Clinical Trial Protocol: POL-Xe-001

Study Title:	Evaluation of Hyperpolarized ^{129}Xe MRI as Compared to ^{133}Xe Scintigraphy for the Assessment of Pulmonary Function in Patients being Evaluated for Possible Lung Resection Surgery
Study Number:	POL-Xe-001
Study Phase:	3
Product Name:	Hyperpolarized ^{129}Xenon (^{129}Xe) Gas in BRANDNAME Device
IND Number:	075,010
Indication:	Diagnostic for the evaluation of pulmonary function and for imaging the lungs.
Investigators:	Multiple Centers
Sponsor:	Polarean, Inc. P.O. Box 14805 Research Triangle Park, NC 27709-4805
Sponsor Contact:	Kenneth P. West kwest@polarean.com 919-206-7900 ext. 102

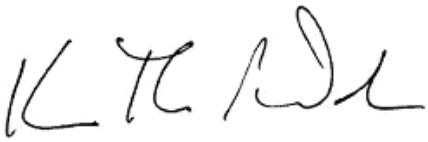
	Date
Original	20 October 2017
Amendment 1	07 November 2017
Amendment 2	29 January 2018
Amendment 3	26 April 2018

Confidentiality Statement

SPONSOR SIGNATURES

Study Title:	Evaluation of Hyperpolarized ^{129}Xe MRI as Compared to ^{133}Xe Scintigraphy for the Assessment of Pulmonary Function in Patients being Evaluated for Possible Lung Resection Surgery
Study Number:	POL-Xe-001
Version:	Amendment 3
Version Date:	26 April 2018

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signature  Date: 26 April 2018

Kenneth P. West
Chief Operating Officer
Polarean, Inc.

Signature Bastiaan Driehuys Digitally signed by Bastiaan Driehuys DN: cn=Bastiaan Driehuys, o=Polarean, Inc., ou, email=bdriehuys@polarean.com, c=US Date: 2018.04.26 10:24:04 -05'00' Date: 26 APR 2018

Bastiaan Driehuys, Ph.D.
Chief Technology Officer
Polarean, Inc.

INVESTIGATOR'S SIGNATURE

Study Title:	Evaluation of Hyperpolarized ^{129}Xe MRI as Compared to ^{133}Xe Scintigraphy for the Assessment of Pulmonary Function in Patients being Evaluated for Possible Lung Resection Surgery
Study Number:	POL-Xe-001
Version:	Amendment 3
Version Date:	26 April 2018

I have read the POL-Xe-001 protocol and agree to conduct the study as outlined.

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

I agree to conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, are consistent with the Good Clinical Practices guidelines of the International Conference on Harmonization, and according to applicable regulatory requirements.

Principal Investigator:_____
(Print Name/Title)

Signature of Principal Investigator: _____

Date:_____

Affiliation/Company: _____

SYNOPSIS

Study Title: Evaluation of Hyperpolarized ^{129}Xe MRI as Compared to ^{133}Xe Scintigraphy for the Assessment of Pulmonary Function in Patients being Evaluated for Possible Lung Resection Surgery	
Name of Finished Product: Hyperpolarized Xenon (^{129}Xe) Gas in BRANDNAME Device	**Name of Active Ingredient:** ^{129}Xe Gas
Study Number: POL-Xe-001	**Study Phase:** 3
Clinical Sites: At least 2 study sites in the United States	
Primary Objective: The primary objective of this study is to demonstrate the equivalence of hyperpolarized ^{129}Xe MRI as compared to ^{133}Xe scintigraphy for the evaluation of pulmonary function.	
Secondary Objectives: The secondary objectives of this study are to: • Assess the safety and tolerability of hyperpolarized ^{129}Xe gas; • Evaluate regional ventilation defects in each of the 6 zones, and • Demonstrate the equivalence of post-operative forced expiratory volume in 1 second (FEV1) values predicted using hyperpolarized ^{129}Xe as compared to ^{133}Xe scintigraphy.	
Study Design: This is a multicenter, randomized, open-label, cross-over Phase 3 study evaluating hyperpolarized ^{129}Xe MRI as compared to ^{133}Xe scintigraphy for the evaluation of pulmonary function. This study is comprised of 4 periods: • Screening: Subjects will be screened for study participation based on inclusion and exclusion criteria. Informed consent will be obtained. • Imaging: Subjects will undergo hyperpolarized ^{129}Xe MRI and ^{133}Xe scintigraphy. During the MRI session, one or more conventional proton MRI scans will also be collected to confirm lung anatomical features. Both ^{133}Xe scintigraphy and hyperpolarized ^{129}Xe MRI will be quantified using commercially available software. Images will be interpreted by central readers who are blinded to the subject's medical history and all study assessments. Information related to any AEs will be collected during this period. • Follow-up: Subjects will be contacted by phone on the day after (+3 days) the completion of all imaging to collect information on any AEs. • Post-op Follow-up: If the subject has lung surgery, approximately 3 months after surgery the subject will come in for a post-operative FEV1 measurement (spirometry). All randomized subjects will be divided between 2 treatment orders in a 1:1 ratio.	
Study Population: *Inclusion Criteria:* Subjects will be eligible for participation in the study only if they meet ALL of the following criteria: 1. Male or female subjects ≥18 years of age. 2. Subject is being evaluated for possible lung resection (e.g., segmentectomy, lobectomy, or pneumonectomy). 3. Able to undergo MRI imaging and able to fit in the MRI coil. 4. Willing and able to comply with all study procedures. 5. Must understand and voluntarily sign an informed consent form (ICF) prior to any study-specific assessments or procedures. *Exclusion Criteria:* Subjects will <u>not</u> be eligible for participation in the study if they meet ANY of the following criteria: 1. Baseline blood oxygen saturation (SpO_2) <90% at rest. For patients requiring routine supplemental oxygen, SpO_2 measurements should be taken with the patient's normal oxygen supplementation.	

2. Female subjects of childbearing potential with a positive serum pregnancy test at screening, or who are not taking (or not willing to take) acceptable birth control measures through the Follow-up Period.

   Adequate birth control methods include with a monogamous partner who was sterilized more than 6 months prior to screening, or measures with a Pearl index of <1 used consistently and correctly (including intrauterine devices, or implantable, injectable, oral, or transdermal contraceptives).

   Women are not considered to be of childbearing potential if they meet at least 1 of the following 2 criteria as documented by the Investigator:

   - They have had a hysterectomy, bilateral salpingectomy, or bilateral oophorectomy at a minimum of 1 menstrual cycle prior to signing the ICF; or
   - They are post-menopausal: for women ≥55 years of age, defined as ≥1 year since their last menstrual period, or for women <55 years of age, defined as ≥1 year since their last menstrual period and have a follicle-stimulating hormone (FSH) level in the laboratory's normal range for post-menopausal phase.

3. Women who are lactating and insist on breast feeding.
4. Subjects who have received any other investigational therapy within 4 weeks prior to Screening.
5. Subjects who require anesthesia or heavy sedation to undergo MRI testing. Mild sedation could be acceptable (i.e. a low dose oral acting anxiolytic medication such as alprazolam) as long as, in the opinion of the investigator, the subject meets Inclusion Criteria #4 and #5.

Planned Number of Subjects:

Completed: 32 subjects having both a ^{129}Xe MRI and ^{133}Xe scintigraphy image.

Subjects who discontinue will be replaced in order to achieve the targeted number of completers.

Test Product, Dose, and Mode of Administration:

The test product is hyperpolarized ^{129}Xe gas and will consist of a volume of hyperpolarized ^{129}Xe inflated with ultra-high purity nitrogen gas to a final volume of 1 L. Subjects will be required to inhale the 1 L of gas mixture and hold their breath for a maximum of 15 seconds (generally 10-15 seconds, based on the length of the scan) while the MRI is acquired.

During the MRI session, one or more conventional proton MRI scans will be collected to confirm lung anatomical features. Subjects will be required to take a full inhalation breath of room air and hold their breath for a maximum of 15 seconds while the MRI is acquired.

Up to 750 mL hyperpolarized ^{129}Xe gas will be administered to the subject while in the MRI scanner. If the resulting scan is determined or thought to be inadequate, then a calibration dose (approximately 100 mL hyperpolarized ^{129}Xe gas) will be administered followed by another full (≤750 mL) dose. The calibration dose is administered to allow for adjustments of MRI parameters, as needed, to ensure best image quality.

Reference Product, Dose, and Mode of Administration:

The reference standard is the anterior and posterior wash-in views of a ^{133}Xe scintigraphy scan. ^{133}Xe is administered by inhalation from a closed respirator system or spirometer in accordance with the Package Insert.

Subjects will be exposed to ^{133}Xe gas for 3-4 minutes, as tolerated.

Duration of Treatment:

Depending on the number of doses required to obtain adequate images, subjects will be exposed to hyperpolarized ^{129}Xe for 15-45 seconds.

Subjects will be exposed to ^{129}Xe gas for approximately 15 seconds per scan.

Duration of Subject Study Participation:

Subjects are expected to participate in this study for a maximum of 15 days unless the subject undergoes resection surgery. For the sub-set of subjects undergoing surgery, the maximum duration for study participation is 4 months.

Efficacy Assessments:

Primary Efficacy Endpoints:

The primary endpoint is the scan predicted post-operative FEV1. The scans will be quantified using commercially available software to report the fraction of activity arising from 6 zones. This will be used to predict post-operative FEV1 by multiplying the percentage of function remaining in the non-operated zones by pre-operative FEV1.

Secondary Efficacy Endpoints:

The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones.

Safety Endpoints:

Safety and tolerability will be assessed based on the incidence and severity of treatment emergent adverse events (AEs) and serious adverse events (SAEs). Additionally, subjects will be monitored before, during, and after each dose to monitor for changes in vital signs.

