CHARACTERIZING THE LONG-TERM CARDIOPULMONARY EFFECTS OF COVID-19 WITH HYPERPOLARIZED XENON AND CARDIAC MRI

Pro00107681

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Regulatory Sponsor:	Duke University Health System Institutional Review Board 2424 Erwin Rd Hock Plaza, Suite 405 Durham, North Carolina, 27710
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Funding Sponsor:	Translating Duke Health Cardiovascular Disease Initiative-COVID-19 Cardiovascular Disease Pilot Research Grant – Duke Health
Study Product:	Hyperpolarized Xenon (^{129}Xe)

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List of Abbreviations

6MWT	6 Minute Walk Test
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CQMP	Clinical Quality Management Program
CRF	Case Report Form
Dx	Diagnose
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
PFT	Pulmonary Function Test
PHI	Protected Health Information
SOC	Standard of Care
Xe	Xenon

Study Summary

Title	Characterizing the Long-Term Cardiopulmonary Effects of COVID-19 with Hyperpolarized Xenon and Cardiac MRI
Short Title	COVID Long Haulers
Protocol Number	Pro00107681
Phase	Phase 2
Study Duration	1 year
Study Center(s)	Single site
Objectives	The study will focus on determining whether subjects with persistent and continued dyspnea after COVID infection exhibit unique imaging signatures on Xenon and Cardiac MRI.
Number of Subjects	30 consent and 23 enrolled.
Diagnosis and Main Inclusion Criteria	Diagnosis of post Coronavirus (COVID-19) Inclusion Criteria a. Age ≥ 18-year-old b. Tested positive for SARS-CoV2 c. Willing and able to give informed consent and adhere to visit/protocol scheduled (consent must be given before any study procedures are performed) Exclusion Criteria a. Prisoners b. Pregnancy, planning pregnancy or lactating c. Conditions that prohibit MRI scanning (metal in eye, claustrophobia, inability to lie supine). d. Medical or psychological conditions which, in the opinion of the investigator, might create undue risk to the subject or interfere with the subject's ability to comply with the protocol requirements
Study Product, Dose, Route, Regimen	Hyperpolarized Xenon (^{129}Xe)
Duration of administration	Open label – Inhalation

Statistical Methodology	^{129}Xe MRI metrics will include RBC/barrier, barrier/gas, RBC/gas and several spectroscopic indices. Continuous data will be presented using the mean ± SD if normally distributed and median with 25th and 75th percentiles otherwise. We are fortunate to be able to compare the findings in our images to a database of approximately 30 healthy reference subjects whom we have scanned to date. These subjects have undergone the same ^{129}Xe MRI protocol and were recruited with no histories of smoking or cardiopulmonary disease, FEV1>80%, FEV1/FVC>0.70 and DLCO>80%. For subjects to be included in the reference cohort they further had an RBC/barrier ratio ≥0.45 (at TR=15 ms), and image SNR >5, based on the Rose criterion. Comparisons between these groups will use analysis of variance (ANOVA0 methods or Kruskal-Wallis test as appropriate.

1 Introduction

The next phase of the COVID-19 pandemic is likely to see a surge in associated chronic cardiopulmonary disease that will challenge health systems. Recovered patients are presenting with persistent dyspnea at our Duke Pulmonary Post-COVID clinic. Evidence is now mounting that recovered patients have significant residual pulmonary disease, while myocardial injury has also been increasingly reported. To optimally care for these patients, we must comprehensively assess and monitor the changes in cardiopulmonary function and relate them to physiologic and quality of life outcomes. We will deploy cutting edge MRI to fully characterize cardiopulmonary function in enrolled 23 subjects post recovery and 6-9 months later. Cardiac MRI will assess myocardial status and right ventricular function, while hyperpolarized 129Xe MRI will provide 3D assessment of pulmonary ventilation, interstitial barrier integrity and pulmonary vascular hemodynamics. The overall objective outlined in this study is to demonstrate feasibility and value of comprehensive longitudinal imaging characterization of cardiopulmonary structure and function in patients recovered from Covid-19.

