Statistical Analysis Plan

Study: PULSE-PAH-004

INHALED NITRIC OXIDE/INOPULSE DEVICE COMBINATION PRODUCT
A PHASE 3, PLACEBO CONTROLLED, DOUBLE-BLIND, RANDOMIZED, CLINICAL STUDY TO DETERMINE EFFICACY, SAFETY AND TOLERABILITY OF PULSED, INHALED NITRIC OXIDE (iNO) VERSUS PLACEBO IN SYMPTOMATIC SUBJECTS WITH PULMONARY ARTERIAL HYPERTENSION (PAH): INOvation-1 (Part I and Part II)

Version No. 2.0.0

[redacted]

Version History	
Version	**Approval date**
V1.0.0	26 APR 2017
V2.0.0	29 DEC 2017

[redacted]

Table of Contents

[redacted]

List of Tables

List of Figures

List of Abbreviations

The following abbreviations are used in this Statistical Analysis Plan (SAP).

Abbreviation	Explanation
6MWD	6 minute walk distance
6MWT	6 minute walk test
AE	adverse event
AESI	adverse event of special interest
ANOVA	Analysis of variance
APAH	Associated pulmonary arterial hypertension
AVT	Acute vasodilator testing
BDS	Borg dyspnea score
BiPAP	Bilevel positive airway pressure
BNP	B-type natriuretic peptide
BP	Blood Pressure
Bpm	beats per minute
BSA	body surface area
CI	Confidence Interval
CMH	Cochran–Mantel–Haenszel
CO	Cardiac output
CPAP	continuous positive airway pressure
CSP	Clinical Study Protocol
CTD	connective tissue disease
CTEPH	chronic thromboembolic pulmonary hypertension
DLCO	diffusing capacity for the lungs measured using carbon monoxide
DMC	Data Monitoring Committee
DBP	diastolic blood pressure
dPAP	diastolic pulmonary artery pressure
eCRF	electronic case report form
ECG	electrocardiogram

Abbreviation	Explanation
EOS	end of study
ERA	Endothelin receptor antagonist
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
HIV	human immunodeficiency virus
HR	Heart Rate
HRQoL	health related quality of life
IBW	ideal body weight
ICF	informed consent form
iNO	inhaled nitric oxide
IPAH	idiopathic pulmonary arterial hypertension
ITT	Intent-to-treat
IRT	Interactive Response Technology
LTOT	long term oxygen therapy
LOCF	Last Observation Carried Forward
LVEDP	left ventricular end diastolic pressure
LVEF	left ventricular ejection fraction
LVSD	left ventricular systolic dysfunction
LVSF	left ventricular shortening fraction
mPAP	Mean pulmonary arterial pressure
MetHgb	methemoglobin
mPCWP	Mean pulmonary capillary wedge pressure
NO	nitric oxide
NT-proBNP	N-terminal of the prohormone brain natriuretic peptide
PAH	pulmonary arterial hypertension
PAP	pulmonary arterial pressure
PCWP	pulmonary capillary wedge pressure
PDE-5	phosphodiesterase type-5
PH	pulmonary hypertension

Abbreviation	Explanation
PPM	parts per million
PP	Per Protocol
PT	Preferred Term
PVR	pulmonary vascular resistance
RAP	right atrial pressure
RHC	right heart catheterization
SAE	Serious Adverse Event
SpO2	oxygen saturation by pulse oximeter
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SOC	System Organ Class
sPAP	systolic pulmonary artery pressure
TEAE	Treatment emergent Adverse event
TLC	total lung capacity
TTCW	time to clinical worsening
WHO	World Health Organization

SAP Change History

Changes Made	
V1.0.0	Initial Version dated 23 April 2017
V1.1.0	Section 2.1: Blinded Treatment Period: Maximum of 18 weeks. Open Label Extension Period: Subjects will be offered open label therapy when the subject completes 18 weeks of blinded drug therapy. All subjects must have completed all assessments in Part 1 and remain on blinded treatment until week 18 before proceeding to Part 2. Section updated as per protocol amendment 3.
	Section 7.3: As primary analysis is MMRM, no imputation will be performed. Text updated to remove imputation proposal for primary analysis. & Imputation Rules added for 6MWD data for sensitivity analysis with ANCOVA model. Section updated as per FDA recommendations and Protocol amendment 3.
	Section 7.3: Additional clarification for imputation rule for subjects who die. All subjects who die will have zero 6MWD imputed for missing data. Section updated as per FDA recommendations and Protocol amendment 3.
	All analysis proposed for other efficacy endpoints changed to ANCOVA, considering planned LOCF imputation for other endpoints

1.0 Objective and Statistical Analysis Plan

This Statistical Analysis Plan (SAP) describes the planned analyses to support completion of the Clinical Study Report (CSR) for protocol PULSE-PAH-004 entitled "A Phase 3, Placebo Controlled, Double-Blind, Randomized, Clinical Study to Determine the Efficacy, Safety and Tolerability of Pulsed, Inhaled Nitric Oxide (iNO) Versus Placebo in Symptomatic Subjects with Pulmonary Arterial Hypertension (PAH): INOvation-1".

This SAP is based on the following study document:

- Original Protocol, 25th August 2015
- Amendment 1, 11th Jan 2017
- Amendment 2, 21st April 2017
- Amendment 3 dated 7th June 2017.

2.0 Introduction

Pulmonary arterial hypertension is a progressive disease characterized by progressive pulmonary vascular arteriopathy leading to elevation in pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP) with right ventricular failure and death if untreated (McLaughlin 2009; Barst 2009; McGoon 2009; Badesch 2007).

The overall current 5-year survival with current standard of care is only 60% (Benza 2010).

There is no cure for PAH and the 5-year survival rate for idiopathic or heritable PAH was approximately 30% prior to the availability of targeted PAH therapies based on Registry data from the 1980s (Anderson 2010).

Currently approved treatments have been demonstrated to have both short- and long-term efficacy (McLaughlin 2006; Barst 2009; McGoon 2009; Badesch 2007; Anderson 2010; Ghofrani 2013). These include:

- Synthetic prostacyclin, i.e., epoprostenol,
- Prostacyclin analog,
- Treprostinil or Iloprost;
- Endothelin receptor antagonist (ERA), i.e., bosentan, ambrisentan, or macitentan;
- Phosphodiesterase type-5 (PDE-5) inhibitors, i.e., sildenafil and tadalafil;
- Soluble guanylate cyclase (sGC) stimulators, i.e., riociguat

These medications may improve symptoms, exercise capacity, and clinical outcome, but may be limited by either parenteral delivery system, the need for frequent laboratory monitoring, frequent dosing schedules, or unwanted side effects.

Because of the availability of several agents working via different mechanisms, interest is developed in add on (i.e., combination) therapy. This strategy has proven successful in improving clinical outcomes in

some subjects. Yet, as stated above, despite these significant advances in the treatment of PAH, the current 5-year survival for Group 1 PAH is ~60% utilizing the available approved PAH therapies (Anderson 2010). Patients who are receiving prostacyclin therapy, ERA, and PDE-5, have no further treatment options other than atrial septostomy and lung transplantation.

PAH is associated with impaired release of nitric oxide at least in part because of reduced expression of nitric oxide synthase in the vascular endothelium of pulmonary arteries (McLaughlin 2009; Barst 2009; McGoon 2009; Badesch 2007; Anderson 2010). Thus, chronic administration of iNO may be viewed as replacement therapy in some subjects with PAH making it a logical choice for clinical evaluation.

Overall, review of the published medical literature and clinical experience indicates that iNO at doses of 0.013 to 0.1 mg/kg per hour for up to 2 years appears safe and suggests efficacy for the treatment of pulmonary hypertension (PH).

Inhaled NO offers an additional treatment option for patients, in particular patients who are already receiving prostacyclin therapy, ERA, and PDE-5, who have no other medical treatment options. Inhaled NO has the advantage of being able to be added to all other available PAH therapies excluding riociguat.

Bellerophon Pulse Technologies recently completed a Phase 2 clinical trial of iNO/INOpulse for PAH in the United States and Canada (Study IK-7001-PAH-201).

The key inclusion criteria for subjects in this trial were: pulmonary hypertension WHO Group I, on at least one other PAH medication for at least 12 weeks prior to treatment with iNO/INOpulse, and demonstrated ability to walk between 100 and 450 meters within 6 minutes.

In Part 1 of the placebo-controlled, double-blind clinical trial, 80 subjects were randomized in a 1:1:1 fashion to placebo or to one of two active iNO doses—25 or 75 mcg/kg IBW/hr for 16 weeks. Subjects who completed Part 1 of the trial were able to enrol in an open-label, long-term extension portion of the study (Part 2), in which all subjects received one of the two iNO doses. The objective in Part 2 was to explore the long-term safety and tolerability of therapy.

The primary endpoint of Part 1 was change in pulmonary vascular resistance (PVR) from baseline at 16 weeks, and the main secondary endpoint was change in placebo-adjusted 6-minute walk distance (6MWD) over the same period.

The baseline characteristics seemed to indicate that subjects in the iNO groups had more severe disease. LTOT use at baseline was 78% and 59% in the 75 and 25 mcg/kg IBW/hr cohorts, respectively, compared to 46% in the placebo cohort. Baseline PVR values were higher in the higher dose group, and 6MWDs were lower in the same group. The results in the ITT population showed a trend toward a lowering of PVR with iNO treatment and an increase in 6MWD, but this did not reach statistical significance.

Inhaled NO was relatively well-tolerated in Part 1 of the trial. More serious adverse events (SAEs) occurred in the 75 mcg/kg IBW/hr dose group (9 events) versus the 25 mcg/kg IBW/hr and placebo groups (4 events each). The excess AEs were related to the respiratory system. All AEs related to the respiratory system will be adjudicated in the Phase 3 trials by an Independent Clinical Events Committee (ICEC).