Statistical Methods:

Sample Size Determination:

The sample size required to demonstrate equivalence is driven by 2 factors: 1) the intra-subject variability of the difference between the predicted post-operative FEV1 from the 2 methods, and 2) the pre-specified equivalence margin. From prior literature, the intra-subject variability for ^{133}Xe scintigraphy leads to an estimated variability in predicted post-operative FEV1 of 0.21 L/sec. Similarly, prior studies have suggested that the equivalence margin between the 2 imaging techniques currently used for resection planning is 0.3 L/sec. Using these assumptions, and based on the use of a two-sided test at the alpha=0.05 level of significance, a sample size of 15 subjects is required for 90% power. However, given limited literature on ^{133}Xe variability, we account for the possibility that it could be higher. If true variability is 0.32 L/sec, then a sample size of 32 subjects will provide 90% power to establish equivalence.

Primary Analysis:

The primary analysis for this study will be to test the mean difference in predicted post-operative FEV1 (measured in L/sec) values as measured using hyperpolarized ^{129}Xe gas MRI relative to ^{133}Xe scintigraphy (reference standard). The primary analysis will be conducted by estimating the 95% confidence interval (CI) for the mean difference in predicted FEV1 from the 2 methods. The scans will be quantified using commercially available software to report the fraction of activity arising from 6 zones. This will be used to predict post-operative FEV1 by multiplying the percentage of function remaining in the non-operated zones by pre-operative FEV1. The CI will be constructed assuming that the within-subject difference between methods is normally distributed. If the 95% CI for the mean difference is contained within the interval (-0.30, +0.30 L/sec), equivalence will have been demonstrated.

Secondary Analysis:

The post-operative secondary analysis for this study will be to test the mean difference in predicted post-operative FEV1 (measured in L/sec) values as measured using hyperpolarized ^{129}Xe gas MRI and ^{133}Xe scintigraphy (reference standard) relative to the final post-operative FEV1 value (spirometry). The secondary equivalence analysis will be conducted using the same methodology and same equivalence margin as specified for the primary analysis.

The 6-zone secondary analysis for this study will be to evaluate the individual percentage function assessments from each of the 6 zone analyses from both the ^{129}Xe and the ^{133}Xe ventilation images. The equivalence margin will be 5 percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval (-5%, +5%).

Safety Analysis:

All AEs will be summarized by system organ class (SOC), preferred term, severity and relationship to study drug. Aes leading to death or to discontinuation from study drug as well as the SAEs will also be tabulated. In the by-subject summary, a subject having the same event more than once will be counted only once and by greatest severity.

Vital sign measurements, including change from baseline, will be summarized. All safety analyses will be performed on the Safety population.
Interim Analyses: No formal interim analyses are planned for this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND TERMS DEFINITIONS

Abbreviation	Definition
^{129}Xe	non-radioactive isotope of xenon (hyperpolarized ^{129}Xe is the drug product)
ADR	adverse drug reaction
AE	adverse event
BP	blood pressure
CFR	Code of Federal Regulations
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRO	Contract Research Organization
eCRF	electronic case report form
FEV1	forced expiratory volume in 1 second
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
He	helium
HR	heart rate
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	intent-to-treat

Lobectomy	Refers to the removal of a lobe (or lobes).
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
N_2	nitrogen
Pneumonectomy	Refers to the removal of an entire lung.
Q	perfusion scan
RBC	red blood cells
Resection	For this study, the definition of resection surgery includes segmentectomies, lobectomies, and pneumonectomies.
SAE	serious adverse event
Segmentectomy	Refers to the removal of an area of the lung that is smaller than a lobe
SNR	signal-to-noise ratio
SOC	system organ class
SpO_2	blood oxygen saturation
^{99m}Tc	Metastable nuclear isomer of technetium-99 isotope
TEAE	treatment-emergent adverse event
US	United States
V	ventilation scan
WHO	World Health Organization
Xe	xenon

1. INTRODUCTION

Polarean, Inc. (Polarean, the Sponsor) has developed a drug/device combination to administer hyperpolarized ^{129}Xe as a diagnostic agent used in conjunction with magnetic resonance imaging (MRI) for the evaluation of pulmonary function and for imaging the lungs. The proposed patient population includes patients requiring imaging evaluation of pulmonary function prior to a variety of lung surgeries. Today, these pre-operative evaluations employ nuclear scintigraphy methods using ^{133}Xe ventilation and/or Technetium (^{99m}Tc) perfusion (the so called ventilation/perfusion [V/Q] scan). The primary objective for this study is to demonstrate the equivalence of hyperpolarized ^{129}Xe MRI as compared to ^{133}Xe scintigraphy.

The population referred for such scans includes patients undergoing evaluation for possible lung resection surgery and transplants. In both circumstances, regional pulmonary function information is used for making surgical decisions. For this study, the population will include patients being evaluated for possible lung resection surgery. The definition of lung resection surgery includes segmentectomies, lobectomies, and pneumonectomies. Segmentectomies refer to the removal of an area of the lung that is smaller than a lobe. A lobectomy is the removal of a lobe (or lobes), whereas a pneumonectomy is the removal of an entire lung (BMC website).

1.1. Hyperpolarized ^{129}Xe in BRANDNAME Device

This device is comprised of 3 different components. Together, these components hyperpolarize, measure the hyperpolarization of ^{129}Xe gas, and permit administration to the patient. Inhalation of the hyperpolarized inert gas allows for clear imaging of its distribution within the lungs when using any multi-nuclear capable MRI scanner. The 3 components are the Hyperpolarizer, the Polarization Measurement Station, and the Gas Delivery Bag (refer to Figure 2).

The drug product is hyperpolarized ^{129}Xe. It is created by passing a gas mixture of xenon (Xe), helium (He), and nitrogen (N_2) through the Hyperpolarizer. Once the Xe is hyperpolarized, it is collected in a Gas Delivery Bag and filled to volume (1 L) with with inert N_2 buffer gas. Within 5 minutes prior to administration to the subject, the degree of hyperpolarization is measured with the Polarization Measurement Station (refer to Section 6.9.1). The standard volume (dose) of hyperpolarized ^{129}Xe is ≤750 mL administered (via inhalation) to the subject while in the MRI (refer to Section 5.2.1).

1.2. Mechanism of Action

Mechanistically, hyperpolarized ^{129}Xe is metabolized in order to achieve its primary purpose. After imaging, the hyperpolarized ^{129}Xe is exhaled from the body during normal respiration.

1.3. Pharmacokinetics

Hyperpolarized ^{129}Xe is chemically and biologically identical to non-polarized Xe, which is a "perfusion-limited" gas and therefore not subject to membrane diffusion (Roughton and Forster, 1957). It can therefore be assumed that the Xe concentration in the venous capillary blood equilibrates instantaneously with the Xe concentration in the alveoli according to its Ostwald solubility. Data have demonstrated that Xe concentration is diminished by more than 1000× prior to achieving 40 breaths. Normal humans breathe at a rate of 12-20 breaths per minute (Guyton and Hall, 2006), therefore the 40-breath mark would be reached at 2-3 minutes. Because blood and airspaces remain in close equilibrium, it is anticipated that the Xe concentration in blood will follow an identical decay curve; however, it will start at a lower concentration. As only one breath of Xe is used for MRI imaging (rather than breathing to equilibrium), it is anticipated that Xe exhalation in patients with lung disease will be similar to that of healthy volunteers.

Detailed information on the pharmacokinetics of Xe and ^{129}Xe is provided in the Investigator's Brochure (IB).

1.4. Nonclinical Evaluations

A single-dose toxicity study was completed in Beagle dogs under IND 075,010 by GE Healthcare (Report No. FY01-015). Results from clinical observations, ophthalmologic examinations, body weights, food consumption, clinical pathology, and pulmonary function all indicated that no biologically significant adverse effects of exposure were observed. The beagle dogs were observed during exposure as well as for 14 days after exposure to depolarized ^{129}Xe.

Expanded, single-dose toxicity studies in rats and dogs have been conducted with Xe gas enriched with ^{129}Xe to 50% v/v. No signs of toxicity, beyond the known physiological effects of Xe gas as an asphyxiant and anesthetic, were observed in these studies.

Literature supports that ^{129}Xe gas does not affect the hepatic and renal systems (Reinelt et al., 2002), is non-reactive, and is excreted quickly via the lung. Toxicity data from repeat-dose reproductive and developmental (Lane et al., 1980; Burov et al., 2002a; Burov et al., 2002b), and immunotoxicological (Burov et al., 2002c; Horn et al., 2001; de Rossi et al., 2001; de Rossi et al., 2002; de Rossi et al., 2004) studies show no evidence that inhalation of Xe gas causes any specific toxic effects.

Detailed information on the nonclinical evaluation of Xe and ^{129}Xe is provided in the IB.

1.5. Clinical Experience

To date, the clinical development program for hyperpolarized ^{129}Xe gas in the BRANDNAME device is comprised of 1 Phase 1 study (GE-141-001) and data reported in the literature. The Phase 1, single-site, open-label, non-randomized study was conducted by GE Healthcare and is reported in the literature by Cleveland et al., 2010; Driehuys et al., 2012; Kaushik et al., 2011; He et al., 2014a; and Virgincar et al., 2013. Additional literature support comes form Kirby et al., 2013; He et al., 2014b; Svenningsen et al., 2013; He et al., 2015; and Stewart et al., 2015.

Across these studies, a total of 55 healthy volunteers, 20 patients with chronic obstructive pulmonary disease (COPD, 10 of which had radiographic evidence of emphysema), 27 patients with asthma, and 1 current smoker (healthy) have been administered 1 or more hyperpolarized ^{129}Xe scan (total exposed to 1 or more doses = 103). No treatment-emergent adverse events (TEAEs) or laboratory changes were reported.

Detailed information on the clinical evaluation of Xe and ^{129}Xe is provided in the IB.

1.6. Rationale for Study

The investigational product is a non-radioactive, 3-dimensional MRI procedure that will allow physicians to clearly and accurately visualize the spatial distribution of a subject's regional pulmonary function. This study was designed to evaluate the ability of hyperpolarized ^{129}Xe to provide clear images as compared to ^{133}Xe scintigraphy for the evaluation of pulmonary function.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of this study is to demonstrate the equivalence of hyperpolarized ^{129}Xe MRI as compared to ^{133}Xe scintigraphy for the evaluation of pulmonary function.