1.1 Background

As patients recover from the SARS-COV-2 virus and present with significant, continued dyspnea and fatigue, there is an urgent need to better characterize their underlying lung and heart function. As the causes for dyspnea are multi-factorial, we must elucidate not only the cardiac and pulmonary causes, but monitor them longitudinally and non-invasively. We now know that the long-term sequelae within the pulmonary system includes diffuse alveolar damage in the setting of ARDS (Bradley '20) as well as significant fibrotic changes (George '20) and pulmonary vascular abnormalities (Ackermann '20). Moreover, recovered patients have been reported to harbor significant myocardial injury on cardiac MRI (CMR) (Puntmann '20). This may be due to: (1) direct myocardial damage from viral infection (Lindner '20) or the effects on the right ventricle from damage to the pulmonary vasculature caused by endothelialitis and thrombosis. Unfortunately, we currently have little understanding of these short- and long-term cardiopulmonary effects in survivors of COVID-19 infection. However, we know they are likely to be multifactorial, with changes to the pulmonary airspaces, interstitium, vasculature and myocardium. Until we can directly and longitudinally measure each of these regional effects on lung and cardiac morphology and function, we will remain blind in our efforts to understand and treat the lingering effects of COVID-19 infection. Here we provide an innovative and non-invasive means to deploy MRI to sensitively and comprehensively characterize the impact of COVID-19 on regional cardiopulmonary function. We will identify the imaging features that can guide the continued care of patients struggling with its enduring effects. We have brought together an interdisciplinary team, ranging from faculty of the Duke pulmonary post-COVID clinic to physicists and engineers who have pioneered hyperpolarized 129Xe pulmonary functional MRI and high-resolution cardiac MRI. This combination of technologies addresses the unmet need by directly, and non-invasively quantifying regional 3D ventilation, alveolar capillary gas exchange, and pulmonary vascular hemodynamics. With the addition of a full cardiac assessment, it can be used to elucidate the causes of dyspnea and monitor progression or resolution of disease non-invasively over time. Our central hypothesis is that noninvasive imaging of the pulmonary airspaces, interstitial membrane and capillary blood volume, combined with cardiac structure/function assessment, provides an unprecedented and

sensitive means to understand the physiologic evolution of COVID-19 infection, its long-term sequalae, and its treatable traits. This approach to characterizing the aftermath of SARS-COV2 infection will improve our understanding of the underlying pathology and provide fundamental insights upon which to design future interventions, such as tailoring treatments to specific patterns of cardiopulmonary impairment.

1.2 Investigational Agent

1.2.1 Hyperpolarized 129Xe

129Xe MRI also provides the unique ability to image the diffusive transfer of gas into the alveolar-capillary barrier tissue and red blood cell (RBC) compartments. Like oxygen, 129Xe must traverse the interstitial barrier to reach capillary blood cells (RBCs). Notably 129Xe in barrier tissues and RBCs exhibits a unique frequency shift, allowing it to be separately imaged in each compartment. It has demonstrated sensitivity to gas exchange impairment in pulmonary fibrosis, and pulmonary vascular disease, without ionizing radiation.

1.2.2 Study Dose

Each xenon dose will be limited to a volume less than 25% of subject lung capacity (TLC), as is the case for all protocols currently carried out under IND 109,490.