Eighty-one percent of subjects elected to enter into the long-term extension trial.

The purpose of this phase III clinical trial is to explore iNO on patients with LTOT.

Subjects with LTOT are more likely to be compliant and may have better outcomes. In this Phase 3 trial, recruitment will be limited to only subjects on LTOT. For the indication of PAH, iNO is being developed as a drug/device combination therapy to be used with the INOpulse delivery device.

The INOpulse device is a complete, integrated system that uses a 0.074 liter aluminum cartridge containing a concentration of 6.0 mg/L (4880 ppm) of NO gas. The device is lightweight and portable, allowing NO to be administered via a nasal cannula to spontaneously breathing subjects. These features make it ideal for use in an ambulatory setting. The main advantage of the INOpulse device for spontaneously breathing subjects lies in its ability to deliver precise, preset NO doses over time, independent of the subject's respiration rate and tidal volume. Prescribed doses of iNO are delivered through the INOpulse according to amount of drug per ideal body weight (IBW) per hour (i.e., mcg/kg IBW/hr). The iNO dose is pulsed over the first half of the subject's inspiration rather than inhaled throughout the entire inspiratory period and the hourly set dose is accurately delivered throughout the hour.

The primary objective in this study is to evaluate the efficacy of iNO on exercise using 6-minute walk distance (6MWD) in subjects with PAH currently receiving background PAH medication and LTOT.

2.1 Study Details

This is a multi-center, randomized, double-blind, placebo-controlled, parallel-group design to determine the efficacy, safety and tolerability of pulsed iNO versus placebo in symptomatic subjects with PAH currently receiving background PAH medication and LTOT (long term oxygen therapy).

This study has two parts as follows:

- Part I: Blinded Treatment Period (Maximum of 18 weeks).
- Part II: Open label (Subjects will be offered open label therapy when the subject completes 18 weeks of blinded therapy)

All subjects must complete Part I, at least 18 weeks of blinded treatment before open label therapy is offered.

After the Screening period, the subjects will be randomized at Week 0 to enter the double blind and run-in period (Week 0 to Week 2) to assess their eligibility to continue in the study by their usage of the INOpulse device for an average ≥ 16 hours (rounded to the nearest hour) per day with no more than 2 days of usage < 8 hours per day.

Those subjects not meeting the 12 hour INOpulse device usage requirement (by Week 2) will be discontinued and ineligible to proceed to the next phase of treatment.

Subjects will be randomized to a treatment assignment as described in Table 1 below. Subjects randomized to active iNO will receive a reduced dose of iNO during run in and subjects randomized to placebo will receive placebo during run in.

Table 1: Dosing Assignments for Run-in, Treatment Periods and Open Label

	Part I Blinded Treatment Period		**Part II Open Label**
Cohort	**Run-in Period Week 0 to Week 2**	**Treatment Period Week 3 to Week 18**	**Open Label Treatment**
Cohort 1: Placebo	Placebo at a dose setting of 15 mcg/kg IBW/hr	Placebo at a dose setting of 75 mcg/kg IBW/hr	iNO 75 mcg/kg IBW/hr
Cohort 2: iNO 75	iNO 15 mcg/kg IBW/hr	iNO 75 mcg/kg IBW/hr	iNO 75 mcg/kg

[redacted]

	Part I Blinded Treatment Period		Part II Open Label
Cohort	**Run-in Period Week 0 to Week 2**	**Treatment Period Week 3 to Week 18**	**Open Label Treatment**
mcg/kg IBW/hr			IBW/hr

Note: Study drug is dosed according to IBW and not actual body weight. IBW is determined by a subject's sex and height, and therefore will not change during the study period.

2.2 Protocol Amendments

Original protocol dated 25th August 2015, Amendment 1, Amendment 2 and Amendment 3 dated 11th Jan 2017, 21st April 2017 and 7th June 2017 respectively were used to develop this SAP.

3.0 Study Objectives

3.1 Part I Blinded Treatment Period

3.1.1 Primary Objective

The primary objective in this study is to evaluate the efficacy of iNO on exercise using 6-minute walk distance (6MWD) in subjects with PAH currently receiving background PAH medication and LTOT.

3.1.2 Secondary Objectives

The secondary objectives in this study are:

- To evaluate the time to clinical worsening (TTCW)
- To evaluate change in World Health Organization (WHO) Functional Class

3.1.3 Tertiary Objectives

The tertiary objectives in this study are:

- To evaluate changes in health-related quality of life using the Short Form-36 (SF-36) version 2 health survey
- To evaluate the impact of iNO on pulmonary hemodynamics in a subset of subjects
- To evaluate the changes in right ventricular (RV) and left ventricular (LV) function as measured by echocardiography, in a subset of subjects
- To evaluate change in N-terminal of the prohormone brain natriuretic peptide (NT-proBNP)
- To evaluate change in Borg dyspnea score immediately following 6-minute walk test (6MWT)
- To evaluate change in 6MWD as related to degree of correlation between drug adherence and clinical efficacy measurement
- To evaluate subjects with unsatisfactory clinical response

- To evaluate the impact of iNO on frequency of heart-lung or lung transplantation, and deaths while awaiting transplantation
- To evaluate the impact of iNO on medical resource utilization

3.1.4 Safety Objectives

The safety objectives in this study are to evaluate the safety and tolerability of iNO.

3.2 Part II Open Label

3.2.1 Primary Objective

To evaluate the long term safety and tolerability.

3.2.2 Secondary Objective

To evaluate the change in exercise tolerance in subjects who switch from placebo to active therapy.

4.0 Study Endpoints

4.1 Primary Efficacy endpoint

6-minute walk distance (6MWD) is the primary endpoint for this study. This primary endpoint is collected at all the visits. Baseline for analysis of 6MWD is the Week 2 (Visit 2 assessments), average for two assessments at Week 2.

The change in 6MWD from baseline to 18 weeks (Table 2) is of primary interest.

Table 2: Change in 6MWD from Baseline to 18 Weeks

Variables	Module	Source of Data	Baseline Definition	Variable specific derivation	Change from Baseline analysis
6MWD	Six Minute Walk Test (SMWT)	CRF	Average of two Week 2 (V2) Assessment	No, CRF data.	Yes

For open label period, Change in 6MWD from Baseline (week 2) and from week 18 will be analysed at month 4, 8 and 12.

4.2 Secondary Efficacy Endpoints

The secondary endpoint in this study includes:

1. Time (in days) to first clinical worsening event (TTCW) measured from baseline to end of treatment period (Week 18).

Where clinical worsening events are defined (Table 3 and Table 4) as follows:

Table 3: Clinical Worsening Event

Criteria #	*Description*
1	Death (all-cause mortality)
2	Atrial septostomy
3	Hospitalization due to worsening of PAH (adjudicated)
4	Start of new specific PAH treatment (endothelin receptor antagonists [ERAs], phosphodiesterase type-5 [PDE-5] inhibitors or prostanoids), an increase in the dose of an ERA or PDE-5, increase in the dose or frequency of an inhaled prostanoids, or an increase in the dose of an intravenous or subcutaneous prostanoids by >10%.
5	Decrease of >15% from baseline or >30% compared with the last study related measurement in 6MWD should be confirmed by a repeat measurement performed at least 14 days later.
6	Worsening of WHO Functional Class (e.g., from Class II to Class III or IV, OR Class III to Class IV); and should be confirmed by a repeat assessment at least 14 days later.

Table 4: Time Clinical Worsening Event Derivation

Variables	Module	Source of Data	Derivation	Comments (to be removed later)
All-cause mortality	End of Therapy Status SAE	CRF	One-One mapping	Standard binary field
Atrial septostomy	End of Therapy Status	CRF	One-One mapping	Standard Binary field
Hospitalization due to worsening of PAH (adjudicated)	Adjudication Module	Non CRF	One-One mapping	Standard Field
New PAH treatment	PAH specific therapies	CRF	One-One mapping	Refer to new PAH Therapy Module
Decrease of >15% from baseline or >30% compared with the last study related measurement in 6MWD should be confirmed by a repeat measurement performed at least 14 days later	6 minute walk test	CRF	1) Decrease of >15% from Baseline **Or** 2) Greater than 30% compared with baseline **And** 3) should be confirmed by repeat	Points (1) and (2) are simple derivations ; Point (3) is linked to visit window mapping, so be careful.

[redacted]

Variables	Module	Source of Data	Derivation	Comments (to be removed later)
			measurement performed at least 14 days	
Worsening of WHO Functional Class (e.g., from Class II to Class III or IV, OR Class III to Class IV); and should be confirmed by a repeat assessment at least 14 days later	WHO functional class assessment done at every visit, choose only post baseline	CRF	(1) Class II to class III (2) Class III to class IV (3) Confirmation by repeat assessment at least 14 days later	Points (1) and (2) are simple derivations ; Point (3) is linked to visit window mapping, so be careful.

2. Change in World Health Organization (WHO) Functional Class (Table 5 and Table 6) from baseline to 18 weeks

Table 5: WHO Functional Class

Class	Subject Symptoms
Class I*	Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope.
Class II	Patients with pulmonary hypertension resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity causes undue dyspnea or fatigue, chest pain, or near syncope.
Class III	Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes undue dyspnea or fatigue, chest pain, or near syncope.
Class IV	Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may even be present at rest. Discomfort is increased by any physical activity. Syncope or near syncope may occur.

*Only subjects with WHO Class II – IV are eligible for the study.

The WHO functional class collected at all the visits.

Table 6: WHO Functional Class

Variables	Module	Source of Data	Baseline Definition	Variable specific derivation	Change from Baseline analysis	Comment (to be removed later)
WHO Functional Class Status	WHO Functional Class assessment	CRF	Week 2 (V2) Assessment	No, CRF data.	Yes	This is not Continuous data

For the second part of the study, change in WHO functional class data from Baseline (week 2) and from week 18 will be analysed at month 4, 8 and 12.