2.2. Secondary Objectives

The secondary objectives of this study are to:

- Assess the safety and tolerability of hyperpolarized ^{129}Xe gas;
- Evaluate regional ventilation defects in each of the 6 zones, and
- Demonstrate the equivalence of post-operative forced expiratory volume in 1 second (FEV1) values predicted using hyperpolarized ^{129}Xe as compared to ^{133}Xe scintigraphy.

3. INVESTIGATIONAL PLAN

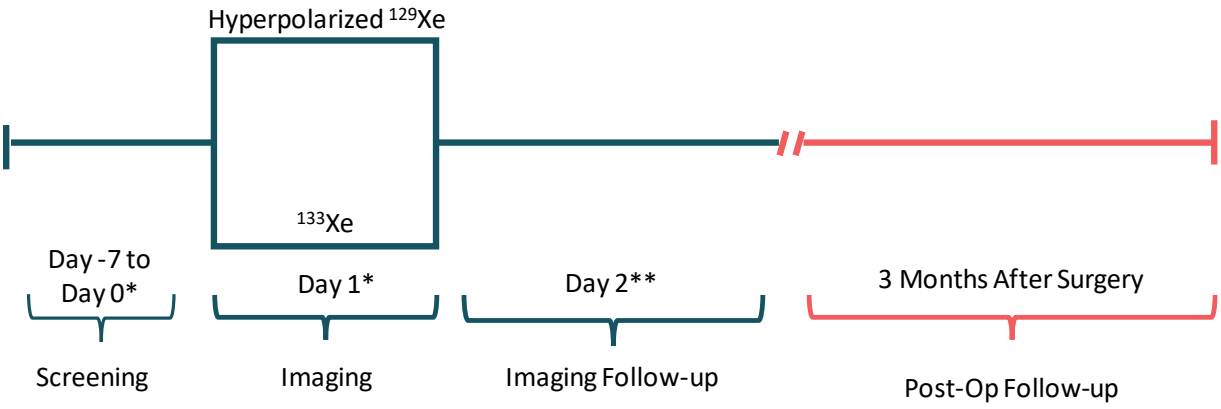
3.1. Overall Study Design and Plan

This is a multicenter, randomized, open-label, cross-over Phase 3 study evaluating hyperpolarized ^{129}Xe gas MRI as compared to ^{133}Xe scintigraphy for the evaluation of pulmonary function. This study will enroll male and female subjects being evaluated for possible lung resection surgery (i.e. segmentectomy, lobectomy, or pneumonectomy).

The planned enrollment is 32 subjects who have completed the study. Completed subjects will have both a ^{129}Xe MRI and ^{133}Xe scintigraphy image. Subjects who discontinue will be replaced in order to achieve the targeted number of completers.

Unless required for safety reasons, all image review will be performed by central readers who are blinded to the subject's medical history and all study assessments. As is shown in Figure 1, this study is composed of 4 periods. All randomized subjects will be divided between 2 treatment orders in a 1:1 ratio.

Figure 1 Illustration of Study Design for Protocol POL-Xe-001



* Screening and Imaging can occur on the same or different days. However, there should be ≤48 hours between administration of the study drug and reference standard scans.
** Can occur up to +3 days after completion of imaging but prior to resection surgery.

The 4 study periods are defined as:

- Screening: Subjects will be screened for study participation based on inclusion and exclusion criteria. Informed consent will be obtained and the investigator's assessment of potential locations of lung resection will be recorded on the electronic case report form (eCRF).
- Imaging: Subjects will undergo hyperpolarized ^{129}Xe MRI and ^{133}Xe scintigraphy. Both ^{133}Xe scintigraphy and hyperpolarized ^{129}Xe MRI will be quantified using commercially

available software. Images will be interpreted by central readers who are blinded to the subject's medical history and all study assessments. Information related to any AEs will be collected during this period.

- Imaging Follow-up: Subjects will be contacted by phone on the day after (+3 days) the completion of all imaging to collect information on any adverse events (AEs).
- Post-op Follow-up: If the subject has lung surgery, approximately 3 months after surgery the subject will return for a post-operative FEV1 measurement (spirometry).

Following completion of screening and baseline eligibility assessments, all subjects will receive both a hyperpolarized ^{129}Xe MRI and ^{133}Xe scintigraphy. During the MRI session, one or more proton MRI scans will also be collected to confirm lung anatomical features. Subjects will receive standard of care at the discretion of the investigator, including appropriate therapies, unless otherwise specified in this protocol. If a subject undergoes resection surgery, an additional study visit to record post-operative FEV1 levels (spirometry) will be required approximately 3 months after surgery.

Safety will be monitored after the administration of both hyperpolarized ^{129}Xe and ^{133}Xe (Day 1) and again on Day 2 during the Imaging Follow-up period.

The Schedule of Assessments is provided in Appendix 1. The study endpoints are provided in Section 8.4.

3.2. Rationale for Study Design and Control

Study Design: This is a multicenter, randomized, open-label, Phase 3 study designed to demonstrate that hyperpolarized ^{129}Xe gas MRI is equivalent to ^{133}Xe scintigraphy for the evaluation of pulmonary function. The vast differences in technique for obtaining hyperpolarized ^{129}Xe MRI as compared to ^{133}Xe scintigraphy make it impossible to blind study procedures. However, all image interpretation will be performed by personnel blinded to the subject's medical history and all study assessments (Section 5.5).

Dose: Dose justification is provided in Section 5.3.2 for hyperpolarized ^{129}Xe and in Section 5.3.3 for ^{133}Xe.

Population Choice: The proposed study population includes subjects requiring imaging evaluation of pulmonary function prior to lung resection surgery. Subjects being considered for surgery normally undergo a full pulmonary evaluation to provide regional lung function information used for surgical decisions.

Control: Regional lung function information is used for making decisions regarding the need for, and predicted success of, resection surgery. Current practice for pre-operative evaluation employs nuclear scintigraphy methods using ^{133}Xe ventilation and/or Technetium (^{99m}Tc)

perfusion (the V/Q scan). This permits the patient's post-operative FEV1 to be estimated based on the amount of function that will be removed by resection surgery. For the purposes of this study, ^{133}Xe will be used as the comparator to evaluate the performance of hyperpolarized ^{129}Xe in patients being considered for lung resection surgery.

3.3. Study Duration and Dates

Subjects are expected to participate in this study for a maximum of 15 days unless the subject undergoes resection surgery. For the subset of subjects undergoing surgery, the maximum study participation is expected to be 4 months.

4. STUDY POPULATION SELECTION

4.1. Study Population

This study will include subjects who are being evaluated for possible lung resection surgery. For the purposes of this study, lung resection surgery will include segmentectomies, lobectomies, and pneumonectomies. Specific inclusion and exclusion criteria are provided below.

At least 32 subjects will be enrolled in the study. Subjects will be recruited primarily via cardio-thoracic surgeons for enrollment at 2 clinical sites in the US.

4.2. Inclusion Criteria

Subjects will be eligible for participation in the study only if they meet ALL of the following criteria:

1. Male or female subjects ≥18 years of age.
2. Subject is being evaluated for possible lung resection (e.g., segmentectomy, lobectomy, or pneumonectomy).
3. Subject is able to undergo MRI imaging and able to fit in the MRI coil.
4. Subject is willing and able to comply with all study procedures.
5. Subject must understand and voluntarily sign an informed consent form (ICF) prior to any study-specific assessments or procedures.

4.3. Exclusion Criteria

Subjects will <u>not</u> be eligible for participation in the study if they meet ANY of the following criteria:

1. Baseline blood oxygen saturation (SpO_2) <90% at rest (refer to Section 6.3.2). For patients requiring routine supplemental oxygen, SpO2 measurements should be taken with the patient's normal oxygen supplementation.

2. Female subjects of childbearing potential with a positive serum pregnancy test at screening, or who are not taking (or not willing to take) acceptable birth control measures through the Follow-up Period. Adequate birth control methods include with a monogamous partner who was sterilized more than 6 months prior to screening, or measures with a Pearl index of <1 used consistently and correctly (including intrauterine devices, or implantable, injectable, oral, or transdermal contraceptives). Women are not considered to be of childbearing potential if they meet at least 1 of the following 2 criteria as documented by the Investigator:
    - They have had a hysterectomy, bilateral salpingectomy, or bilateral oophorectomy at a minimum of 1 menstrual cycle prior to signing the ICF; or
    - They are post-menopausal: for women ≥55 years of age, defined as ≥1 year since their last menstrual period, or for women <55 years of age, defined as ≥1 year since their last menstrual period and have a follicle-stimulating hormone (FSH) level in the laboratory's normal range for post-menopausal phase.
3. Women who are lactating and insist on breast feeding.
4. Subjects who have received any other investigational therapy within 4 weeks prior to Screening.
5. Subjects who require anesthesia or heavy sedation to undergo MRI testing. Mild sedation could be acceptable (i.e. a low dose oral acting anxiolytic medication such as alprazolam) as long as, in the opinion of the investigator, the subject meets Inclusion Criteria #4 and #5.

5. STUDY TREATMENTS

5.1. Description of Treatments

5.1.1. Study Drug

The study drug is hyperpolarized ^{129}Xe gas generated from gas cylinders containing Xe/He/N_2 (Section 6.9.1).

5.1.2. Active Comparator

The active comparator (reference standard) is ^{133}Xe gas.

5.2. Treatments Administered

5.2.1. Study Drug

Up to 750 mL hyperpolarized ^{129}Xe gas will be inflated with ultra-high purity nitrogen (N_2) gas to a final volume of 1 L. To ensure that each dose has sufficient polarization (>10%), the polarization level will be measured at the Polarization Measurement Station within 5 minutes prior to administration.

The hyperpolarized ^{129}Xe gas (in N_2) will be administered to the subject while in the MRI scanner. Subjects will be required to inhale the 1 L of gas mixture and hold their breath for a maximum of 15 seconds (generally 10-15 seconds, based on the length of the scan) while the MRI is administered.