1.3 Risk and Benefits

1.3.1 Potential Risk

1. Inhalation of hyperpolarized 129Xe may carry some minor risks. Xenon is a general anesthetic when breathed continuously at concentrations greater than 70% for extended periods of time. In the proposed study, Xenon will be delivered in a single breath, with alveolar concentrations below 25%. At these concentrations, subjects may experience transient effects, including dizziness, slight tingling or numbness of the extremities, nausea, smelling of flowers, or a feeling of well-being and euphoria. These effects will wane within 1-2 minutes of exhaling the Xenon and are documented in the consent forms.
2. The risks of participating in the MRI component of the study are considered minimal. MRI is a noninvasive imaging modality that involves no ionizing radiation. At the time of recruitment/consenting and again before the MRI session, all subjects will complete a standard questionnaire to screen for contraindications to MRI imaging (e.g., presence of metal in the eye).
3. There is also the potential risk of loss of confidentiality.
4. Administering HP ^{129}Xe without oxygen - This is necessary to preserve good image quality because O2 is paramagnetic and depolarizes the HP ^{129}Xe. Therefore, ^{129}Xe is not mixed with O2 within the dose delivery bag, but only once it is inhaled. Administration of a single anoxic 1-liter breath has been well tolerated by subjects undergoing both ^{3}He MRI and ^{129}Xe MRI. After a single breath, the residual oxygen in the subject's lungs is sufficient to maintain blood O2 saturation during the breath-hold. For each subject, their blood-oxygenation will be monitored throughout the time they are in the MRI

scanner. To reduce risk, a baseline resting oxygen saturation of 90% on supplemental oxygen will be required. Furthermore, supplemental oxygen will be provided during the MRI exam, as indicated.

5. The IV contrast material that is used routinely during the cardiac MRI procedure. However, as with any foreign material in the body, whether food or medicine, there is always the risk of an allergic reaction. Allergic reactions occur uncommonly but may be severe and can result in death. Other specific risks from the contrast agent relate to leaking of the agent at the injection site and side effects or reactions to contrast agents.

1.3.2 MRI Risk

MRI uses a magnet and radio waves to make diagnostic medical images of the body. There have been no ill effects reported from exposure to the magnetism or radio waves used in this test. However, it is possible that harmful effects could be recognized in the future. A known risk is that the magnet could attract certain kinds of metal. Therefore, the technologist will carefully ask the subject about metal within the subject's body (this includes certain dyes found in tattoos). If there is any question about potentially hazardous metal within the subject's body, we will be excluding the subject from participation in this research study. We will also keep the exam room locked so that no one carrying metal objects can enter while the subject is in the scanner.

The study involves entering a large room in which a magnet is present. The subject will be placed on a narrow bed and then slid into a small tunnel approximately 6 feet in length and 25 inches in diameter. The subject will be asked to lie still for about one hour on this bed. The subject will hear a loud machine-like noise. The subject may be asked to have a harmless monitoring device applied during the study. During the study, the subject can have voice contact and physical contact with someone in attendance if desired.

1.3.3 *Standard dose CT (SDCT) Risk*

SDCT is a standard medical procedure in lung imaging, widely used clinically and in research. Risks to participants are minimal. The study will involve a small amount of radiation exposure to the subjects. There will be one SDCT scan performed during the study. The scan protocol will be adjusted based on the participant weight. The average weight adult radiation dose is 4 mSv per scan, (8mSv) for the study. This compares to the US limit on radiation workers which is 50 mSv.
Risk: The SDCT (lung scan) will be reviewed by a qualified person. There is a possibility that while reviewing the SDCT an incidental finding will be identified.
Managing risk: The patient will be informed about the incidental finding. With permission from the patient, the information about the incidental finding will be forwarded to the primary doctor or the patient can be referred to an appropriate doctor for further evaluation.
The patient will be provided the following information:

- An incidental finding may cause you to feel anxious.
- Since an incidental finding will be part of your medical record, it may affect your current or future life or health insurance coverage. This risk will vary depending on the type of insurance plan involved.

- The costs for any care that will be needed to diagnose or treat an incidental finding would not be paid for by this research study. Any additional tests or treatments will be your choice; you or your insurer will be responsible for additional costs.

1.3.4 Risk of Incidental Findings

Since the MRI methods being tested are experimental, the MRI images will not be formally reviewed for incidental findings. However, if there is something that is of concern to the PI, then the PI will approach the IRB for guidance on how to proceed on a case-by-case basis. The consent will clearly state that the MRI images will not be evaluated for incidental imaging findings.