4.3 Tertiary Efficacy endpoint

I. SF-36 V2

Change in health-related quality of life (using SF-36 version 2 health survey) from baseline to 18 weeks.

SF-36 v2 is collected at baseline (v1), Week 18 and at early discontinuation visit during double blind period of study.

The following eight categories are derived from SF-36 v2 (Table 7and Table 8) as follows.

Table 7: SF-36 V2 Categories

	Category
1	Physical Functioning (PF)
2	Role- Physical (RP)
3	Bodily Pain (BP)
4	General Health (GH)
5	Vitality (VT)
6	Social Functioning (SF)
7	Role-Emotional (RE)
8	Mental Health (MH)

Table 8: SF-36 V2

Variables	Module	Source of Data	Baseline Definition	Variable specific derivation
SF-36 v2 questionnaire	SF-36	Non-CRF Data	Baseline (V1)	No, non - CRF data.

II. Right Heart Catheterization (RHC)

Change in pulmonary hemodynamics measured by right heart catheterization (RHC) (Table 9) from baseline to 18 weeks, in a subset of subjects (approximately 50), at selected site:

1. Cardiac output [CO],
2. Cardiac index [CI] (to be derived),
3. Mean pulmonary artery pressure [mPAP],
4. Mean pulmonary capillary wedge pressure [mPCWP],
5. Systolic pulmonary artery pressure [sPAP],
6. Diastolic pulmonary artery pressure [dPAP],
7. Pulmonary vascular resistance [PVR],
8. Pulmonary vascular resistance [PVR] Index,
9. Oxygen saturation by pulse oximeter [SpO_2],
10. Mixed venous O_2
11. Right atrial pressure [RAP],

Table 9: Pulmonary Hemodynamic Measured by Right Heart Catheterization (RHC)

Variables	Module	Source	Continuous/ Categorical	Baseline Definition	Variable specific derivation	Change from Baseline analysis
Cardiac Output (CO)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Cardiac Index (CI)	RHC	CRF	Continuous	Baseline (V1)	CI = CO/BSA ; BSA = 0.007184*(weight^0.425)*(Height^0.725)	Yes
Mean Pulmonary artery pressure (mPAP)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Mean Pulmonary Capillary wedge pressure (mPCWP)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Systolic pulmonary artery pressure	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes

Variables	Module	Source	Continuous/ Categorical	Baseline Definition	Variable specific derivation	Change from Baseline analysis
(sPAP)						
Diastolic pulmonary artery pressure (dPAP)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Pulmonary vascular resistance (PVR),	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Pulmonary vascular resistance [PVR] Index	RHC	CRF	Continuous	Baseline (V1)	PVR (Index) = 80 (mPAP - mPCWP) ÷ CI)	
Oxygen saturation by pulse oximeter (SpO2)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Mixed venous O2	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes
Right atrial pressure (RAP)	RHC	CRF	Continuous	Baseline (V1)	No, CRF data.	Yes

III. Echocardiogram

Changes in the right and left ventricular function by echocardiography will be analysed by cardiac imaging core laboratory at the Brigham and Women's Hospital.

IV. NT-proBNP:

Change in NT-proBNP (Table 10) from screening to 18 weeks.

Table 10: NT-proBNP

Variables	Module	Source of Data	Continuous/ Categorical	Baseline Definition	Variable specific derivation	Change from Baseline analysis
NT-proBNP	Lab	Non CRF	Continuous	Screening (V0)	No, CRF data.	Yes

V. Borg Dyspnea Score:

Change in Borg dyspnea score (Table 11 and Table 12) immediately following 6MWT from baseline to 18 weeks for blinded period and change from baseline and from week 18 at month 4, 8 and 12 during open label period of study.

The Borg Dyspnea score defined as follows:

Table 11: Borg Dyspnea Score

Score	Severity
0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	--
7	Very Severe
8	--
9	--
10	Very, very severe (maximal)

Table 12: Borg Dyspnea Score

Variables	Module	Source of Data	Baseline Definition	Variable specific derivation	Change from Baseline analysis
Borg dyspnea score	Six Minute Walk Test (SMWT)	CRF	Week 2 (V2) Assessment	No, CRF data.	Yes

VI. 6MWD and Drug Adherence:

Change in 6MWD as related to degree of drug adherence (Table 13) from baseline to 18 weeks.

The drug adherence categories defined as follows:

1) Average Compliance < 8 hours per day
2) Average Compliance ≥ 8 hours per day
3) Average compliance < 12 hours per day
4) Average Compliance ≥12 hours per day
5) Average Compliance ≥16 hours per day

Table 13: 6MWD and Drug Adherence

Variables	Module	Source of Data	Baseline Definition	Variable specific derivation	Change from Baseline analysis
6MWD	Six Minute Walk Test (SMWT)	CRF	Week 2 (V2) Assessment	Derived variable for average compliance	Yes
Drug Adherence	Drug Compliance assessment from Y-Prime	Non CRF			

VII. Clinical Response:

Number of subjects with unsatisfactory clinical response (Table 14), (WHO Functional Class III or IV symptoms with no improvement in 6MWD), with iNO as compared with Placebo, from baseline to 18 weeks.

Table 14: Clinical Response

Variables	Module	Source of Data	Variable specific derivation	Combined Derivation
WHO Functional Class Status	WHO Functional Class assessment	CRF	No, CRF data.	At week 18 WHO status is III or IV.
6MWD	Six Minute Walk Test (SMWT)	CRF	No, CRF data.	1) Decrease of >15% from baseline **Or** 2) Greater than 30% compared with baseline **And** should be confirmed by repeat measurement performed at least 14 days

VIII. Lung Transplants:

The following variables will be summarized and analyzed in context to lung transplant (Table 15) between the treatments, from baseline to 18 weeks.

1) Number of subjects undergoing heart-lung or lung transplant
2) Number of deaths while awaiting transplantation (at the beginning of the study)
3) Number of subjects on transplant list at the beginning of study

4) Number of subjects on transplant list during the study

Table 15: Lung Transplants

Variables	Module	Source of Data	Variable specific derivation	Derivation	Comments(to be deleted later)
Lung Transplant status	Total Lung Capacity (TLC)	CRF	No, CRF data.	No.	

IX. Number of Hospitalizations:

The number of PAH specific hospitalizations (Table 16) will be summarized and analyzed between the treatment.

Table 16: Number of Hospitalizations

Variables	Module	Source of Data	Variable specific derivation	Derivation	Comments(to be deleted later)
Hospitalization Status	Additional Inpatient Hospitalization (AIPH)	CRF	No, CRF data.	No.	

X. Medical Resource Utilization:

Analysis of Medical Resource Utilization from MDRU panel.

4.4 Safety Endpoint

The secondary endpoint in this study part I includes:

1) Incidence and severity of adverse events (AEs)
2) Incidence of device malfunction and/or device failure leading to an AE
3) Incidence of rebound pulmonary hypertension (PH)
4) Clinically significant changes in:
   a. Clinical laboratory tests
   b. Pulmonary function tests
   c. Vital signs

For study part II primary endpoint is incidence of AEs and SAEs.

5.0 Study Design

5.1 General Design and Plan

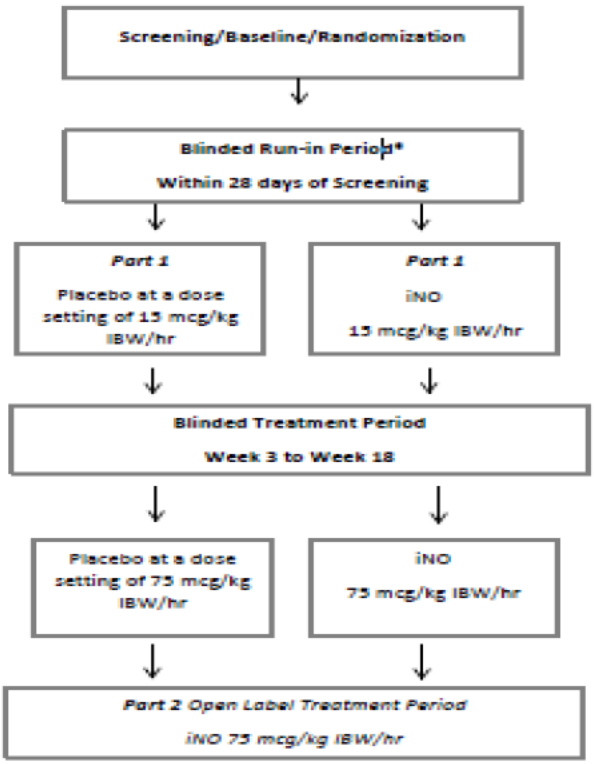
This is a multi-center, randomized, double-blind, placebo-controlled, parallel-group design to determine the efficacy, safety and tolerability of pulsed iNO versus placebo in symptomatic subjects with PAH currently receiving background PAH medication and LTOT.

The study consists of two parts Part 1, the Blinded Treatment Period, and Part 2, the Open Label Extension Period. The duration of treatment in double blind period will be a maximum of 18 weeks Subjects will be offered open label therapy when a subject completes 18 weeks of blinded drug therapy.

Screen failures will be defined as subjects who sign the ICF but fail to meet the eligibility criteria and/or do not qualify for randomization.

Subjects who do not complete their scheduled visits, have not died, and have not officially withdrawn from the study are considered lost to follow-up.

Figure 1: Study Design



*All subjects during the Run-in period must be willing to comply with usage of INOpulse device for ≥ 12 hours (rounded to the nearest hour) per day, with no more than 2 days of usage < 8 hours per day. Subjects randomized to iNO will receive 15 mcg/kg IBW/hr for the first 2 weeks (Run-in phase) followed by iNO at 75 mcg/kg IBW/hr dose for the Treatment Period. Subjects randomized to Placebo will receive Placebo product 15 mcg/kg IBW/hr at a delivery setting matching the treatment groups.