If the resulting scan is determined, or thought to be, inadequate, then a calibration dose (approximately 100 mL hyperpolarized ^{129}Xe gas) will be administered followed by another full (≤750 mL) dose. The calibration dose will be administered to allow for adjustments of MRI parameters, as needed, to ensure best image quality. If calibration is required, subjects will be exposed to hyperpolarized ^{129}Xe for a maximum of 45 seconds.

During the MRI session, one ore more conventional proton MRI scans will be collected to confirm lung anatomical features. Subjects will be required to take a full inhalation breath of room air and hold their breath for a maximum of 15 seconds while the MRI is acquired.

Detailed information for preparation and administration of hyperpolarized ^{129}Xe gas is provided in the IMP Handling and Labeling Instruction Manual for this study.

5.2.2. Active Comparator

The active comparator, ^{133}Xe, will be administered by inhalation from a closed respirator system or spirometer in accordance with the Package Insert. Subjects will be exposed to ^{133}Xe gas for 3-4 minutes as tolerated.

Detailed information for preparation and administration of ^{133}Xe is provided in the IMP Handling and Labeling Instruction Manual for this study as well as the ^{133}Xe Package Insert.

5.3. Selection and Timing of Dose for Each Patient

5.3.1. Timing for Performing Scans

Imaging should be performed within the established 48 hour window (refer to the Schedule of Events in Appendix 1).

5.3.2. Hyperpolarized ^{129}Xe Dose Selection

Since this is a diagnostic drug-device combination, dosing is performed only at the time of imaging. To ensure that each dose has sufficient polarization (>10%) for imaging, the polarization will be measured within 5 minutes prior to administration.

The dose range for hyperpolarized ^{129}Xe for this study was calculated based on a typical fast gradient echo image acquisition signal-to-noise ratio (SNR) and voxel volumes from studies conducted to date. The calculated hyperpolarized ^{129}Xe dose equivalent is 75 mL in order to provide scans with adequate SNR. This dose equivalent represents the product of Xe volume,

isotopic enrichment, and polarization. Assuming polarization levels >10% and isotopically enriched ^{129}Xe at ~85%, the calculated Xe volume for evaluation is ≤750 mL.

Information regarding safety and nonclinical evaluations of hyperpolarized ^{129}Xe is provided in the IB.

5.3.3. ^{133}Xe Dose Selection

^{133}Xe will be dosed according to the Package Insert. Please refer to the IMP Handling and Labeling Instuction Manual for additional details.

5.4. Method of Assigning Subjects to Treatment Groups

All randomized subjects will be divided between 2 treatment orders in a 1:1 ratio. Subjects will be randomized to receive either ^{129}Xe followed by ^{133}Xe, or ^{133}Xe followed by ^{129}Xe. All subjects will receive at least 1 dose of hyperpolarized ^{129}Xe and 1 dose of ^{133}Xe during study participation.

5.5. Blinding

This is an open-label study (refer to Section 3.2 for rationale).

Images generated from this study will be interpreted by central readers blinded to the subject's medical history and all study assessments. The images will be uploaded in a blinded manner to a computer for review. Both ^{133}Xe scintigraphy and hyperpolarized ^{129}Xe MRI will be quantified using commercially available software.

5.6. Concomitant Therapy

There are no restrictions with respect to concomitant therapies for this study. All concomitant medications should be listed in the electronic case report form (eCRF) along with the date therapy was initiated, and if applicable, terminated.

For patients taking inhalers, there are no restrictions with respect to timing of dose and imaging. However, the last dose administered prior to imaging should be recorded on the eCRF.

For patients requiring supplemental oxygen, there are no restrictions with respect to timing of dose and imaging. However, the oxygen flow rate should be recorded on the eCRF.

5.7. Restrictions

There are no restrictions for this protocol with respect to prior therapies, or food and fluid intake.

5.8. Treatment Compliance

Site personnel will administer both hyperpolarized ^{129}Xe and ^{133}Xe while the subject is at the site. All administration information will be recorded on the eCRF.

5.9. Packaging and Labeling

Polarean will provide each of the 3 components of the device (Hyperpolarizer, Polarization Measurement Station, and Gas Delivery Bag) to the site. Gas cylinders containing the Xe/He/N_2 gas blend and the ultra-high purity N_2 gas will be provided to the site from a commercial vendor. All cylinders will have normally required labeling upon site receipt.

Labeling instructions for the hyperpolarized ^{129}Xe in the Gas Delivery Bag are provided in the IMP Handling and Labeling Instruction Manual.

5.10. Storage and Accountability

The components of the device (Hyperpolarizer and Hyperpolarization Measurement Station) will be stored in an area located in a separate area (or room) close to the MRI suite.

The Investigator (or delegate) will maintain accountability records for the receipt, dispensing, and return of all study products (including the gas cylinders used to make the study drug). Records will include (but not limited to):

- The receipt of the gas cylinders to be used for study drug preparation;
- Updated inventory of the gas cylinders, and
- The return of empty or unused cylinders.

5.11. Investigational Product Retention at Study Site

During and at the end of the study, the number of gas cylinders will be reconciled against current inventory and dispensing records as part of routine monitoring visits. After reconciliation, unused (full) gas cylinders will be returned to the Sponsor.

Additionally, during and at the end of the study, the use of tedlar bags, tubing, and mouth pieces will be reconciled against current inventory and dispensing records as part of routine monitoring visits. Items that have been used should be disposed of in a biohazard bag. After reconciliation, all unused components will be returned to the Sponsor.

6. STUDY PROCEDURES

The Schedule of Assessments is provided in Appendix 1. All assessments and treatments are to be performed either at a hospital or in a doctor's office.

6.1. Informed Consent

Written informed consent must be obtained from each subject or legal representative (if subject is unable to sign) after the nature of the study has been fully explained in accordance with International Conference on Harmonization (ICH) Good Clinical Practices (GCP). Informed consent must be obtained prior to performing any study-specific procedures.

6.2. Demographics, Medical History, and Concomitant Medications

Demographic information (age, race, gender, weight, height, smoking history, etc.) will be collected during the Screening Period. Medical history, including significant medical conditions, pulmonary function values, and surgical history, will also be collected during the Screening Period. Additionally, any medications taken as well as baseline signs and symptoms occurring within 2 weeks of screening will be recorded.

On the day(s) of the Imaging Period, any changes to the subject's condition that affect inclusion/exclusion criteria will be documented, as well as any additional medications taken. Subject must meet all inclusion/exclusion criteria upon entering the Imaging Period.

6.3. Vital Signs

Vital signs will include blood pressure (BP, systolic and diastolic measurements), heart rate (HR), respiration rate, blood oxygen saturation (SpO_2) and temperature. Vital signs will be assessed during the Screening Period as well as before and after each scanning session (both ^{129}Xe and ^{133}Xe) during the Imaging Period. Subjects will have a 5-minute rest in a supine position before vital signs are assessed.

The subject will be monitored for the duration of the MRI exam by qualified medical professionals.

6.3.1. Heart Rate

HR will be assessed during the Screening Period as well as before and after each scanning session (both ^{129}Xe and ^{133}Xe) during the Imaging Period. Changes in HR of >20% are considered significant. The next dose of any study treatment will not be administered until the HR is within 20% of the baseline value. If the subject has received their last dose, they will be observed until the HR is within 20% of the baseline value.

6.3.2. Blood Oxygen Saturation

SpO_2 will be assessed during the Screening Period as well as before (within 5 minutes) and after (within 1 minute) each scanning session (both ^{129}Xe and ^{133}Xe) during the Imaging Period. An absolute decrease of SpO_2 by >10% is considered significant. The next dose of any study treatment will not be administered until the SpO_2 is within 10% of the baseline value. If the

subject has received their last dose, they will be observed until the SpO_2 is within 10% of the baseline value.

If the subject's SpO_2 value does not return to within 10% of the baseline value within a few minutes (2-5 minutes), supplemental oxygen should be provided until values are within range. Once the value is within range, the subject can proceed to the next dose.

6.4. Baseline Physical Exam

A standard physical exam will be performed during the Screening Period. Baseline findings will be recorded on the eCRF.

6.5. Clinical Laboratory Tests

A negative serum pregnancy test must be confirmed at Screening for all females of childbearing potential. A blood sample for serum pregnancy testing will be taken from all females of childbearing potential at Screening. If the serum pregnancy test is positive, the patient must be discontinued from study treatment, unless subject is post-partum.

6.6. MRI-suitable Screening

As indicated in exclusion criteria #5 (Section 4.3), a subject who requires anesthesia or heavy sedation in order to undergo MRI testing cannot participate in this study. However, mild sedation (i.e. a low dose oral acting anxiolytic medication such as alprazolam) could be acceptable if, in the investigator's opinion, the subject is still able to comply with all study procedures.

There are no additional protocol specific requirements or restrictions with respect to a subject being suitable for MRI testing.

6.7. Spirometry Evaluation of FEV1

Pulmonary function will be evaluated by measuring FEV1 using spirometry prior to imaging on Day 1 (Imaging Period). For the sub-set of subjects undergoing surgery, FEV1 will also be measured during the Post-op Follow-up Period.

6.8. Image Interpretation

As indicated in Section 5.5, image interpretation will be performed by central readers. Details for performing image evaluation are provided in the Imaging Manual.

6.9. Dispensing Study Drug

6.9.1. Study Drug – Hyperpolarized ^{129}Xe Gas Administration

The hyperpolarized ^{129}Xe gas will be prepared by a technician using the BRANDNAME device located in a separate area (or room) close to the MRI suite. The device is comprised of 3 different components that hyperpolarize, measure the hyperpolarization of the ^{129}Xe gas, and administer it to the patient (Figure 2). The technicians will be trained on the use of each of the device components prior to study initiation. In addition, the Operator's Manuals will be available during study execution.

Figure 2 Depiction of the Hyperpolarization Process and Device Components

The system functions by attaching a gas cylinder containing the Xe/He/N_2 gas blend to the Hyperpolarizer. This system hyperpolarizes high purity, inert, ^{129}Xe gas, which is collected as a solid in a cryogenic trap (a cold finger), then thawed, and dispensed into a 1 L delivery bag.