1.3.5 Protection Against Risk:

1. Subjects will stay on their prescribed oxygen levels while participating in the study. Oxygen will be discontinued briefly for 2 preparatory breaths prior to xenon inhalation, and then re-started after that.
2. Each subject will be allowed to read the consent form and ask questions. After all, the study subject questions are answered, and before any protocol-specified procedures are initiated, each subject will sign and date the consent form. A copy of the signed consent will be provided to the subject
3. Every effort will be made to keep your information confidential; however, this cannot be guaranteed
4. Duke University has formal education and certification procedures regarding research ethics (https://irb.duhs.duke.edu/training-and-education). All research personnel will complete these in full before entry into the protocol; the study's nature and risks will be reviewed with each subjects.

2 Study Objectives

The proposed research builds on the established sensitivity of Hyperpolarized ^{129}Xe MRI to obstructive (Ebner'17 , restrictive (Rankine'20) and pulmonary vascular lung disease (Wang '19), features expected to represented in the Covid-19 cohort. It further incorporates new understand of the possible role of myocardial injury in these recovered patients by combining cutting edge pulmonary and cardiac MRI.

Although the initial presentation of patients with moderate to severe symptoms of COVID19 infections is dominated by respiratory symptoms, 10% go on to develop persistent post-infection symptoms which are thought to have an inflammatory etiology. Evidence suggests that pathologic activation of the inflammasome persists beyond the acute initial presentation that contributes to the persistent disabling symptoms characterized as "long-haul COVID". For this trial, subjects will be eligible for enrollment if they are outpatients with a history of a laboratory confirmed diagnosis of COVID-19 infection, and after 60 days or longer. We will enroll 13 subjects who continue to have respiratory symptoms (i.e., cough, shortness of breath, dyspnea on exertion). An additional 10 subjects engaged in competitive sports and diagnosed with COVID-19 will also be enrolled. These subjects can be asymptomatic or mildly to moderately. Although rare, cardiac impairment has been documented in <2% of these

individuals. To our knowledge, To our knowledge, XeMRI has not yet been studied in this specific patient subset.

The 6-minute walk test and PFTs will not be used to guide enrollment, but rather will be used in combination with XeMRI/cMRI to assess degree of functional impairment. We will stratify our results based on ethnicity and spirometric abnormalities from those subjects without these findings.

Existing data highlight significant racial and ethnic disparities with historically underserved minority populations (i.e., Black, LatinX) suffering disproportionately higher infection rates and more severe illness compared to Whites. This is reflected by the population enrolled in our RedCAP database and biorepository. As such 30% of subjects enrolled will be required to be from underserved communities.

2.1 *Primary Study Aims*

- Aim 1 - Determine the cardiopulmonary structure-function abnormalities that characterize early phase COVID-19 recovery. We anticipate conducting 129Xe and cardiac MRI in 13 recovering COVID patients recruited at their clinic visit 60 days' or longer post-discharge from their acute infection. We will recruit patients with new onset persistent and continued dyspnea and to characterize the interstitial, pulmonary vascular and myocardial abnormalities relative to a control population.
- Aim 2 – Characterize the evolution of cardiopulmonary abnormalities over 9 months. Patients will undergo repeat scanning 9 months later to assess changes in all regional parameters acquired from pulmonary and cardiac MRI, directly addressing the possible reversibility of cardiopulmonary limitations arising from "long COVID."
- Aim 3 – Identify MRI features that predict physiologic outcomes and can alter treatment decisions. Patients will undergo PFTs and quality of life evaluations at 3, 6 and 9 months, as well as detailed clinical histories. These data will be used to understand which baseline imaging features are predictive of long-term quality of life and physiology outcomes as well as those that represent "treatable traits" that can be targeted for therapeutic intervention.

3 Study Design

3.1 General Design

The study is open label and will enroll 23 subjects (consenting up to 30 subjects).