Additional details regarding assessments done at each visit can be found in section 11.1 of CSP.

Inclusion and Exclusion Criteria:

There are a total of 11 inclusion criteria's and 26 exclusion criteria's for double blind period of study. They can be found in protocol deviation specifications.

Inclusion Criteria:

1. Signed Informed Consent Form prior to the initiation of any study mandated procedures or assessments.
2. A confirmed diagnosis of PAH Group 1 who have either idiopathic PAH (IPAH), heritable PAH, drug and toxin-induced PAH, associated PAH (APAH) with connective tissue disease (CTD), APAH with repaired simple congenital systemic to pulmonary shunt (i.e., atrial septal defect, ventricular septal defect and/or patent ductus arteriosus; complete repair at least 1 year prior to Screening), APAH with human immunodeficiency virus (HIV), or APAH with portal hypertension.
3. Subjects receiving at least one PAH specific therapy (ERA or PDE-5 inhibitor, or inhaled, subcutaneous, or intravenous prostacyclin or a prostacyclin analog) with the same type of therapy for at least 3 months with stable dosing 4 weeks prior to Screening. (Subjects should be receiving optimal therapy according to the disease severity).
4. Subjects using oxygen therapy by nasal cannula for at least 4 weeks prior to Screening.
5. PAH diagnosis confirmed by RHC within the previous 5 years, according to the following definitions:
   - PVR $\geq$ 400 dynes.sec.cm-5 (5 Wood units)
   - mPAP $\geq$ 25 mmHg
   - PCWP or LVEDP $\leq$ 15 mmHg
   - Subjects who otherwise meet all the inclusion criteria and none of the exclusion criteria but have not undergone a RHC within the previous 5 years may be considered eligible for the study if they undergo a RHC and then meet the pulmonary hemodynamics criterion.
6. 6MWD $\geq$ 100 meters and $\leq$ 450 meters prior to randomization.
7. WHO Functional Class II-IV. Subjects with WHO Functional Class IV should be treated with prostacyclin or a prostacyclin analog (subcutaneous or intravenous), plus at least one PULSE-PAH-004 Phase 3 Protocol additional PAH specific therapy (ERA or PDE-5), if available to the subject and reimbursed by health insurance.
8. Age between 18 and 80 years (inclusive).
9. Willingness to use INOpulse delivery device for at least 12 hours per day.
10. Willingness to continue on study drug until the subject has completed Week 18 assessments.
11. Female subjects of childbearing potential must have a negative pre-treatment pregnancy test (serum or urine). All female subjects should take adequate precaution to avoid pregnancy.

Exclusion Criteria:

1. Subjects with known HIV infection who have a history within the past 3 months of any opportunistic pulmonary disease (e.g., tuberculosis, Pneumocystis carinii pneumonia, or other pneumonias) at the time of Screening.
2. PAH associated with, untreated thyroid disorders, glycogen storage disease, Gaucher's disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders or splenectomy.

3. Subjects with pulmonary conditions that may contribute to PAH including, but not limited to, chronic bronchiectasis, cystic fibrosis, or other pulmonary condition that the Investigator may deem to contribute to the severity of the disease or impair the delivery of iNO due to airway disease.
4. Subjects receiving riociguat.
5. Subjects receiving oral prostanoids as monotherapy.
6. <Exclusion deleted via Protocol Amendment #3>
7. PAH associated with significant venous or capillary involvement, known or suspected pulmonary veno-occlusive disease, or pulmonary capillary hemangiomatosis.
8. Any subject with WHO PH Groups 2, 3, 4 or 5
9. Subjects with any of the following cardiac abnormalities:
    a. Underlying cardiomyopathy or clinically significant aortic or mitral valve disease in the opinion of the investigator
    b. Left ventricular systolic dysfunction (LVSD), i.e., left ventricular ejection fraction (LVEF) < 40% or left ventricular shortening fraction (LVSF) < 22%, as determined by local reading
    c. Current symptomatic coronary artery disease, myocardial infarction within 1 year, or any coronary artery interventions within 6 months.
10. Systemic hypertension defined as systolic blood pressure (SBP) > 160 mmHg and/or diastolic blood pressure (DBP) > 100 mmHg persistent at Screening after a period of rest (treated or untreated).
11. Subjects with a history of deep vein thrombosis, pulmonary embolism/infarction or prothrombotic disorder must have had chronic thromboembolic pulmonary hypertension (CTEPH) excluded by ventilation/perfusion lung (V/Q) scan.
12. Severe obstructive lung disease defined as both a forced expiratory volume in 1 second/forced vital capacity (FEV1/FVC) < 70% and FEV1 < 55% of predicted value.
13. Moderate to severe restrictive lung disease: total lung capacity (TLC) < 60% of predicted; if TLC 60% to 70% predicted, a high resolution CT scan showing diffuse disease or more than mild patchy disease
14. Any subject who develops or has developed a PCWP > 20 mmHg during acute vasodilator testing (AVT).
15. Systemic hypotension defined as SBP < 90 mmHg persistent at Screening after a period of rest.
16. Moderate to severe hepatic impairment, i.e., Child-Pugh Class B or C.
17. On dialysis.
18. Acute or chronic physical impairment (other than dyspnea due to PAH) that would limit the ability to comply with study procedures or adherence to therapy (i.e., 6MWT), including carrying and wearing the pulsed delivery device per study protocol, or medical problem(s) likely to preclude completion of the study.
19. Pregnant or breastfeeding females at Screening.
20. Administered L-arginine within 1 month prior to Screening.
21. Known concomitant life-threatening disease with a life expectancy less than 1 year.
22. Atrial septostomy within 3 months preceding randomization.

23. The concurrent use of the INOpulse device with a continuous positive airway pressure (CPAP), bilevel positive airway pressure BiPAP, or any other positive pressure device.
24. Use of investigational drugs or devices within 1 month prior to Screening (other than acute vasodilator testing with iNO).
25. Any underlying medical or psychiatric condition that, in the opinion of the Investigator, makes the subject an unsuitable candidate for the study.
26. Any subject who has been enrolled in any previous clinical study with inhaled NO administered through pulse delivery.

5.2 Visit Schedule

This study has several scheduled visits for part I & II (Table 17 and Table 18) as follows:

Table 17: Visit Schedule – Part I

Visit Name	Number	Duration	Target day	Window	Day Range
Screening	V0	NA	NA	NA	NA
Baseline	V1	NA	1	28 Days of Screening	≤ 1
Week 2	V2	Month 0.5	14	±3 Days	11-17
Week 6	V3	Month 1	42	±7 Days	35-49
Week 10	V4	Month 2	70	±7 Days	63-77
Week 14	V5	Month 3	98	±7 Days	90-105
Week 18 (End of blinded phase)	V6	Month 4	126	±7 Days	118-133

Table 18: Visit Schedule – Part II

Visit Name	Number	Duration	Target day	Window	Day Range
Week 0	V7	NA	126	±7 Days	118-133

Visit Name	Number	Duration	Target day	Window	Day Range
(week 18 of part I)					
Week 2	V8	Month 0.5 for Part II	140	±3 Days	137-143
Week 16	V9	Month 4 for Part II	238	±14 Days	224-252
Week 32	V10	Month 8 for part II	350	±14 Days	336-364
Every 4 months until EOS	V11 onwards	Every 4 months in Part II	NA	±14 Days	As appropriate

All efficacy analysis will follow above given study assessment window range.

Visit Window mapping for Safety as follows:

Application Of Bisection Rule:

Step (1) Difference between the date mentioned on the CRF and the randomization date will be calculated.

$$\text{Difference day} = \begin{cases} \text{Date of assessment} - \text{date of randomization}, \text{Assessments prior to randomization} \\ \text{Date of assessment} - \text{date of randomization} + 1, \text{Otherwise} \end{cases}$$

Step (2)

There are seven pre-assigned nominal (planned) visit days according to the protocol.

- Screening (i.e. V0),
- Baseline(Safety Baseline) (Day 1) (i.e.V1)
- Week 2 (Efficacy Baseline) (Day 14)(i.e.V2)
- Week 6 (Day 42) (i.e.V3)
- Week 10 (Day 70) (i.e.V4)
- Week 14 (Day 98) (i.e.V5)
- Week 18 (Day 126)(i.e.V6)We choose intervals as follows (Table 19):

Table 19: Visit Schedule (Part I)

Visit	Visit Description	Target day	Range	
Visit 0	Screening	Screening	-28	0
Visit 1	Week 0	7	1	11

Visit	Visit Description	Target day	Range	
Visit 2	Week 2#	14	12	28
Visit 3	Week 6	42	29	56
Visit 4	Week 10	70	57	84
Visit 5	Week 14	98	85	112
Visit 6	Week 18	126	113	141
Visit Y*	Every 4 Months	X	142	[X +((X-Target day of previous visit)/2)]

*: Subsequent visit in Part II
#: Baseline for efficacy endpoints

Step (3)

When there are multiple assessments in a particular visit window, the following rules are applied to select one value representing the subject in summary statistics.

- When there are multiple assessments falling in a particular visit window then the one which is closest to the scheduled visit day will be chosen. For safety assessments like Laboratory and Vitals consider average of observations.
- The visit will be missing if no assessment was reported within the specified visit window around the scheduled visit day.
- If the difference between two duplicate records is more than or equal to 14 days, then the former duplicate record will be assigned to previous visit. The difference of 14 days is chosen because the difference between all the visits is 28 days. For safety assessments like Laboratory and Vitals consider average of observations.