After dispensing an appropriate volume of Xe into the bag (based on dose required, e.g., full or calibration dose), the remaining bag volume is filled with ultra-high purity N_2 buffer gas. Afterwards, the degree of ^{129}Xe hyperpolarization is measured with the Polarization Measurement Station. The gas sample can be temporarily stored on the Polarization Measurement Station platform until it is needed for patient administration. To ensure that each

dose has sufficient polarization, the polarization will be measured within 5 minutes prior to administration to ensure the value is >10%.

Additional information for preparation and administration of hyperpolarized ^{129}Xe gas are provided in Section 5.2.1 as well as the IMP Handling and Labeling Instuction Manual for this study.

6.9.2. Anatomical Proton MRI (MRI Session Only)

During the MRI session, one ore more conventional proton MRI scans will be collected to confirm lung anatomical features. Subjects will be required to take a full inhalation breath of room air and hold their breath for a maximum of 15 seconds while the MRI is acquired.

6.9.3. Active Comparator – ^{133}Xe Administration

The active comparator, ^{133}Xe, will be administered in accordance with the Package Insert. Detailed information for preparation and administration of ^{133}Xe gas are provided in the IMP Handling and Labeling Instruction Manual for this study.

6.10. Adverse Events Assessments

AEs will be reported using the current version of the Medical Dictionary for Regulatory Activities (MedDRA).

The investigator (and any sub-investigators) is responsible for the appropriate medical care of subjects during the study. The investigator remains responsible for following, through an appropriate health care option, serious adverse events (SAEs) or AEs that caused the subject to discontinue before completing the study. The subject should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

6.10.1. Adverse Events

An AE is any untoward medical occurrence in a subject participating in a clinical investigation/study that has received treatment with a drug or biologic (medicinal) product. The event does not necessarily have a causal relationship with the administration of the study drug. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. This includes any side effect, injury, toxicity, or sensitivity reaction, and may include a single symptom or sign, a set of related symptoms or signs, or a disease. An AE may also be any laboratory abnormality judged to be clinically significant by the Investigator (or sub-investigator) that worsened when compared to baseline.

An abnormal laboratory value is not an AE unless it is considered to be clinically significant. AEs include the following:

- All suspected adverse drug reactions.
- All reactions from medication overdose, abuse, withdrawal, sensitivity, or toxicity.
- Apparently unrelated illnesses, including the worsening of a pre-existing illness.
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (e.g., a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate AEs.
- Abnormalities in physiological testing or physical examination (findings that require clinical intervention or further investigation beyond ordering a repeat [confirmatory] test).
- Laboratory abnormalities that are clinically significant and require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event.

An AE **does not** include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE.
- Pre-existing diseases or conditions present or detected at screening/baseline that do not worsen in severity or frequency.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
- Overdose of either study drug or concomitant medication without any signs or symptoms.

Throughout the course of the study, every effort should be made to remain alert to possible AEs. Subjects should be encouraged to report AEs spontaneously or in response to general, non-directed questioning.

With the occurrence of an AE, the primary concern is the safety of the subject. If necessary, appropriate medical intervention should be provided, and the study drug discontinued. Urgent safety issues may be discussed with physicians at the Contract Research Organization (CRO).

6.10.1.1. Adverse Events of Special Interest

Subjective sensation of residual anesthesia (e.g., lightheadedness, loss of sensation or awareness, loss of memory, or unconsciousness) is an AE of special interest for this study. The next dose of any study treatment will not be administered until any sensation of residual analgesia has resolved. If the subject has received their last dose, they will be observed until the sensation of residual analgesia has resolved.

6.10.1.2. Serious Adverse Events

An SAE is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes:

- Death.
- A life-threatening AE (i.e., immediate risk of death)
  Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which, hypothetically, might have caused death if it were more severe.
- Prolongation of existing hospitalization or re-hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the AE may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

6.10.1.3. Unexpected Adverse Event

An unexpected AE is any AE that is not identified in nature, severity, or frequency in the current IB or product information.

6.10.1.4. Unexpected Adverse Drug Reaction

An adverse drug reaction (ADR) is an adverse reaction, the severity of which is not consistent with the applicable product information or IB. All noxious and unintended responses to a medical product related to any dose should be considered an ADR.

- The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility (meaning the relationship cannot be ruled out).
- The expression "causal relationship" is meant to convey that, in general, there are facts, evidence, or arguments to suggest a reasonable causal relationship. All serious and unexpected ADRs will have expedited reporting to regulatory agencies following ICH requirements.

6.10.2. Adverse Event Reporting Period

The AE reporting period for this study begins upon receiving the first dose of study treatment during the Imaging Period and ends during the Imaging Follow-up Period. If a subject experiences an AE after signing the informed consent, but prior to receiving study drug, the event will NOT be collected unless the investigator feels the event may have been caused by a protocol procedure. Likewise, if a subject experiences an AE during the Post-Op Follow-up Period, the event will NOT be collected unless the investigator feels the event may have been caused by a protocol procedure.

All AEs (both serious and non-serious) must be assessed through the Imaging Follow-up Period. All measures required for AE management and the ultimate outcome of the AE must be recorded in the eCRF and reported to the Sponsor. All study-drug related AEs must be followed until resolution, the return to baseline, or until the event is deemed stable or irreversible.

In addition, any known untoward event that occurs subsequent to the AE-reporting period that the investigator assesses as related to the study drug should also be reported as an AE and must be recorded in the eCRF.

6.10.3. Recording Adverse Events

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event. The Investigator will then record all relevant information regarding an AE/SAE in the eCRF. All details of any treatments initiated due to the AE should also be recorded in the subjects' notes and the eCRF.

All AEs will be documented in the appropriate section of the eCRF. Among these AEs, all SAEs (refer to Section 6.10.1.2) will be additionally documented in an SAE Report. The following will be recorded for each event on the eCRF:

- A description of the AE in medical terms, not as reported by the subject. Whenever possible, a diagnosis should be given when signs and symptoms are due to common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection").
- The date of onset (start date).
- The date of resolution (stop date).
- The severity as assessed by the investigator according to the definitions in Section 6.10.4.
- The causal relationship to study drug as assessed by the investigator; the decisive factor in the documentation is the temporal relation between the AE and the study drug (refer to Section 6.10.5).

- Action taken for study drug (none, study drug discontinued, study drug dose reduction, study drug delayed).
- Other action(s) taken (none, concomitant medication given, new or prolonged hospitalization, procedural surgery).
- The outcome according to the following definitions:
    - Recovered with sequelae;
    - Recovered without sequelae;
    - Ongoing, no therapy;
    - Ongoing, therapy;
    - Died, or
    - Change in toxicity grade/severity.
- Serious Adverse Event (as defined in Section 6.10.1.2): yes or no.

If, in any subject, the same AE occurs on several occasions (unless the AE is continuous and of stable grade), then the AE in question will be documented and assessed each time. For an SAE, the information listed above to be recorded in the eCRF will also be recorded in the SAE Report. Only AEs that fulfill the criteria for SAEs (refer to Section 6.10.1.2) should be recorded in the SAE Report.

6.10.4. Assessing Adverse Event Severity

To ensure there is no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

> The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache).
>
> This is not the same as "serious", which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

All AEs are to be evaluated with respect to severity using:

- Mild;
- Moderate, or
- Severe.

6.10.5. Assessing Adverse Event Relationship to Study Drug

The investigator (or sub-investigator) must record his/her opinion concerning the relationship of the AE to study treatment on the eCRF. Investigators will determine relatedness of an event to

study drug based on a temporal relationship as well as if the event is unanticipated or unexplained given the subject's clinical course, previous medical conditions, and concomitant medications. An event should be recorded as "drug related" if the investigator believes it to be reasonably related to study drug.

6.10.6. Reporting Adverse Events

Each AE is to be classified by the investigator as serious or non-serious. This classification determines the reporting procedures to be followed (Table 1).

Table 1 Reporting Requirements for Adverse Events

Gravity	Reporting Time	Type of Report
Serious	Within 24 hours	Complete AE eCRF
Non-serious	Per eCRF submission procedure	Appropriate AE eCRF

6.10.7. Reporting Serious or Unexpected Adverse Events

Any SAE, irrespective of the relationship to Study Drug, that occurs during the course of the study (from the first dose of study treatment during the Imaging Period up to and including the Imaging Follow-up Period) will be reported on the eCRF as soon as possible (within 24 hours after the site becomes aware of the event).

The investigator is encouraged to discuss with the Pharmacovigilance group any AE for which the issue of reportability is unclear or questioned.

An SAE Report should be prepared, containing as much available information concerning the event as possible, so that a written report can be filed with the appropriate regulatory authorities. If causality cannot be definitively assessed at the time of the SAE, it is important to notify the Sponsor, or its designee, within the timelines stated in Table 1. When new significant information is obtained, and the outcome and attribution of the event is known, the investigator will communicate this on the eCRF. The relevant information will be provided in a timely manner to allow reporting to regulatory authorities within the required reporting period.

For a follow-up report to the regulatory authorities, the Sponsor may be required to collect further information for a detailed description and a final evaluation of the case, including copies of hospital reports, autopsy reports, or other relevant documents. Any SAE follow-up information requested by the Sponsor (or its designee) should be provided in a timely manner to ensure that the SAE report is made to regulatory authorities within the requested timeframe.

The Investigator, or Sponsor where applicable, will notify the relevant Institutional Review Borad (IRB) of any SAEs and safety reports according to local regulation requirements.

6.10.8. Follow-Up of Adverse Events

All AEs (both serious and non-serious) should be followed through the end of the observation period or until resolution, whichever is longer. All measures required for AE management, as well as the ultimate outcome of the AE, must be recorded in the source document and reported to the Sponsor.

6.11. Removal of Subjects from the Study or Study Drug

The investigator may withdraw a subject from study participation for any of the following reasons:

- A significant protocol violation occurs;
- A serious or intolerable AE occurs;
- The sponsor or investigator terminates the study, or
- The subject requests to be discontinued from the study.

6.12. Discontinuation of Study Sites

Study site participation may be discontinued if the Sponsor, investigator, or the IRB of the study site judges it necessary for any reason.