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Diagnosis of post Coronavirus (COVID-19)
Inclusion Criteria
a. Age ≥ 18-year-old

b. Tested positive for SARS-CoV2
c. Willing and able to give informed consent and adhere to visit/protocol scheduled (consent must be given before any study procedures are performed)

4.2 Exclusion Criteria

a. Prisoners
b. Pregnant, planning pregnancy or lactating
c. Conditions that prohibit MRI scanning (metal in eye, claustrophobia, inability to lie supine).
d. Medical or psychological conditions which, in the opinion of the investigator, might create undue risk to the subject or interfere with the subject's ability to comply with the protocol requirements

4.3 Subject Recruitment and Screening

Potential subjects will be identified in the Duke post-COVID clinic by clinical staff at their standard of care (SOC) at 6-12 wks. after their initials COVID-19 diagnosis. The clinical staff will focus on identifying subjects with persistent and continued dyspnea after COVID-19 infection. At initial SOC visit (post-diagnosis of COVID positive), subjects will undergo evaluation in pulmonary clinic. Pulmonary function testing (PFTs) 6-minute walk test (6MWT), and relevant labs will be ordered as indicated by the pulmonary physician. Subjects will be invited to participate in the post-COVID Pulmonary Biorepository and the COVID Long-Haulers pilot study. A subject can enroll in the COVID Long Haulers pilot without enrollment in the Biorepository.

4.4 *Early Withdrawal of Subjects*

4.4.1 When and How to Withdraw Subjects

A participant will be withdrawn from the study for any of the following reasons:

- Lost to follow-up.
- Withdrawal of consent.
- Death.
- If a participant is poorly compliant with study procedures, visits, and assessments, withdrawal should preferably take place after evaluation and discussion.
- If the investigator considers that continued participation in the study would be contrary to the best interests of the participant.
- Principal investigator's decision for any reason, including, but not limited to, premature termination or suspension of the study.

When a subject withdraws before completion of their study activities, the reason for withdrawal is to be documented in the eCRF and in the source document.

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. A subject cannot be deemed lost to follow-up until all reasonable efforts made by the study site personnel to contact the subject are deemed futile. The following actions must be taken if a participant fail to return to the study site for a required study visit:

- The study site personnel must attempt to contact the participant to reschedule the missed visit as soon as possible, to counsel the participant on the importance of maintaining the assigned visit schedule, to ascertain whether the participant wishes to or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every reasonable effort to regain contact with the participant (where possible, 3 telephone calls, e-mails and a certified letter to the participant's last known mailing address). These contact attempts should be documented in the participant's research records.

Should the subject continue to be unreachable, they will be considered to have withdrawn from the study.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Participation in this study is voluntary and subjects are free to withdraw. If a subject withdraws from all study activities before the study is completed for any reason (except for death or withdrawal of consent), every attempt should be made to schedule the end of study telephone call to assess the safety and well-being of the subject. Attempts should also be made to obtain permission to record at least survival data up to the protocol-described end of subject follow-up period.

4.4.3 Replacement of Early Complete Withdrawal(s)

Subjects who withdraw from the study prior to completing study activities will have the data collected about them used in the primary and secondary endpoint analyses. A new subject will be consented to replace the one withdrawn to ensure data analysis of 23 subjects who have completed all interventions and completed end of study.

5 Standard of Care drugs

Subjects will take their standard of care medication during the necessary time points in the study. There will be no restrictions on concomitant medications.

6 Study Procedures

6.1 Study Visits

Potentials subjects will be identified in the Duke Post-COVID at their SOC visit after their initial COVID-19 diagnosis. At the subject initial SOC visit, if indicated, subjects may undergo SOC pulmonary function testing, 6-minute walk test, laboratory blood testing, chest X-ray and cardiac MRI. Subjects will undergo ^{129}Xe MRI at the baseline visit and ^{129}Xe MRI and cardiac MRI at the 6-9-month visit.