The details of the different assessments (Table 20 and Table 21) to be done at each visit during the trial as follows:

Table 20: Schedule of Assessment (Part I)

Assessment	Screening	Run-in		Blinded Treatment Period				
		Baseline (Within 28 Days of Screening) Week 0	Week 2 (± 3 Days)	Week 6 Month 1 (± 7 Days)	Week 10 Month 2 (± 7 Days)	Week 14 Month 3 (± 7 Days)	Week 18 Month 4 (± 7 Days)	EOS (Early Discontinuation)
Informed Consent	X							
Inclusion/ Exclusion	X							
Medical History	X							
Prior and Concomitant Medication	X	X	X	X	X	X	X	X
WHO Functional Class	X	X	X	X	X	X	X	X
6 Min Walk Test	X	X[a]	X[a]	X	X	X	X	X
Borg Dyspnea, Heart Rate and SpO2	X		X	X	X	X	X	X
Vital Signs (SpO2 via Pulse Oximetry)	X	X	X	X	X	X	X	X
Total Lung Capacity (TLC)	X[b]							
Spirometry Testing (and DLCO)	X[c]						X	X
Physical Examination	X[d]						X	X
Pregnancy Test	X						X	X
MetHgb	X		X	X				X

[redacted]

Assessment	Screening	Run-in		Blinded Treatment Period				
Hematology, Chemistry	X						X	X
NT-proBNP	X						X	
Eligibility for INOpulse (Average Daily Use ≥ 12 hr/day)			X[h]					
Randomization		X						
Right Heart Catheterization (RHC) (Subset of ~ 50 Subjects)		X					X	X[i]
Right Heart Catheterization (RHC) (all subjects not enrolled in subset)	X[j]							
Echocardiogram for central reading (subset of ~50 subjects)		X			X		X	X[i]
Echocardiogram for local Reading only (all subjects not enrolled in subset)		X						X[i]
Evaluation of Symptomatic Rebound pH		X[k]	X					
Patient Reported Outcome (SF-36)		X					X	X
AE's/SAE's/AESI's		X	X	X	X	X	X	X
Drug Dispensing for Run-in period		X[l]						
Drug Dispensing for Treatment period			X[l]	X[l]	X[l]	X[l]	X[l]	

[redacted]

Assessment	Screening	Run-in		Blinded Treatment Period				
Drug Device Usage[n]			X[n]	X[n]	X[n]	X[n]	X[n]	X[n]
INOpulse questionnaire							X[m]	X
Survival Assessment			X	X	X	X	X	X
Medical Resource Utilization	X	X	X	X	X	X	X	X
Lung Transplantation Listing Status	X	X	X	X	X	X	X	X

a: A Screening 6MWT may be obtained at baseline if the subject is known to have a 6MWD ≥ 100 meters and ≤ 450 meters. The average of two 6MWD at Week 2 after run-in will be used at the baseline.
b: Total lung capacity test to be done if not performed within 6 months of screening.
c: DLCO (Diffusing Capacity for Lungs Measured Using Carbon Monoxide) will be performed at Screening Only.
d: Height and Weight to be performed at Screening.
e: N/A-Footnote no longer applicable as of Amendment #3
f: Pregnancy test to be performed at the discretion of investigator.
g: N/A-Footnote no longer applicable as of Amendment #3.
h: Subjects will be required to demonstrate an average daily usage ≥ 12 hours (rounded to the nearest hour) with no more than 2 days of usage < 8 hours per day of the INOpulse device during the initial 2 weeks of the study in order to be eligible to continue to the treatment phase.
i: RHC should be obtained at early discontinuation only for subjects participating in RHC substudies ECHO should be obtained for all subjects at early discontinuation regardless if they are participating in the ECHO substudy.
j: A RHC will be conducted at Screening for subjects who are not included in this subset who have not had a RHC within the previous 5 years that meet eligibility criteria and are otherwise eligible.
k: After randomization at Week 0 and at end of Run-in, Week 2, all subjects will remain in the hospital or clinic for 1 hour after starting study drug and for 1 hour after study drug is discontinued for observation for signs and symptoms of rebound PH. Vital signs, including heart rate, respiratory rate, blood pressure, and SpO2 will be recorded in the supine position every 15 minutes for 1 hour, and then again every 15 minutes for another hour after discontinuation of study drug. After the 1 hour observation period off study drug, if the subject's status is unchanged with respect to vital signs and symptoms and subject does not exhibit signs or symptoms of rebound PH, they will restart their blinded study drug treatment and be sent home.
l: One week of drug dispensed to site at Baseline and Week 2; all other drug dispensed to subject's home via home delivery.
m: At Week 18 or prior to study discontinuation.
n: Site personnel should review INOpulse use with the subjects during study visits to encourage use as close to 24 hours per day as possible. Reasons for device interruptions should also be reviewed with the subject.

Table 21: Schedule of Assessment (Part II)

Assessment	Open Label Long Term Extension Period						
	Week 0 If subject enters Part 2, Week 0 assessments are done at Week 18 of Part 1	Week 2 (± 3 Days)	Week 16 Month 4 (± 14 Days)	Week 32 Month 8 (± 14 Days)	Week 52 Month 12 (± 14 Days)	Every 4 Months Until EOS (± 14 Days)	EOS (Early Discontinuation)
Informed Consent	X[1]						
Inclusion/ Exclusion	X[2]						
Review of Prior and Concomitant Medications		X	X	X	X	X	X
WHO Functional Class			X	X	X		
6 Min Walk Test			X	X	X		
Borg Dyspnea, Heart Rate and SpO2			X	X	X		
Vital Signs(SpO2 via Pulse Oximetry)			X	X	X	X	X
Telephone contact		X					
Pregnancy Test	X		X[4]	X[4]	X[4]	X[4]	X[4]
MetHgb	X				X	X[5]	X
Hematology, Chemistry					X	X[5]	X

Assessment	Open Label Long Term Extension Period						
	Week 0 If subject enters Part 2, Week 0 assessments are done at Week 18 of Part 1	Week 2 (± 3 Days)	Week 16 Month 4 (± 14 Days)	Week 32 Month 8 (± 14 Days)	Week 52 Month 12 (± 14 Days)	Every 4 Months Until EOS (± 14 Days)	EOS (Early Discontinuation)
INOpulse Compliance and Check for Interruptions	X		X	X	X	X	X
Evaluation for Symptomatic Rebound PH	X[6]		X	X	X		
AEs/SAEs/ AESIs	X	X	X	X	X	X	X
Drug Dispensing	X[1]		X[7]	X[7]	X[7]	X[7]	
Drug/Device Usage			X[8]	X[8]	X[8]	X[8]	X[8]

1 Ensure the subject has agreed to participate in both parts of the PAH-004 study by checking and initializing Part 1 & Part 2 participation in the ICF
2 Subject must have completed 18 weeks of blinded treatment
3 N/A-Footnote no longer applicable as of Amendment #3.
4 Pregnancy testing to be performed at the discretion of the Investigator.
5 Hematology and chemistry labs and MetHgb will be performed on a yearly basis.
6 Assessment Symptomatic Rebound PH: all subjects will remain in the hospital or clinic under medical supervision for 1 hour after starting study drug and for 1 hour after study drug is discontinued for observation for signs and symptoms of rebound PH. Vital signs, including heart rate, respiratory rate, blood pressure, and SpO2 will be recorded in the supine position every 15 minutes for 1 hour, and then again every 15 minutes for another hour after discontinuation of study drug. After the 1 hour observation period off study drug, if the subject's status is unchanged with respect to vital signs and symptoms and subject does not exhibit signs or symptoms of rebound PH, they will restart their blinded study drug treatment and be sent home.
7 Open Label Drug dispensed by site at Week 0; all other drug dispensed to subject's home via home delivery every 4 weeks thereafter
8 Site personnel should review INOpulse use with the subjects during study visits to encourage use as close to 24 hours per day as possible. Reasons for device interruptions should also be reviewed with the subject.

5.3 Treatment Assignment

Dosing regimen during the treatment phase will be via the INOpulse device.

Cohort 1: Placebo

[redacted]

Placebo at a dose setting of 75 mcg/kg IBW/hr for up to 24 hr/day 99.999% Nitrogen [N2] cartridge; change approximately every 12 hours

Cohort 1: iNO 75 mcg/kg IBW/hr

iNO at a dose setting of 75 mcg/kg IBW/hr for up to 24 hr/day 6.0 mg/L [4800 ppm] NO cartridge; change approximately every 12 hours.

5.4 Sample Size

The sample size calculation for this study was performed assuming one interim analysis. The purpose of the interim analysis is to stop the trial for efficacy or futility conclusion or to continue the trial to 150 or 188 patients.

The sample size calculations were performed using following two designs:

1) Design1: Group sequential early stopping criteria and
2) Design 2: Sample size re-estimation methodology

Design 1: Traditional group sequential design (GSD) alpha spending function Gamma=-3 (Hwang, Shih, deCani, 1990) is used to control the overall type 1 error at 0.025, 1-sided.

Design 2: This is the "promising zone" sample size re-estimation (SSR) design of Mehta & Pocock (2011), which uses the Cui, Hung, Wang (1999) method of analysis to combine the test statistics for the separate analyses of the IA and post-IA parts of the trial, if the trial does not stop at IA.

The final analysis is via weighted Z statistic using equal weights for the IA and post IA test statistics. It requires $p<0.0229$ 1-sided for statistical significance to control the overall type 1 error level at 0.025 1-sided with gamma=-3 error spending function (Hwang, Shih, deCani 1990).

A sample size of 150 patients (75 in each group) with simulations assuming true underlying mean treatment difference in 6MWD ranging from 30-70 meters and standard deviation from 50-70 meters yielded > 87% power for both designs (for all cases except the most extreme case (mean difference of 30 meters and S.D 75, power for this combination was 71%).

The estimated conditional power (CP) and predictive power (PP) based on interim estimated SD and mean differences observed on 75 subjects with a planned final sample size of 150 is shown in (Table 22).