6.13. Discontinuation of the Study

The study will be discontinued if the Sponsor judges it necessary for any reason.

6.14. Appropriateness of Measurements

All procedures and assessments used to evaluate the safety and efficacy of hyperpolarized ^{129}Xe are widely used and generally regarded as reliable, accurate, and relevant. Pulmonary function tests are used to evaluate potential post-operative risks and complications in patients considering lung surgery (Wang, 2004). The specific tests used in this protocol to assess pulmonary function, FEV1, and ventilation assessments, are both commonly used assessments in this patient population.

7. QUALITY CONTROL AND ASSURANCE

A study start-up/investigator training will be held for all sites prior to first subject enrollment. To ensure accurate, complete, and reliable data, the Sponsor or its representatives will:

- Provide instructional material to the study sites, as appropriate.

- Conduct a start-up training session to instruct the investigators and study coordinators. This session will give instruction on the protocol, the completion of the eCRF, and study procedures.
- Make periodic visits to the study site.
- Be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
- Review and evaluate eCRF data and use standard computer edits to detect errors in data collection.
- Conduct a quality review of the database.

In addition, the Sponsor or its representatives may periodically check a sample of subject data recorded against source documents at the study site. The study may be audited by the Sponsor or its representatives, and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of participants in the study, and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and medical records in the subject's files as original source documents for the study. If requested, the Investigator will provide the sponsor, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

8. PLANNED STATISTICAL METHODS

8.1. Determination of Sample Size

The sample size required to demonstrate equivalence is driven by 2 factors: 1) the intra-subject variability of the difference between the predicted post-operative FEV1 from the 2 methods, and 2) the pre-specified equivalence margin. From prior literature, the intra-subject variability for ^{133}Xe scintigraphy leads to an estimated variability in predicted post-operative FEV1 of 0.21 L/sec. Similarly, prior studies have suggested that the equivalence margin between the 2 imaging techniques currently used for resection planning is 0.3 L/sec. Using these assumptions, and based on the use of a two-sided test at the alpha=0.05 level of significance, a sample size of 15 subjects is required for 90% power. However, given limited literature on ^{133}Xe variability, we have accounted for the possibility that it could be higher. If true variability is 0.32 L/sec, then a sample size of 32 subjects will provide 90% power to establish equivalence.

8.2. Analysis Populations

This study will evaluate 2 different subject populations:

- Efficacy Population: The efficacy analysis set will consist of all subjects that have both a ^{129}Xe MRI scan and a ^{133}Xe scintigraphy scan. Primary and the 6-zone analysis secondary efficacy endpoints will be analyzed using the efficacy analysis set.

- Post-operative Population: This analysis set will consist of all subjects that have both a ^{129}Xe MRI scan and a ^{133}Xe scintigraphy scan, and have a post-operative FEV1 (spirometry) value.

- Safety: The safety analysis set will consist of all subjects who received at least 1 dose of either hyperpolarized ^{129}Xe or ^{133}Xe. Secondary safety endpoints will be analyzed using the Safety analysis set.

8.3. Demographics and Baseline Characteristics

Patients' age, height, weight, and baseline characteristics (including smoking history) will be summarized using descriptive statistics, while gender, race, and other categorical variables will be provided using frequency tabulations. Medical history will be summarized using frequency tabulations by system organ class (SOC) and preferred term.

8.4. Study Endpoints

8.4.1. Primary Efficacy Endpoint

The primary endpoint is the scan predicted post-operative FEV1. The scans will be quantified using commercially available software to report the fraction of activity arising from 6 zones. This will be used to predict post-operative FEV1 by multiplying the percentage of function remaining in the non-operated zones by pre-operative FEV1.

8.4.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones.

8.4.3. Secondary Safety Endpoints

Safety and tolerability will be assessed based on the incidence and severity of treatment emergent AEs and SAEs. Additionally, subjects will be monitored before, during, and after each dose to monitor for changes in vital signs.

8.5. Study Disposition

The number of enrolled, completed, and discontinued subjects, with the reason for discontinuation, will be summarized descriptively.

8.6. Primary Efficacy Analysis

The primary analysis for this study will be to test the mean difference in predicted post-operative FEV1 (measured in L/sec) values as measured using hyperpolarized ^{129}Xe gas MRI relative to ^{133}Xe scintigraphy (reference standard). The primary analysis will be conducted by estimating the 95% confidence interval (CI) for the mean difference in predicted FEV1 from the 2 methods. The scans will be quantified using commercially available software to report the fraction of activity arising from 6 zones. This will be used to predict post-operative FEV1 by multiplying the percentage of function remaining in the non-operated zones by pre-operative FEV1. The CI will be constructed assuming that the within-subject difference between methods is normally distributed. If the 95% CI for the mean difference is contained within the interval (-0.30, +0.30 L/sec), equivalence will have been demonstrated.

8.7. Secondary Efficacy Analysis

The post-operative secondary analysis for this study will be to test the mean difference in predicted post-operative FEV1 (measured in L/sec) values as measured using hyperpolarized ^{129}Xe gas MRI and ^{133}Xe scintigraphy (reference standard) relative to the final post-operative FEV1 value (spirometry). The secondary equivalence analysis will be conducted using the same methodology and same equivalence margin as specified for the primary analysis.

The 6-zone secondary analysis for this study will be to evaluate the individual percentage function assessments from each of the 6 zone analyses from both the ^{129}Xe and the ^{133}Xe ventilation images. The equivalence margin will be 5 percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval (-5%, +5%).

8.8. Safety Analysis

All safety analyses will be performed on the Safety population, unless otherwise stated.

AEs will be coded using MedDRA terminology. Events are defined as AEs if they start or worsen after a subject's administration of study drug. All AEs will be summarized by SOC, preferred term, severity and relationship to study drug. AEs leading to death or to discontinuation from study drug and SAEs will also be tabulated. In the by-subject summary, a subject having the same event more than once will be counted only once and by greatest severity.

Vital signs, including change from baseline, will be summarized.

Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug dictionary. Concomitant medications will be summarized descriptively. The summary tables will show the number and percentage of subjects by preferred term. Subjects who take the same medication (in terms of the preferred term) more than once will only be counted once for that medication. Prior and concomitant medications will be listed separately.

8.9. Interim Analysis

No formal interim analyses are planned for this study.

9. ADMINISTRATIVE CONSIDERATIONS

9.1. Institutional Review Board Approval

The study protocol and ICF, as well as any amendments, will be approved by the appropriate ethical review board (IRB) prior to initiation of the study at a particular site. All subjects will sign an ICF prior to any study-specific procedures. Site performance during the study will be routinely monitored by a study monitor (refer to Section 9.5).

Documentation of ethical review board approval of the protocol and ICF must be provided to the Sponsor *before* the study may begin at the study sites. The IRB will review the protocol and ICF as required in accordance with the ICH GCP guidelines. Any member of the IRB who is directly affiliated with this study as an Investigator or as site personnel must abstain from the vote on the approval of the protocol.

In the event of an SAE, the Investigator, or Sponsor where applicable, will notify the relevant IRB of any SAEs and safety reports according to local regulation requirements (refer to Section 6.10.7 for more information regarding reporting of SAEs).

9.2. Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with GCPs and the applicable laws and regulations. The Investigator, head of the medical institution, or designee will promptly submit the protocol to applicable ethical review boards.

This investigational product is being studied in the US under an IND application. Study sites will include approximately 2 sites in the US.

All or some of the obligations of the Sponsor will be assigned to CROs.

An identification code assigned for each subject will be used in lieu of the subject's name to protect the subject's identity when reporting AEs and/or other study-related data.

9.3. Patient Information and Consent

Written informed consent must be obtained from each subject or legal representative (if subject is unable to sign) after the nature of the study has been fully explained, in accordance with ICH GCPs. Informed consent must be obtained during the Screening Period prior to performing any study-specific procedures. The consent form that is used must be approved by both the reviewing IRB and by the Sponsor.

The subject (or legal representative) and the individual explaining the study will sign the current IRB-approved version of the consent form. A copy of the signed consent form will be given to the subject. The date that consent was obtained will be recorded in the eCRF as well as the subject's chart.

A copy of the IRB approved version of the consent form will be provided to the Sponsor. The original signed consent form must be maintained at the site and made available for inspection, as appropriate.

9.4. Patient Confidentiality

The anonymity of subjects participating in this study must be maintained. Subjects will be identified by their assigned subject number in all written communications between the Investigator and Sponsor or its designees. Site documents that are not submitted to the Sponsor or its designees and that identify the subject (e.g., signed informed consent; source documents/charts) will be made available to the Sponsor (or its designees) or regulatory authorities for inspections, but will be otherwise maintained in confidence.

All study-related information provided by the Sponsor or its designees to the Investigator and not previously published (including but not limited to the active study agent identity, the IB, the study protocol, verbal and written communication, study data, assay methods and scientific data), will be considered confidential. In addition, all information developed during the conduct of the clinical investigation of the study agent is also considered confidential. Neither the Investigator nor any of his/her employees or agents shall disclose or use this information for any purpose other than the performance of the clinical study. Such information shall remain the confidential and proprietary property of the Sponsor, and disclosure to others will be limited to other physicians who are conducting studies with the same active study agent, the IRB, and the applicable regulatory authorities except by prior written permission of the Sponsor or its agents. At such time, that information becomes widely and publicly available through no fault of the Investigator, the obligation of nondisclosure toward that particular information will cease.

9.5. Study Monitoring

The Sponsor's clinical research physician will monitor safety data throughout the course of the program. All SAEs will be reviewed within time frames mandated by company procedures and local regulatory requirements.

All deaths and SAE reports will be reviewed by the clinical team during the program. These reports will be reviewed to assure completeness and accuracy.

To ensure the safety of participants in the program, and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the program. If requested, the Investigator will provide the Sponsor, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

The Sponsor or designee will assure the accuracy of data, the selection of qualified Investigators, appropriate program centers, and review protocol procedures with the Investigators and associated personnel prior to the program and during periodic monitoring visits. The Sponsor or a designee will review CRFs for accuracy and completeness during on-site monitoring visits and after their return from the clinical site. Discrepancies will be resolved with the Investigator as appropriate.