6.2 *Scheduled of Event*

Protocol Activities	SOC Visit Baseline (V0)	SOC Visit Visit 1 (V1)
Months	Post COVID	6-9 Months
Informed Consent	X	
History re: Disease, exposure, secondary diagnoses	X	X
Medical history	X	
Concomitant Medications[6]	X	X
Pregnancy Test (serum/urine)[1]	X	X
Pulmonary Function Testing with DLCO[6]	X	X
Labs[7]	X	X
6MWT [7]	X	X
MRI Screening[2] Form	X	X
Imaging Session (Cardiac MRI)[4]	X[7]	X[8]
Imaging Session (^{129}Xe MRIs) [3]	X	X
Adverse Events[5]		X
Standard dose CT	X	X
Vital Signs Assessment with optional noninvasive, optical Hemoglobin measurement	X	X

1 For women of childbearing potential, a serum pregnancy test will be performed prior to the cardiac and ^{129}XeMRI studies. The test must be negative before the MRI. The pregnancy test will be conducted and interpreted by study personnel who have completed competency training from the Duke Office of Clinical Research (DOCR).

2 An MRI screening form will be completed at the screening session and verified before starting the imaging session. If an MRI is performed on a different day from the screening session, the subject will need to complete a new MRI screen form for each imaging session.

3 The ^{129}MRI session will be conducted on the CAMRD research MRI scanner. Hyperpolarized ^{129}Xe is produced and delivered by the study team. During MRI session, a qualified medical professional (MD, DO, PA, LNP, RN, RT, or MT) will be on hand to monitor subjects during MRI and note any symptoms related to Xenon MRI.

4 The Cardiac MRI session will be conducted at the Duke Cardiovascular Magnetic Resonance Center (DCMRC).

5 Based on the known pharmacokinetics of Xenon, no additional effects are expected after the subject is released from the imaging study.

6 7, These procedures are considered the standard of care

8 The Research Cardiac MRI will be conduct during visit 2, will use Dotarem (IV contrast) 0.15 mmol//kg.

6.2.1 Prescreening

If the subject is willing to participate in the study, the study coordinator will contact the subject by phone to prescreen the subject (screened for MRI contraindications and obtain verbal consent to schedule their consent/screening visit). Our study coordinators will approach potential subject who meet inclusion/exclusion criteria.

6.2.2 Baseline (V0) (post COVID DX)

Informed consent will contain a statement that indicates that if a subject does not qualify to continue in the research study after the end of the screening, he/she will be withdrawn from

the study. Subjects that sign consent but do not pass the screening are considered enrolled in the research study and counted as withdrawn.

- After obtaining informed consent, the following data may be collected and documented on the source (paper or subject's electronic medical record):
    - Documentation of relevant patient history and symptoms – SOC
    - Medical History (including comorbid conditions) – SOC
    - Vital signs (temperature, heart rate, blood pressure, respiratory rate, and pulse oximetry) including weight and height - SOC
        - Including optional noninvasive, optical hemoglobin measurement
    - Concomitant Medications – SOC
    - PFT's - SOC
    - 6MWTs – SOC
    - Labs- SOC
    - Chest X-ray-SOC
    - MRI Imaging -SOC
        - Cardiac MRI
    - Completion of MRI screening form - research
    - MRI Imaging session – research
        - ^{129}Xe MRI
        - Urine pregnancy test (if applicate) performed outside the 48hrs window
    - Adverse event reporting – research
    - Standard dose CT - research

6.2.3 Visit 1 (V1) (6-9 months)

The following data may be collected and documented on the source (paper or subject's an electronic medical record):

- Documentation of relevant patient history and symptoms – SOC
- Medical History (including comorbid conditions) – SOC
- Vital signs (temperature, heart rate, blood pressure, respiratory rate, and pulse oximetry) including weight and height - SOC
    - Including optional noninvasive, optical hemoglobin measurement
- Concomitant Medications – SOC
- PFT's - SOC
- 6MWTs – SOC
- Completion of MRI screening form - research
- MRI Imaging session – research
    - Cardiac MRI
    - ^{129}Xe MRI
- Serum Pregnancy testing for a female of childbearing potential - research
    - Urine pregnancy test (if applicate) performed outside the 48hrs window
- Adverse event reporting – research
- Standard dose CT - research

7 Statistical Plan

7.1 *Sample Size Determination*

Power calculation: Based on the ^{129}Xe MRI metrics from our study (Wang, et al., 2019), we expect that we could detect an absolute difference of high barrier uptake of 10% between the post-COVID subjects and an existing cohort of healthy controls. We assume that with a standard deviation of 10% high barrier uptake within patients, and requiring a power of 90%, that a two-sided test would require a sample size of 23 to detect a difference. However, given that high-barrier uptake is expected only in the post-COVID group, a one-sided test suggests the proposed sample size has 99% power to detect a significant difference.