Table 22: Conditional and Predictive Power

S.D	Mean Difference	Interim Z Statistics	Conditional Power (CP)	Predictive Power (PP) with vague prior#	Predictive Power (PP) with informative prior*
50	30	2.598	0.991	0.951	0.994
	40	3.464	>0.999	0.998	>0.999
	50	4.33	>0.999	>0.999	>0.999
	60	5.196	>0.999	>0.999	>0.999
	70	6.062	>0.999	>0.999	>0.999
60	30	2.165	0.934	0.853	0.974
	40	2.887	0.998	0.981	0.998
	50	3.608	>0.999	0.999	>0.999
	60	4.33	>0.999	>0.999	>0.999

[redacted]

S.D	Mean Difference	Interim Z Statistics	Conditional Power (CP)	Predictive Power (PP) with vague prior#	Predictive Power (PP) with informative prior*
	70	5.052	>0.999	>0.999	>0.999
75	30	1.732	0.739	0.669	0.912
	40	2.309	0.964	0.895	0.983
	50	2.887	0.998	0.981	0.998
	60	3.464	>0.999	0.998	>0.999
	70	4.041	>0.999	>0.999	>0.999

*: The informative prior distribution on the treatment effect is based on estimates from a prior PAH study, in which analysis of change from baseline in 6 minute walk distance for 20 patients (7 controls, 13 treated) receiving oxygen for at least 12 hours a day yields an estimated treatment difference of 69.6 and pooled standard deviation estimate of 38.7.
#: Vague prior distribution which assumes no difference between groups.

All the sample size calculations were performed using EAST 6.4.

Using the above estimates yielded a planned sample size of 75 evaluable subjects per treatment arm (in total 150 subjects).

Further, in case the planned final analysis at N=150 (2 groups combined) is found not to have sufficient power, sample size increase could be made at IA up to a maximum of N=188 total patients (2 groups combined).

Assuming a 20% drop out rate, a total of 188 subjects (94 per treatment arm) are planned to support 150 at final analysis, with the ability to go up to 226 subjects (113 per treatment arm) to support 188 at final analysis, based on the sample size reassessment at the interim analysis.

As subjects must achieve a satisfactory adherence to the INOpulse delivery device, a dynamic randomization system will be used to ensure that approximately 94 (or 113) subjects per treatment cohort comply with the adherence criteria at Week 2. Enough subjects will be recruited such that a minimum sample size of 150 (or 188) subjects will complete 18 weeks of treatment on the INOpulse delivery device.

Subjects who do not comply with the required device adherence, as well as who do not meet the study entry criteria at Week 2, will be withdrawn from the study and replaced.

5.5 Randomization and Blinding

5.5.1 Randomization

All eligible subjects will be screened for enrollment. All eligible and consented subjects will be randomized into 1 of 2 treatment groups in a ratio of 1:1.Randomization will be performed using an Interactive Response Technology (IRT) system.

Randomization will occur ONLY after the subject has signed the informed consent, has been enrolled into the study, and has met all Inclusion and Exclusion criteria. Study drug should be started after randomization during the Run-in visit.

Interactive Response Technology (IRT)

Selected individuals at each study center, along with subjects, will be authorized by pre-coded identification numbers to have password protected access to the designated portions of the system. Authorized individuals will interact with the IRT in accordance with the user manual. The IRT will be used by the site to assign subjects randomization numbers, dosing cohorts, and drug/device supplies.

The IRT will also be used for functions such as: accountability (by site and subjects), dispensing, reordering or replacement of INOpulse device, cartridges or ancillary supplies, programming of INOpulse devices, and emergency unblinding of subject treatment.

5.5.2 Blinding

All study drug cartridges will be labeled with informative language that each cartridge contains up to 6.0 mg/L (4880 ppm) NO gas to allow for compliance with compressed gas transportation regulations while maintaining a blinded label. All study labels will indicate the kit number and all drug cartridges will be labeled with a blinded unique identification number prior to shipment to the investigative site or the subject's home. Drug cartridges are intended for use by a single subject who will be assigned the corresponding kit number on the study label by the IRT. Similarly, all devices will be uniquely identified, and tracked and controlled by the IRT.

Breaking of Blinded Codes

The blind could be broken only if specific urgent treatment would be dictated by knowing the treatment status of the subject. In such cases, the Investigator or designee will be able to call or login to the IRT to unblind a subject. It is strongly recommended to discuss the case with the Sponsor's medical monitor before unblinding.

The date, time, and reason for the unblinding must be documented in the eCRF, and the Sponsor's medical monitor must be informed as soon as possible.

6.0 Data Considerations

6.1 Protocol Violations

The following protocol violations will be considered major violations and will lead to the exclusion of subjects from the Per-Protocol (PP) analysis set. This list is not exhaustive and will be expanded at the discretion of the study team.

- Major Protocol Violations.
- Subjects whose compliance to study product and/or study procedures have been insufficient.
- Details of all the protocol deviations will further be elaborated in separate protocol deviation specification document.

Summary table by treatment group for number of subjects encountering protocol violations will be produced.

6.2 Product Compliance (Device Adherence)

Subjects will be supplied with products at randomization visit. The product adherence data is not captured on CRF and will be supplied via the IRT by Y Prime.

The following fields are agreed.

- Patient number
- Report Start Date
- Report End Date
- Day of Usage
- Number of Minutes of Use
- Average Usage for Period

The subject will be deemed compliant if their average daily usage is more than 12 hours.

6.3 Handling of Missing Data

Missing data imputation for primary endpoint:

For primary analysis, i.e. change from baseline at Week 18, missing data will not be imputed.

The following missing data imputation techniques will be employed for sensitivity analyses of primary endpoint.

1) Last Observation Carry Forward (LOCF) approach will be applied for subjects not withdrawing trial due to adverse events, for subjects withdrawing trial due to adverse events (including deaths), worst value (for e.g. zero for 6MWD) will be assigned at 18 weeks visit.
2) Worst-case imputation approach I (i.e. impute the missing value for a subject with worst value (within the subject) for all subjects who withdraw prematurely for any reason. The value of zero for 6MWD will be imputed for all deaths.
3) Worst-case imputation approach II (i.e., impute the missing value for a subject in the active and Placebo dose group by the worst result of all subjects with non-missing assessments in their respective assigned dose groups). The value of zero for 6MWD will be imputed for all deaths.
4) Worst-case imputation approach III (i.e. impute the missing value for a subject in the active dose group by the worst result of all subjects with non-missing assessments in the same active dose groups and impute the missing value for a subject in placebo group by his/her last available assessment prior to the missing assessment. The value of zero for 6MWD will be imputed for all deaths.

Missing data imputation for secondary endpoint:

For all the other efficacy endpoints, the last observation carry forward (LOCF) approach will be used in handling the missing data as that used in the EARLY study (Galiè 2008).

6.4 Baseline Definition

The Week 2 assessment is considered as baseline for all the efficacy analyses. For SF-36, week 0 assessment is used as baseline.

7.0 Statistical Considerations

For statistical analysis reporting, all categorical (or discrete) variables will be summarized with frequency counts and percentage, and continuous variables will be summarized showing mean, geometric mean (appropriate for log transformed variables), standard deviation, median, minimum and maximum. Within each cell of a summary table, number should be integer, and percentage should be rounded with one decimal point. Mean and median should be rounded to one decimal point, minimum and maximum may be integer, but can have no more than one decimal point. A standard deviation or standard error should be rounded to two decimal points.

The p-value will be in the format of "#.xxx", in which "#" stands for 0 or 1 and "xxx" for any numbers from 0 to 9 each. If "#" is "1", "xxx" must be "000". However, if the p-value is less than 0.001, it will be indicated "< 0.001" instead.

Decimal points as recorded in the database should be used to calculate any derived variable/s. rounding for significant digits, if applicable, will be applied only after the analysis has been performed.

7.1 Definition of Analysis Sets

All study data will be analyzed using the subjects' populations as defined below.

7.1.1 Enrolled Analysis Set

All subjects enrolled in the study will comprise of enrolled population. There will be no safety or efficacy analysis performed on this set.

7.1.2 Randomized Analysis Set

All subjects randomized in the study will comprise of randomized population.

7.1.3 Intent to Treat (ITT) Analysis Set

The ITT analysis set will include all randomized subjects who remained eligible after the Run-in period. It will compare the treatment groups on the basis of randomized treatment rather than treatment actually received.

7.1.4 Modified Intent to Treat (mITT) Analysis Set

The mITT analysis set will include all randomized subjects who remained eligible after the Run-in period and have more than average 12 hours of device usage. It will compare the treatment groups on the basis of randomized treatment rather than treatment actually received.

7.1.5 Per-Protocol (PP) Analysis Set

The Per Protocol (PP) analysis set will consist of all subjects included in the ITT population who adhere to all protocol requirements without causing any major violation. Major protocol violations are defined in section 6.1 of SAP.

Primary population for the analysis is ITT. Primary analysis will also be performed on PP analysis set.

7.1.6 Safety Analysis Set

The safety analysis set will include all randomized subjects who received the study treatment. Safety analysis will be based on the treatment actually received and not randomized.

7.1.7 Modified Safety Analysis Set

The safety analysis set will include all randomized subjects who received the study treatment and became eligible after run in period. Safety analysis will be based on the treatment actually received and not randomized.

Adverse events (AEs and SAEs) and concomitant medication data will be analysed and summarized based on Safety Analysis set and Modified Safety analysis set.

7.2 Evaluation of Baseline and Demographic Characteristics

7.2.1 Screening

All screening variables will be summarized on enrolled population.

7.2.2 Demographic

All demographic characteristics variables as captured on CRF will be summarized on randomized population and ITT.

These characteristics will also be summarized for following subgroups:

1) Continuous positive airway pressure (CPAP),
2) Bilevel positive airway pressure (BiPAP)

7.2.3 Baseline Variables

All the baseline characteristics variables as captured on CRF will be summarized on randomized population and ITT. Baseline characteristics will also be summarized for subjects discontinuing before 18 weeks.