The Sponsor or its designee will monitor the program using any of the following methods:

- Telephone contacts;
- Site visits, and
- Review of original patient records, CRFs, drug accountability, storage, and general program documentation.

So that the program may be adequately monitored, the Investigator will cooperate in providing the Sponsor's designee with all program documents (e.g., subject charts and program files) and responding to inquiries that may arise as a result of the document review.

Review of these documents will usually occur during a routine monitoring visit, but may also be required during a visit by a quality assurance auditor. The Sponsor reserves the right to terminate the program if access to source documentation of work performed in this program is denied to the Sponsor or regulatory representatives.

9.6. Case Report Forms and Study Records

An eCRF will be provided for each study subject. Data collected through the completion of study procedures required by this protocol will be recorded in the subject's chart as source documentation. This data will then be transcribed onto the eCRF.

All required study data will be entered onto the Sponsor-provided eCRF. All information in the eCRFs must be supported by original data in the subject's medical records. All medical records, laboratory printouts, notes made by the physician, and other materials, such as x-rays, will be considered source data and must be available for inspection by the Sponsor, the Sponsor's delegates, or government representatives.

Appropriate training prior to study initiation at that site will be conducted to assist with making entries and corrections to data entered into an eCRF. The Investigator remains responsible for the accuracy and adequacy of all data entered on the eCRF.

Data will be monitored as described in Section 9.5. Under direction of the clinical monitor, eCRFs will be verified, locked, and submitted for data entry and analysis. A copy of each eCRF page must remain at the investigative site in the appropriate subject's eCRF file. Data entered into eCRFs will be monitored by monitors that are adequately trained on the system.

Upon further data processing, queries may be generated and sent to the Investigator for clarification or correction. The Investigator will address any queries and forward resolutions as directed by the site monitor.

9.7. Data Disclosure

The Investigator agrees by his/her participation that the results of this study may be used for submission to national and/or international registration and supervising authorities. As required, authorities will be provided with the names of the Investigators, their addresses, qualifications, and extent of involvement. The Investigator is required to provide the Sponsor, or its designee, with all study data, complete reports, and access to all study records.

Data generated by this study must be available for inspection by the FDA and other regulatory authorities, the Sponsor or its designee, and the IRB, as appropriate. At a subject's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Subject medical information obtained during the course of this study is confidential and disclosure to third parties other than those noted above is prohibited.

9.8. Retention of Data

Source documents are the original documents, data, and records from which the subject's eCRF data are obtained. These include, but are not limited to, hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence. eCRF entries may be considered source data if the eCRF is the site of the original data recording (i.e. no other written or electronic record of the data exists). All source documents and study documentation will be

kept by the Investigator for the appropriate retention period as stipulated by the site IRB or FDA guidelines, whichever is longer.

US IND regulations (21 CFR 312.62) require that records and documents pertaining to the conduct of this study and the distribution of investigational drugs including eCRFs, consent forms, laboratory test results, and medication inventory records be kept on file by the Investigator for 2 years after a marketing application is approved for the drug for the indication for which it is being studied. If no application is filed or approved, these records must be kept for 2 years after the investigation has been discontinued and the FDA has been notified. ICH guidelines indicate that documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. If there is a country or institutional policy that specific records and documents be retained for a longer period than described above, the applicable sites must comply with those policies in addition to US and ICH policies. No study records should be destroyed without prior authorization from Polarean.

9.9. Financial Disclosure

The FDA Financial Disclosure by Clinical Investigators (21 CFR 54) regulations require sponsors to obtain certain financial information from Investigators participating in covered clinical studies. The Principal Investigator is required to provide the necessary financial information before participating in the study and to promptly update Polarean with any relevant changes to their financial information throughout the course of the clinical study and for up to 1 year after its completion. This rule applies to all principal investigators participating in covered clinical studies to be submitted to the FDA in support of an application for market approval.

9.10. Publication and Disclosure Policy

All data generated from this program are the property of the Sponsor and shall be held in strict confidence along with all information furnished by the Sponsor. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of the Sponsor.

Any formal presentation or publication of data from this study will be considered as a joint publication by the Investigator(s) and appropriate Polarean personnel. Authorship will be determined by mutual agreement. For multicenter studies it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol and not by individual Investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to in writing. Written permission to Investigators to publish results will be

contingent on review by Polarean of the methodology and statistical analyses and any publication or presentation will provide for nondisclosure of Polarean confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties at least 60 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

Further details on the publication process are provided in individual contractual agreements signed by the Investigators and Polarean.

10. REFERENCE LIST

BMC website: http://www.bmc.org/thoraciconcology/treatments/lung-resection.htm accessed on 28 March 2016.

Burov NE, Arzamastev EV, Kornienko LI and Kudimova LA. Influence of xenon on reproductive function. Anaesteziol Reanimatol. 2002a Jul-Aug;(4):71-2.

Burov NE, Arzamastev EV, Kornienko LI and Kudimova LA. Investigation of the teratogenic and embryotoxic action of xenon. Anaesteziol Reanimatol, 2002b Jul-Aug;(4):69-70.

Burov NE, Arzamastev EV, Kornienko LI, Terekhova OA and Eliseeva, IL. Study of immunodepressive and allergic effects of xenon. Anaesteziol Reanimatol. 2002c May-Jun;(3):71-2.

Cleveland ZI, Cofer GP, Metx G, Beaver D, Nouls J, Kaushik SS, et al. Hyperpolarized Xe MR imaging of alveolar gas uptake in humans. PLoS One. 2010 Aug;5(8):e12192.

de Rossi LW, Horn NA, Baumert JH, Gutensohn K, Hutschenreuter G, Rossaint R. Xenon does not affect human platelet function in vitro. Anesth Analg. 2001 Sept;93(3):635-40.

de Rossi LW, Gott K, Horn N, Hecker K, Hutschenreuter G, Rossaint R. Xenon preserves neutrophil and monocyte function in human whole blood. Can J Anesth. 2002 Nov;49(9):942-5.

de Rossi LW, Horn NA, Stevanovic A, Buhre W, Hutschenreuter G, Rossaint R. Xenon modulates neutrophil adhesion molecule expression in vitro. Eur J Anesthesiol. 2004 Feb;21(2):139-43.

Driehuys, B, Martinez-Jimenez S, Cleveland ZI, Metz GM, Beaver DM, Nouls JC, et al. Chronic obstructive pulmonary disease: safety and tolerability of hyperpolarized 129Xe MR imaging in healthy volunteers and patients. Radiol. 2012 Jan;262(1):279-89.

Guyton AC, Hall JE. Textbook of Medical Physiology. 11th ed. Philadelphia: Elsevier; 2006, Chapter 37, Pullmonary Ventilation; p.471-90.

Horn NA, Hecker KE, Bongers B, Baumert HJ, Reyle-Hahn SM, Rossaint R. Coagulation assessment in healthy pigs undergoing single xenon anaesthesia and combinations with isoflurane and sevoflurane. Acta Anaesthesiol Scand. 2001 May;45(5):634-8.

He M, Heacock T, Kaushik SS, Robertson SH, Freeman MS, McAdams HP, et al. Hyperpolarized 129Xe MRI to quantify regional ventilation differences in older versus younger asthmatics. Am J Respir Crit Care Med. 2014a May;189:A6370.

He M, Kauskik SS, Robertson SH, Freeman MS, Virgincar RS McAdams HP, Driehuys B. Extending semiautomatic ventilation defect analysis for hyperpolarized 129Xe ventilation MRI. Acad Radiol. 2014b Dec;21(12):1530–41.

He M, Robertson SH, Kaushik SS, Freeman MS, Virgincar RS, Davies J, Stiles J, Foster WM, McAdams HP, Driehuys B. Dose and Pulse Sequence Considerations for Hyperpolarized 129Xe Ventilation MRI. Magn Reson Imaging 2015;33(7):877-885.

Kaushik SS, Cleveland ZI, Cofer GP, Metz G, Beaver D, Nouls J, et al. Diffusion-weighted hyperpolarized 129Xe MRI in healthy volunteers and subjects with chronic obstructive pulmonary disease. Magn. Reson. Med. 2011 Apr;65(4):1154-65.

Kirby M, Coxson HO, Parraga G. Pulmonary functional magnetic resonance imaging for paediatric lung disease. Ped Respiratory reviews. 2013 Sept;14(3):180-9.

Lane GA, Nahrwold ML, Tait AR, Taylor-Busch M, Cohen PJ, Beaudoin AR. Anesthetics as teratogens: nitrous oxide is fetotoxic, xenon is not. Science. 1980 Nov;210(4472);899-901.

Reinelt H, Marx T, Kotzerke J, Topalidis P, Luederwald S, Armbruster S, et al. Hepatic function during xenon anesthesia in pigs. Acta Anaesthesiol Scand. 2002 Jul;46(6):713-6.

Roughton FTW, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 1957;11:290-302.

Stewart NJ, Norquay G, Griffiths PD, Wild JM. Feasibility of human lung ventilation imaging using highly polarized naturally abundant xenon and optimized three-dimensional steady-state free precession. Magn Reson Med. 2015 Aug;74(2):346–52.

Svenningsen A, Kirby M, Starr D, Leary D, Wheatley A, Maksym GN, et al. Hyperpolarized 3He and 129Xe MRI: differences in asthma before bronchodilation. JMRI. 2013 Feb;38:1521–30.

Virgincar RS, Cleveland ZI, Kaushik SS, Freeman MS, Nouls J, Cofer GP, et al. Quantitative analysis of hyperpolarized 129Xe ventilation imaging in healthy volunteers and subjects with chronic obstructive pulmonary disease. NMR Biomed. 2013 Apr;26(4):424-35.

Wang JS. Pulmonary function tests in preoperative pulmonary evaluation. Respir Med. 2004 July;98(7):598-605.