7.2 *Statistical Methods*

^{129}Xe MRI metrics will include RBC/barrier, barrier/gas, RBC/gas,
and several spectroscopic indices. Continuous data will be presented using the mean ± SD if normally distributed and median with 25th and 75th percentiles otherwise. We are fortunate to be able to compare the findings in our images to a database of approximately 30 healthy reference subjects whom we have scanned to date. These subjects have undergone the same 129Xe MRI protocol and were recruited with no histories of smoking or cardiopulmonary disease, FEV1>80%, FEV1/FVC>0.70 and DLCO >80%. For subjects to be included in the reference cohort they further had a ventilation defect percentage ≤6%, (upper limit of normal VDP (Ebner '19)) as well as RBC/barrier ≥0.45 (at TR=15 ms**)**, and image SNR>5, based on the Rose criterion. Comparisons between these groups will use analysis of variance (ANOVA) methods or Kruskal-Wallis test as appropriate.

8 Safety and Adverse Events

8.1 **Overview**

As the sponsor of the study, Duke University Medical Center and the Principal Investigator shall be solely responsible for complying, within the required timelines, any safety reporting obligation to competent Health Authorities, IRB and any participating (co or sub) investigators, as defined in applicable laws and regulations. For this protocol, safety data include adverse events and special situations, including pregnancies.

8.2 **Management of Safety data**

This study has been designated as an interventional study. As such, all adverse events, special situations including pregnancies and product quality complaints will be reported as described in this protocol from the time a subject has signed and dated an Informed Consent Form (ICF) until 30 days after the last documented use of the study drugs (i.e., end of study (9 months)).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness, or experience that develops or worsens in severity during the study. Intercurrent diseases or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- is associated with Abnormal lab values
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE, that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events may not be immediately life-threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in an in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non serious adverse events***.

NOTE: DEATH FOR ANY REASON WILL BE REPORTED AS A SERIOUS ADVERSE EVENT.

Post-study Adverse Event
The investigator should follow all unresolved adverse events until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the resident, or the subject's physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of cancer development or a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in

this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the adverse event criteria.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery is reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should *not* be reported as an outcome of an adverse event if the surgery's purpose was elective or diagnostic, and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the study's target disease, unless it is a worsening or increase in hospital admissions frequency as judged by the clinical investigator.

8.3 **Life-Threatening Conditions**

The cause of death of a subject in a study within 30 days of the end of the study, whether or not the event is expected or associated with the investigation, is considered a serious adverse event.

8.4 **Recording of Adverse Events**

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the medical record and noted in our REDCAP database. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. Each event's clinical course should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed to determine the outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.5 **Study Closure**

Following the completion of the studies, the sponsor will be responsible for ensuring the following activities:

- Data clarification and resolution
- Review of Site study records for completeness

8.6 ***IRB Notification by Investigator***

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within ten working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.7 Stopping Rules

No stopping rules are needed for this study. If patients want to withdraw. They can at any time.

8.8 Termination Criteria

Premature termination of these clinical trials may occur because of a regulatory authority decision, a change in opinion of the IRB, or the FDA's discretion. DUMC also reserves the right to discontinue the trial before inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons.

8.9 CQMP Monitoring

The Duke School of Medicine Clinical quality management program (CQMP) may conduct confidential audits to evaluate compliance with the protocols and GCP principles. The PI agrees to allow the CQMP reviewer direct access to all relevant documents and allocate his/her time and the time of the study team to the CQMP reviewer to discuss findings and any relevant issues. CQMP audits are designed to protect the rights and well-being of human research subjects.