These characteristics will also be summarized for following subgroups:

1) Continuous positive airway pressure (CPAP),
2) Bilevel positive airway pressure (BiPAP)

7.3 Primary analysis

7.3.1 Change in 6MWD

The primary endpoint for double blinded of study is change from baseline in 6MWD at Week 18.

The average of the two 6MWDs at Week 2 (after run-in) will be used as the baseline value.

This endpoint is collected at all the visits including screening.

The change from baseline in 6MWD will be analysed using Mixed Model Repeated Measures (MMRM) (Table 23).

Table 23 Factors/Covariates for Analysis

Factor/Covariates	Random/Fixed	Type

Factor/Covariates	Random/Fixed	Type
Treatment Group	Fixed	Categorical(Two)
Visit	Fixed	Categorical(Four)
Prostanoids usage status	Fixed	Categories(Two)
Baseline 6MWD	Fixed	Continuous
Treatment By Visit Interaction	Fixed	Categorical (Eight)

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} > \mu_{Placebo}$ for at least one realization.

The following treatment comparisons will be assessed on change from baseline.

1) *iNO 75 mcg/kg IBW/hr versus matching Placebo (Primary interest) at Week 18.*

Missing Data Imputation Rules:

Missing data will not be imputed for primary analysis.

The results will be presented in terms of LS means and the difference between LS means, with associated confidence interval and p-value.

Standard descriptive statistics for two separate categories ((1) increase in 6MWD from baseline at Week 18 and decrease in 6MWD from baseline at Week 18) will be presented along with the visit-wise 6MWD.

This analysis will be performed on Intention to treat (ITT), Per-Protocol (PP) and Modified Intention to treat (mITT).

Analysis performed on ITT will be considered as primary.

The within-subject error is assumed to have an unstructured (UN) covariance matrix. However, if the fit of the unstructured (UN) covariance structure fails to converge, then the following options will be tried in order until convergence is reached.

1) Toeplitz with Heterogeneity (TOEPH)
2) Autoregressive with Heterogeneity (ARH(1))
3) Toeplitz (TOEP)
4) Autoregressive (AR(1))
5) Compound Symmetry (CS)

If model still doesn't converge then this will be highlighted and discussed in the BDR meeting and an appropriate covariance structure to be used will be agreed. The denominator degrees of freedom will be estimated using a Kenward-Roger approximation. The pairwise treatment differences will be estimated as differences between the least-squares means and tested using an F-test based on the Type III sums of squares.

The SAS code for the MMRM analysis is provided below for interim analysis.

[redacted]

PROC MIXED Data= 6MWD;

CLASS PATID TREAT STRATA VISIT;

MODEL CHG = 6MWDBASE TREAT STRATA VISIT TREAT*VISIT / DDFM=KR OUTPRED= RESID;

REPEATED VISIT / SUBJECT=PATID (TREAT) GROUP = TREAT type=UN;

LSMEANS TREAT*VISIT / PDIFF cl;

ODS OUTPUT DIFFS=DIFFS1;

ODS OUTPUT LSMEANS=LSMEANS1;

RUN;

If the blinded data is used to determine the sample size then Z statistics by CHW will not be used. This decision will be taken before interim analysis.

If the unblinded data is used to determine the sample size then Z statistics by CHW will be used.

For final analysis the Z statistics will be calculated using the CHW method (Cui L, Hung HM, Wang SJ, 1999).

If the sample size does not increase, the test statistics at the final analysis, denoted by Z_N is weighted sum of the test statistics from the two stages Z_n (Test statistics at interim) and Z_{N-n} (Menon and Zinc).

$$Z_N = \sqrt{\frac{n}{N}} Z_n + \sqrt{\frac{N-n}{N}} Z_{N-n}$$

If after interim analysis, the sample size is increased to M (M>N) per group, the same statistic becomes: .

$$Z_M = \sqrt{\frac{n}{N}} Z_n + \sqrt{\frac{M-n}{M}} Z_{M-n}$$

This may inflate the type one error and hence CHW proposed a new test statistics as follows:

$$Z'_M = \sqrt{\frac{n}{N}} Z_n + \sqrt{\frac{N-n}{N}} Z_{M-n}$$

Pooling of centers:

NA.

Direction of Difference: Higher is better.

Positive difference in LS means (*iNO 75 mcg/kg IBW/hr versus matching Placebo*) for change from baseline in 6MWD at Week 18.

Heteroscedasticity Assumption:

The square of residuals will be modelled using PROC MIXED and overall F test will indicate the presence or absence of heteroscedasticity.

Normality Assumption:

Normality assumption for Change from baseline in 6MWD will be assessed using the following:

1. Shapiro-Wilk statistics and
2. Visual examination of Q-Q plots.

PROC UNIVARIATE procedure in SAS will be used to check for the normality assumption. The normal probability plot (normal Q-Q plot) of residuals will also be generated and used to assess the normality. If

the residuals generated come from normal distribution then the normal probability plots should approximate straight lines.

Following SAS code will be used to generate Wilk's Shapiro and Q-Q plots.

PROC UNIVARIATE Data= Residual NORMAL;

VAR RESID;

QQPLOT RESID /NORMAL (MU=EST SIGMA=EST COLOR=RED L=1);

RUN;

Normality results will be discussed during model diagnostics post unblinding and analysis approach will be finalized.

Analysis of Non-Normal Data:

In case of normality issues, the analysis will be performed using van Elteren test for stratified response (change from baseline).

The SAS code applied as follows:

Proc Npar1way data=data;

Strata strata;

Class treatment;

Var change;

Run;

In case, we encounter normality issues, the results needs to be interpreted accordingly.

Sensitivity Analysis:

No formal normality assumption tests will be performed for this sensitivity analysis (Table 24).

Table 24: Sensitivity Analysis

No.	Sensitivity Analysis
1	Primary analysis on Per Protocol Population
2	Primary Analysis on Modified Intention To Treat Population
3	Analysis will be performed with Last Observation Carry Forward (LOCF) approach will be applied for subjects not withdrawing trial due to adverse events, for subjects withdrawing trial due to adverse events (including deaths), worst value (zero for 6MWD) will be assigned at EOS visit. The analysis will be performed on ITT population.
4	Analysis will be performed with worst-case imputation approach I (i.e. impute the missing value for a subject with worst value (within the subject) for all subjects who withdraw prematurely for any reason. The value of zero for 6MWD will be imputed for all deaths.

[redacted]

No.	Sensitivity Analysis
	The analysis will be performed on ITT population.
5	Analysis will be performed with worst-case imputation approach II (i.e., impute the missing value for a subject in the active and Placebo dose group by the worst result of all subjects with non-missing assessments in their respective assigned dose groups). The value of zero for 6MWD will be imputed for all deaths. The analysis will be performed on ITT population.
6	Analysis will be performed with worst-case imputation approach III (i.e. impute the missing value for a subject in the active dose group by the worst result of all subjects with non-missing assessments in the same active dose groups and impute the missing value for a subject in placebo group by his/her last available assessment prior to the missing assessment. The value of zero for 6MWD will be imputed for all deaths. The analysis will be performed on ITT population.
7	Change from Baseline in 6MWD analyzed using the same model as primary for all subjects completing the 18 weeks treatment in ITT and mITT population.
8	Change from baseline (defined as week 1 assessment) in 6MWD analyzed using the same model as primary on ITT population
9	Change from baseline analyzed using the same model as primary for all subjects on IV/SC prostacyclin +/- orals in ITT population.
10	The Change from baseline analyzed using the same model as primary on ITT population for all subjects on one oral treatment (no IV, Inhaled and SC) of either prostacyclin or Endothelin Receptor Antagonists (ERAs) or Phosphodiesterase Inhibitors (PDE 5 Inhibitors)
11	The Change from baseline analyzed using the same model as primary on ITT population for all subjects on at least two oral treatment (no IV, Inhaled and SC) of either prostacyclin or Endothelin Receptor Antagonists (ERAs) or Phosphodiesterase Inhibitors (PDE 5 Inhibitors)
12	If the normality and homogeneity of variance assumptions are significantly violated, then the baseline and change from baseline parameters are first ranked separately, using the combined data from all treatment groups. Tied values will receive the mean value (midranks) of the corresponding ranks. The primary analysis MMRM model on ranked values will be performed on ITT population.
13	Responder analysis for patients achieving ≥ 30 meters 6MWD response from baseline at Week 18 will be performed using the proportions test on ITT population
14	Change from Baseline in 6MWD analyzed using the same model as primary for all subjects having concurrent use of INOpulse device with a continuous positive airway pressure (CPAP), bilevel positive airway pressure BiPAP, or any other positive pressure device.