Appendix 1 Schedule of Events

	Screening[1]	Imaging[1]	Imaging Follow-up[2]	Post-Op Follow-up
Evaluation	**Day -7 to Day 0**	**Day 1**	**Day 2**	**1-2 Months Post-Op**
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
Demographics and Medical History[3]	X	X		
Physical Exam	X			
Concomitant Medications Review	X	X		
MRI-suitable Screening	X			
Recording of potential resection location on eCRF	X			
Spirometry (FEV1)[4]		X		X
^{129}Xe Administration[1]		X		
Anatomical proton MRI (MRI session only)		X		
^{133}Xe Administration[1]		X		
Vital Signs[5,6]	X	X		
SpO_2[7]	X	X		
Adverse Events (AE)[8]		X	X	
Local Laboratory Tests				
Serum Pregnancy Test[9]	X			

Abbreviations: Abbreviations: AE = adverse event; eCRF = electronic case report form; FEV1 = forced expiratory volume; MRI = magnetic resonance imaging; SpO_2 = arterial oxygen saturation.

[1] Screening and Imaging study periods can occur on the same day or on different days, however there should be ≤48 hours between administration of the study drug and reference standard scans.

[2] The Follow-Up period will occur on the day after (+3 days) the completion of all imaging and prior to resection surgery.

[3] Medical history will include any pulmonary function values (i.e. FEV1) from the subject files as well as smoking history.

[4] FEV1 measurement should occur prior to administration of the study drug and reference standard scans.

[5] Vital Signs (including heart rate, respiration rate, temperature, and blood pressure) will be assessed before and after each scanning session (both ^{129}Xe and ^{133}Xe). Subjects will have a 5-minute rest in a supine position before vital signs are assessed.

[6] Changes in heart rate of greater than ±20% are considered significant. If the subject is to receive another dose, the next dose will not be administered until the heart rate is within 20% of its baseline value. If the subject has received their last dose, they will be observed until their heart rate is within 20% of its baseline value. The subject will be monitored for the duration of the MRI exam by a qualified medical professional.

[7] SpO_2 is measured at baseline as well as before and after each scanning session (both ^{129}Xe and ^{133}Xe). An absolute decrease of SpO_2 by greater than 10% is considered significant. If the subject is to receive another dose, the next dose will not be administered until the SpO_2 is within 10% of its baseline value. If the subject has received their last dose, they will be observed until the SpO_2 is within 10% of its baseline value. The subject will be monitored for the duration of the MRI exam by a qualified medical professional.

[8] Subjects will be monitored for any subjective sensation of residual anesthesia (e.g., lightheadedness, loss of sensation or awareness, loss of memory, or unconsciousness). If the subject is to receive another dose, the next dose will not be administered until the sensation has resolved. If the subject has received their last dose, they will be observed until the

sensation has resolved. The subject will be monitored for the duration of the MRI exam by a qualified medical professional.

[9] Eligibility assessment will include, if applicable, a negative pregnancy test.

Appendix 2 Summary of Changes from Previous Version

Summary and Justification of Changes

The protocol Amendment 2, dated 29 January 2018, has been amended. This amendment includes revisions to:

- Refine the equivalence margin for the secondary efficacy endpoint comparing each individual zone within the 6-zone analysis between ^{133}Xe and ^{129}Xe.
- Include potential resection location recording on the eCRF at the screening visit.
- Provide correct location of ^{129}Xe and ^{133}Xe handling and labeling instructions.

The individual changes are provided below for each impacted section. For each section, the previously approved protocol verbiage is provided with all deleted text marked by using ~~strikethrough~~. The updated verbiage from the protocol amendment is also provided with all added test marked by using **bold**. An ellipsis (…) is used to indicate the omission of unchanged text for concise representation of the changes made.

Individual Changes and Justification for Change

Original Protocol Information			Protocol Amendment	
Page No.	**Section No.**	**Previous Text**	**Amended Text**	**Justification for Change**
2 - End	Header	Clinical Study Protocol: POL-Xe-001 Amendment ~~2~~	Clinical Study Protocol: POL-Xe-001 Amendment **3**	Amended protocol.
1	Cover Page	Amendment 2 ~~Date: 29 January 2018~~ ~~Replaces Amendment 2, released 07 November 2017~~	**Original 20 October 2017** **Amendment 1 07 November 2017** Amendment 2 **29 January 2018** **Amendment 3 26 April 2018**	Amended protocol, revised cover page format.
2	Sponsor Signature Page	Version: Amendment ~~2~~ Version Date: ~~29 January~~ 2018	Version: Amendment **3** Version Date: **26 April 2018**	Amended protocol
3	Investigator Signature Page	Version: Amendment 2 Version Date: ~~29 January~~ 2018	Version: Amendment **3** Version Date: **26 April 2018**	Amended protocol
6	Synopsis: Efficacy Assessments:	Secondary Efficacy Endpoints: The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones. ~~For the post-operative FEV1 endpoint we seek to demonstrate an equivalence margin of 0.3 L/sec. For the 6-zone percentage function endpoint, we seek to demonstrate an equivalence margin of 4% for each of the zones.~~	Secondary Efficacy Endpoints: The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones.	Deletion of redundant text.
6	Synopsis: Statistical Methods	Secondary Analysis: … The equivalence margin will be ~~4~~ percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval ~~(-4%, +4%).~~	Secondary Analysis: … The equivalence margin will be **5** percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval **(-5%, +5%).**	Adjustment of secondary efficacy endpoint equivalence margin.

Original Protocol Information			Protocol Amendment	
Page No.	**Section No.**	**Previous Text**	**Amended Text**	**Justification for Change**
15	3.1	… The 4 study periods are defined as: • Screening: Subjects will be screened for study participation based on inclusion and exclusion criteria. Informed consent will be obtained.	… The 4 study periods are defined as: • Screening: Subjects will be screened for study participation based on inclusion and exclusion criteria.Informed consent will be obtained **and the investigator's assessment of potential lcations of lung resection will be recorded on the electronic case report form (eCRF).**	Clarify instruction to investigators about recording of potential sections of lung to be resected.
20, 26	5.2.1, 5.2.2, and 6.9.3	… Detailed information for preparation and administration of hyperpolarized ^{129}Xe gas is provided in the ~~Imaging Manual~~.	… Detailed information for preparation and administration of hyperpolarized ^{129}Xe gas is provided in the **IMP Handling and Labeling Instruction Manual**.	Correction of location of ^{129}Xe handling and labeling instructions.
21	5.3.3	… ^{133}Xe will be dosed according to the Pacage Insert. Please refer to the ~~Imaging Manual~~ for additional details.	… ^{133}Xe will be dosed according to the Pacage Insert. Please refer to the the **IMP Handling and Labeling Instruction Manual** for additional details.	Correction of location of ^{133}Xe handling and labeling instructions.
22	5.9	… Labeling instructions for the hyperpolarized ^{129}Xe in the Gas Delivery Bag are provided in the ~~Imaging Manual~~.	… Labeling instructions for the hyperpolarized ^{129}Xe in the Gas Delivery Bag are provided in the **IMP Handling and Labeling Instruction Manual**.	Correction of location of instructions for labeling gas delivery bag.
26	6.9.1	… Additional information for preparation and administration of hyperpolarized ^{129}Xe gas are provided in Section 5.2.1 as well as the ~~Imaging Manual~~ for this study.	… Additional information for preparation and administration of hyperpolarized ^{129}Xe gas are provided in Section 5.2.1 as well as the **IMP Handling and Labeling Instuction Manual** for this study.	Correction of location of ^{129}Xe handling and labeling instructions.
34	8.4.2	The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones. ~~For the post-operative FEV1 endpoint we seek to demonstrate an equivalence margin of 0.3 L/sec. For the 6-zone percentage function endpoint, we seek to demonstrate an equivalence margin of 4% for each of the zones.~~	The secondary efficacy endpoints include the post-operative FEV1 value (spirometry) and the percentage function contributed by each of the individual 6 lung zones.	Deletion of redundant text.

Original Protocol Information			Protocol Amendment	
Page No.	**Section No.**	**Previous Text**	**Amended Text**	**Justification for Change**
35	8.7	… The equivalence margin will be ~~-4~~ percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval ~~(-4%, +4%)~~.	… The equivalence margin will be **5** percentage points. Thus, for each of the six zones, equivalence will have been demonstrated if the 95% CI for the mean difference is contained within the interval (-**5**%, +**5**%).	Adjustment of secondary efficacy endpoint equivalence margin.
44	Appendix 1	See previous Schedule of Events table below.	See updated Schedule of Events table below.	Provide instruction on timing of eCRF recording of potential resection locations.

Previous Schedule of Events Table:

	Screening[1]	**Imaging[1]**	**Imaging Follow-up[2]**	**Post-Op Follow-up**
Evaluation	**Day -7 to Day 0**	**Day 1**	**Day 2**	**1-2 Months Post-Op**
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
Demographics and Medical History[3]	X	X		
Physical Exam	X			
Concomitant Medications Review	X	X		
MRI-suitable Screening	X			
Spirometry (FEV1)[4]		X		X
^{129}Xe Administration[1]		X		
Anatomical proton MRI (MRI session only)		X		
^{133}Xe Administration[1]		X		
Vital Signs[5,6]	X	X		
SpO_2[7]	X	X		
Adverse Events (AE)[8]		X	X	
Local Laboratory Tests				
Serum Pregnancy Test[9]	X			

Updated Schedule of Events Table:

	Screening[1]	**Imaging[1]**	**Imaging Follow-up[2]**	**Post-Op Follow-up**
Evaluation	**Day -7 to Day 0**	**Day 1**	**Day 2**	**1-2 Months Post-Op**
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
Demographics and Medical History[3]	X	X		
Physical Exam	X			
Concomitant Medications Review	X	X		
MRI-suitable Screening	X			
Recording of potential resection location on eCRF	**X**			
Spirometry (FEV1)[4]		X		X
^{129}Xe Administration[1]		X		
Anatomical proton MRI (MRI session only)		X		
^{133}Xe Administration[1]		X		
Vital Signs[5,6]	X	X		
SpO_2[7]	X	X		
Adverse Events (AE)[8]		X	X	
Local Laboratory Tests				
Serum Pregnancy Test[9]	X			