8.10 *Consent Process*

The Duke IRB must approve the informed consent form and comply with ICH, GCP, local regulatory requirements, and federal laws. The investigator must ensure that each trial subject is fully informed about the trial's nature and objectives and possible risks associated with participation.

The investigator will obtain written informed consent from each subject before any study-specific activity is performed. The IRB must prospectively approve the informed consent form used in this trial, and any changes made during the trial before use. Additionally, the FDA will be notified of any changes before implementation as appropriate. The subject must sign this consent form, and the investigator-designated research professional obtaining the consent. The investigator will retain a copy of each subject's signed consent form.

9 Subject's Capacity to Give Legally Effective Consent

Subjects without the capacity to give consent will not be recruited into this study.

10 Data Handling and Record Keeping

10.1 *Data Handling*

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded REDCAP database.

10.2 *Source Documents*

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary to reconstruct and evaluate the trial. Source data are contained in source documents Examples of these original documents and data records include hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy

dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.3 *Case Report Forms*

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this trial. A CRF is required and should be completed for each included subject. The completed original CRFs are the sole property of Duke. They should not be made available in any form to third parties, except for authorized representatives of Duke or appropriate regulatory authorities, without written permission from Duke.

It is the investigator's responsibility to ensure completion and to review and approve all CRFs. CRFs must be electronically signed by the investigator or by an authorized staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the investigator has final, personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs

All consent and case report forms will be stored in a locked filing cabinet in the study coordinator or principal investigator's office. Any other digital data (images, image analysis) will be associated only with the subject identification number and the date and time of the ^{129}Xe MRI. Image data will be retrieved and analyzed only by study personnel. Data will be captured in a RedCap database. After all, manuscripts have been published, the key to the code will be destroyed.

10.4 *RedCap*

Data will be captured in a RedCap© database. The database will be reviewed and discussed before database closure and closed only after resolving all remaining queries. An audit trail will be kept of all subsequent changes to the data. Only Key personnel will have access to the database.

10.5 *Records Retention*

As required by law and to enable evaluations and audits from regulatory authorities, the investigator agrees to keep records, including the identity of all participating subject's (i.e., sufficient information to link records to identify), all original signed informed consent forms, copies of all CRFs, other source documents, and detailed descriptions of treatment disposition

11 Study Monitoring, Auditing, and Inspecting

11.1 *Study Monitoring Plan*

This clinical research study will be monitored internally by the sponsor and monitored institutionally by the CQMP. In terms of the internal review, the sponsor will continuously

monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If unexpected frequencies of events occur, action appropriate to the nature and frequency of these adverse events will be taken depending on their nature. This may require a protocol amendment, medication change, or potential closure of the study. The sponsor of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Stopping rules are met;
- The risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of AEs and outcomes is done;
- Over-accrual does not occur;
- Under accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner

11.2 *Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

12.1 *Subject information and Consent*

The Duke IRB must approve the informed consent form and comply with ICH, GCP, local regulatory requirements, and federal laws. The investigator must ensure that each trial subject is fully informed about the trial's nature and objectives and possible risks associated with participation.

The investigator will obtain written informed consent from each subject before any study-specific activity is performed. The IRB must prospectively approve the informed consent form used in this

trial, and any changes made during the trial before use. Additionally, the FDA will be notified of any changes before implementation as appropriate. The subject must sign this consent form, and the investigator-designated research professional obtaining the consent. The investigator will retain a copy of each subject's signed consent form.

The subjects will be asked to provide contact information for their primary care physician (or other physicians) to be notified of any potentially clinically relevant findings obtained from the standard clinical procedure (e.g., Lab, PFT, 6MW).

13 Publication Plan

The principal investigator will plan to publish the results in a peer-review journal with abstract presentation beforehand at a recognized major cardiology or pulmonary conference.

14 References

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