site staff will be suggested and the need for additional review will be assessed. A close out visit will be conducted once all participants have completed, withdrawn or been declared lost to follow-up and all data queries have been resolved. Any outstanding issues from previous monitoring reports will be reconciled.

- The sponsor will be provided copies of monitoring reports after allowing sites sufficient time to respond/resolve discrepancies from the list of findings provided to the site immediately following the review. The monitoring reports will include a visit summary, any issues for follow-up, and any pertinent conversations with study staff surrounding monitoring activities.

- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

15. ASSESSMENT OF SAFETY

The participants will be monitored before, immediately following and after each inhalation of ^{129}Xe, per study procedures above, to assess for adverse events and changes in vital signs.

Parameters monitored may also include the following:

- Participant assessment of anesthetic/analgesic effects, heart rate, and SPO_2. The participant's sense of analgesia is assessed by in inquiring about how the participant feels after administration of the xenon dose. The participant will be asked to describe how they feel as well as well as about symptoms such as: dizziness, light-headedness, numbness, euphoria, and tingling.
- Heart rate and SpO2 will be measured while the subject is supine in the imaging chamber prior to any imaging to establish a baseline by which to compare subsequent measurements after inhalation of the Xenon gas. SpO_2 is monitored by percentage based on absolute percent change (ie, 95%-90% is a 5% drop in value).
- Heart rate will also be measured immediately following each inhalation and through a minimum of two minutes after the inhalation. Changes in heart rate of with increases by 40 bpm are considered significant. If the participant is to receive another dose, the next

dose will not be administered until the heart rate is within 20bpm of its baseline monitoring value. If the participant has received their last dose, they will be observed for 2 minutes post-inhalation or until their heart rate has returned to within 20 bpm of the base line value.

- In addition to baseline, SPO_2 is also measured immediately following and through 2 minutes of each xenon dose. A decrease of SPO_2 by greater than 5% is considered significant. If the participant is to receive another dose, the next dose will not be administered until the SPO_2 is within 5% of its baseline monitoring value. If the participant has received their last dose, they will be observed for 2 minutes post-inhalation or until the SPO_2 is within 5% of its baseline value or until the end of the observation period, whichever is longer.
- The participant will be monitored for the duration of the xenon treatment and post procedural period by a trained medical professional. Additional assessments of adverse events will be conducted by study staff on Day 1, preferably prior to the clinical bronchoscopy, but if not feasible, a +3 day window is allowed. Follow up will also occur on Day 7 (+3/-1).
- Data for each subject collected during the study, including medical history, physical assessment results, data during the MRI, any protocol deviations as well as Adverse Events and Serious Adverse Events will be reviewed by delegated medical study staff and will also be reviewed by the study PI at the end of the subject's follow up, at a minimum, for acknowledgement and awareness of study data and subject safety. Serious Adverse events related to the study or study drug will be reported to the PI within 24 hours of learning of the event.

Adverse Events Defined

Adverse events for this study will be defined as any change from baseline that occurs from admission to IRC to follow-up phone call after each study visit from enrollment until completion or withdrawal from study.

1. Unexpected versus Expected Adverse Events
   An *unexpected* adverse event is one that has not been listed previously in the protocol, consent form, or Investigator's Brochure and is not associated with known risks of bronchial biopsy or the subject's underlying disease.

   Expected events as outlined in study documents will not be recorded as adverse events unless they become moderate and require medical intervention or discontinuation from the study.

2. Relatedness (Attributions)

Attribution	Description
Unrelated	The event is unrelated.
Unlikely	The event is unlikely related.
Possibly Related	The event or severity of event is not usually associated; there is no strong evidence to link the event.

Probably Related	The event or severity of event is such that it can likely be correlated.
Definitely Related	There is a strong correlation.

3. Severity Descriptors

Severity	Numerical Value	Description
Mild	1	Aware of sign, symptom, or event, but easily tolerated; does not interfere with daily routine
Moderate	2	Discomfort enough to interfere with daily routine and may require some therapeutic intervention
Severe	3	Incapacitating, significantly affects clinical status; requires therapeutic intervention
Life Threatening	4	Life-threatening; immediate intervention required
Death	5	Adverse event causes death.

Adverse Event Reporting

All adverse events that meet criteria for expedited reporting to the FDA will be reported per 21 CFR §312.32(c). This includes events that are determined to be at least possibly related to the use of the drug, unexpected, and serious. Serious adverse events are defined as:

- Death
- Life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly/birth defect
- Medical events that when, based upon appropriate medical judgment, may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Serious events that are fatal or life-threatening will be reported as soon as possible but in no event later than 7 calendars days after the sponsor's initial receipt of information. All other serious events shall be reported as soon as possible but in no event later than 15 calendar days after the sponsor's initial receipt of the information.

All adverse events that are unexpected and related to the study or involve an accidental release of PHI will be reported to the IRB as a Reportable Event. Since all subjects will have clinical biopsies along with additional research biopsies, it may not be possible to distinguish bronchoscopic adverse events due to research or clinical care. The PI will report to the IRB as a Reportable Event within 48 hours of being made aware the following events:

- Death within 48 hours of bronchoscopy where research biopsy samples were obtained.
- Hemorrhage requiring intervention such as transfusion, surgery, bedside cauterization within 48 hours of bronchoscopy where research biopsy samples were obtained.
- Pneumothorax within 48 hours of bronchoscopy where research biopsy samples were obtained.

Adverse events that do not meet the criteria for Reportable Events will be submitted in table form at the next continuing review.

16. PRIVACY and CONFIDENTIALITY:

All subjects enrolled in this study will be identified by a unique study number only. Personal identifiers such as name and medical record number will be removed from each case as the study subject's information is entered into a secure database. Protected health information **other than dates (date of birth and visit date)** will not be retained. A single copy of a list linking medical record number to study number will be kept locked and available only to study investigators. All records will be kept in a locked cabinet at CCHMC and maintained by the study investigator. All computerized records will be kept on the CCHMC secure network.

Case report forms will be created for this study. Study specific data will be recorded directly on to the case report forms; therefore, the case report form will serve as a source document. Electronic case report forms (eCRF) will be retained in a secure database.

In order to protect the privacy of the medical information, access to the identifying information will be limited to research personnel. No individual identifiers will be used in publications or reports resulting from the study. After the study is completed and results are published, the code linking the records to PHI will be destroyed and databases will be reviewed to confirm they are de-identified except for dates.

Future use:
Specimens will be stored in the Heart Institute Bio-Repository (IRB#: 2011-2856). Specimens will be labelled and stored with the date and study ID. The code linking the study ID to PHI will be maintained separately from the specimens. A separate anonymized database containing study data (PHI will include dates of procedures) will link with the specimen ID. Longitudinal data will be acquired from subjects for up to 10 years to follow transplant outcome or until re-transplantation or death.

Subjects and families will be given contact information with instructions on how to withdraw consent for future use.

17. COST OF PARTICIPATION:
There will be no cost to the subjects for the MRI.

18. PAYMENT FOR PARTICIPATION:
Subjects will be compensated for their participation in the study. Compensation will be provided to the participant via ClinCard.
$25.00 per MRI visit.
$25.00 per biopsy visit

19. STUDY COMPLIANCE

This study will be conducted as outlined by the protocol approved by the CCHMC IRB, and according to Good Clinical Practice standards. No changes to the protocol will be implemented without the approval by the Institutional Review Board and notification of the FDA, except if following the protocol would endanger the health and safety of the research participant. Should this occur, the protocol alteration will be reported to the IRB and submitted to our IND

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