Table 25: Summary of Primary Analysis

Variable	Model	Population	Factors	Primary /Sensitivity	Imputation technique /algorithm
The Change from baseline in 6MWD	MMRM	ITT	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Primary	No Imputation
The Change from baseline in 6MWD	MMRM	PP & mITT	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation
The Change from baseline in 6MWD	ANCOVA at week 18	ITT	Treatment Group, Prostanoid Usage and Baseline 6MWD	Sensitivity	LOCF and Worst value to all subjects who withdraw prematurely due to AE or death
The Change from baseline in 6MWD	ANCOVA at week 18	ITT	Treatment Group,Prostanoid Usage and Baseline 6MWD	Sensitivity	Worst case Imputation Approach (I, II & III)
The Change from baseline in 6MWD	ANCOVA at week 18	ITT	Treatment Group, Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation
Change from Baseline in 6MWD	MMRM	ITT (all subjects completed 18 weeks of treatment)	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation
Change from Baseline in 6MWD	MMRM	mITT (all subjects completed 18 weeks of treatment)	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation
Change from baseline (defined as week 1 assessment) in 6MWD analyzed using the same	MMRM	ITT	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation

[redacted]

Variable	Model	Population	Factors	Primary /Sensitivity	Imputation technique /algorithm
model as primary.					
Change from baseline in 6MWD for subjects with PAH SPECIFIC THERAPIES (PHST) [PHST] and algorithm to filter the Prostacyclin IV and Oral therapy.	MMRM	ITT (all subjects on IV/SC prostacyclin +/- orals)	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No imputation
Change from baseline in 6MWD for subjects with PAH SPECIFIC THERAPIES (PHST) [PHST] and algorithm to filter for oral treatments.	MMRM	ITT (all subjects on one oral treatment of either prostacyclin or Endothelin Receptor Antagonists (ERAs) or Phosphodiesterase Inhibitors (PDE 5 Inhibitors)	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No imputation
Change from baseline in 6MWD for subjects with with PAH SPECIFIC THERAPIES (PHST) [PHST] and algorithm to filter for oral treatments.	MMRM	ITT (all subjects on at least two oral treatment of either prostacyclin or Endothelin Receptor Antagonists (ERAs) or Phosphodiesterase Inhibitors	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No imputation

Variable	Model	Population	Factors	Primary /Sensitivity	Imputation technique /algorithm
		(PDE 5 Inhibitors)			
Change from baseline in 6MWD ranks analyzed using same model as primary.	MMRM	ITT	Treatment Group, Visit, Treat*Visit Prostanoid Usage and Baseline 6MWD	Sensitivity	No Imputation
Responder for patients achieving ≥ 30 meters 6MWD from baseline at Week 18	Proportions test	ITT	Treatment	Sensitivity	NA

For open label period of study change in 6MWD at month 4, 8 and 12 from baseline (week 2) and week 18 will be analysed for all subjects participated in open label and summarized separately for subjects who switched from placebo to active treatment.

Change in 6MWD at week 18, during blinded period, from baseline will be analyse for subjects who completed 6 months and 12 months of study treatment using mITT population.

7.4 Secondary Analysis

All secondary analysis will be performed on ITT analysis set. All missing data will be imputed using Last Observation Carried Forward Rule (LOCF).

For all secondary continuous endpoints if normality assumptions fail then log transformed analysis will be performed. If the normality assumptions still fail, then analysis will be performed on the ranked data.

7.4.1 Time (in days) to First Clinical Worsening Event (TTCW)

Time to First Clinical worsening event from baseline to week 18 will be analyzed with log-rank test stratified by the prostanoid usage status (Table 26) for blinded period of study. All Individual criteria's to be analyzed using the same model as TTCW.

The censoring time points for the analyses include the time points at which subjects drop out of the study or complete the study blinded treatment period (week 18) without any of the specified events.

Time (in days) to first clinical worsening (TTCW) will be defined as time from randomization to either of the below mentioned events:

i. Death (all-cause mortality)

ii. Atrial septostomy

iii. Hospitalization due to worsening of PAH (adjudicated)

iv. Start of new specific PAH treatment (endothelin receptor antagonists [ERAs], phosphodiesterase type-5 [PDE-5] inhibitors or prostanoids), an increase in the dose of an ERA or PDE-5, increase in the dose or frequency of an inhaled prostanoids, or an increase in the dose of an intravenous or subcutaneous prostanoids by >10%.

v. Decrease of >15% from baseline or >30% compared with the last study related measurement in 6MWD should be confirmed by a repeat measurement performed at least 14 days later

vi. Worsening of WHO Functional Class (e.g., from Class II to Class III or IV, OR Class III to Class IV); and should be confirmed by a repeat assessment at least 14 days later

Patients who did not experience clinical worsening at the time of statistical analysis will be censored at the time of subject's last visit in part I phase, this includes patients who are lost to follow-up or have withdrawn consent.

Time to first clinical worsening (TTCW) Calculation:

1. Always calculated from date of randomization
2. If a patient discontinues study treatment prior to any of the above mentioned events then patient's last visit will be considered for censoring.
3. Patients who have not experienced TTCW will be censored at the last evaluable assessment. This includes patients who are lost to follow up or have withdrawn consent.
4. If a patient has no evaluable baseline assessment then TTCW will be censored at 0 days.
5. If a patient has no evaluable post baseline assessment then TTCW will be censored at 0 days.
6. Censoring variables for each of the individual criteria's will same as above.

TTCW will be analyzed using log rank test stratified by prostanoid usage status. The following SAS code will be used for analysis:

Hypothesis:

The hypothesis to be tested is as follows;

H_0: $S_{1(t)} = S_{2(t)}$ versus H_1: $S_{1(t)} > S_{2(t)}$ for at least one realization.

Where, $S_1(t)$: Survival Function for iNO 75 mcg/kg IBW/hr ; $S_2(t)$: Survival Function for matching Placebo

The following SAS code will be used for analysis:

```
PROC LIFETEST Data = TTCW notable;
    TIME Days*Censor (0);
    STRATA PROSSTATUS/GROUP = TREAT;
RUN;
```

Missing Data: Please refer to Section 6.3 for missing data imputation. Missing values will be imputed using the LOCF.

Table 26: Summary of Clinical Worsening Analysis

Variable	Model	Population	Factors
Time to clinical Worsening	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to clinical Worsening	Log Rank	mITT	Treatment Group and stratification by Prostanoid usage.
Time to Death (All-cause mortality)	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to Atrial septostomy	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to Hospitalization due to worsening of PAH (Adjudicated)	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to PAH specific therapy	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to decrease in Decrease of >15% from baseline or >30% compared with the last study related measurement in 6MWD	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.
Time to Worsening of WHO Functional Class (e.g., from Class II to Class III or IV, OR Class III to Class IV)	Log Rank	ITT	Treatment Group and stratification by Prostanoid usage.

7.4.2 Change in WHO functional Class

The change from baseline for WHO Functional Class at Week 18 will be analysed by using ANCOVA with treatment group and stratification factor prostanoid usage as factor and baseline WHO functional score as covariates.

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} < \mu_{Placebo}$ for at least one realization.

SAS code: Same as primary

Direction of difference: Smaller the Better.

Missing Data: Please refer to Section 6.3 for missing data imputation. Missing values will be imputed using the LOCF.

No.	Sensitivity Analysis
1	Analysis on Modified Intention to treat Population
2	Analysis on mITT population for all the subjects completing the 18 weeks treatment period

Analysis: Similar to primary for WHO functional class (Table 27).

Table 27: Summary of WHO Analysis

Variable	Model	Population/Criteria	Factors
The Change from baseline in WHO functional class	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in WHO functional class	ANCOVA	mITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in WHO functional class	ANCOVA	mITT (All 18 weeks completers)	Treatment Group, Prostanoid Usage and Baseline Score.

Shift table for WHO categories will also be presented.

For open label period change in WHO Functional Class at month 4, 8 and 12 from baseline and week 18 will be analysed using ANCOVA as defined above.

7.5 Multiplicity Adjustment

To control the Type-1 error rate at a level of 0.05, a hierarchal gate keeping procedure will be applied to test the significant difference between the treatment groups in the primary and secondary endpoints in the following order:

- 6MWD
- TTCW
- WHO Functional Class

Each test will be evaluated at the appropriate p-value as per the alpha spending function. The procedure will stop if lack of significance is found for any of the variables and the remaining test(s) in the sequence (as mentioned above) will also be concluded as non-significant.

7.6 Tertiary Analysis

All tertiary analysis will be performed on ITT analysis set. No multiplicity adjustments will be done for any of the tertiary endpoints analysis. Missing data will be imputed using Last Observation Carried Forward Rule (LOCF).

For all tertiary continuous endpoints if normality assumptions fail then log transformed analysis will be performed. If the normality assumptions still fails then analysis will be performed on the ranked data.

7.6.1 Quality of Life (SF-36 v2)

All the SF-36 continuous components will be analyzed using ANCOVA model at Week 18 with the same factors/covariates as given for secondary parameter WHO functional class (Table 28). All the tests will be one sided.

In addition, the separate proportions analysis on responders (defined as subjects having improved 6MWD post baseline) will be performed on each component. The same analysis will also be replicated for non-responders.

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} > \mu_{Placebo}$ for at least one realization.

Direction of Difference: Higher the Better. Higher the score better the health related quality of life (HRQol)

	Component
1	Physical Functioning (PF)
2	Role- Physical (RP)
3	Bodily Pain (BP)
4	General Health (GH)
5	Vitality (VT)
6	Social Functioning (SF)
7	Role-Emotional (RE)
8	Mental Health (MH)

Table 28: Summary of SF 36 Analysis

Variable	Model	Population/ Criteria	Factors
The Change from baseline in Physical Functioning (PF)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in Role-Physical (RP)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in Bodily Pain (BP)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in General Health (GH)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in Vitality (VT)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in Social Functioning (SF)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.
The Change from baseline in Role-Emotional (RE)	ANCOVA at Week 18	ITT	Treatment Group,Prostanoid Usage and Baseline Score.

Variable	Model	Population/ Criteria	Factors
The Change from baseline in Mental Health (MH)	ANCOVA at Week 18	ITT	Treatment Group, Prostanoid Usage and Baseline Score.

7.6.2 Change in Pulmonary Hemodynamics Measured by Right Heart Catheterization (RHC)

For study part I, the change from baseline for the following at Week 18 will be analyzed using ANCOVA at Week 18 with the same factors/covariates as given for secondary parameter WHO functional class (section 7.4.2).

1. Cardiac output [CO],
2. Cardiac index [CI],
3. Mean pulmonary artery pressure [mPAP],
4. Mean pulmonary capillary wedge pressure [mPCWP],
5. Systolic pulmonary artery pressure [sPAP],
6. Diastolic pulmonary artery pressure [dPAP],
7. Pulmonary vascular resistance [PVR],
8. PVR Index
9. Oxygen saturation by pulse oximeter [SpO2],
10. Mixed venous O2, and
11. Right atrial pressure [RAP]

Table 29: Summary of RHC Analysis

Variable	Model	Population	Factors
The Change from baseline in Cardiac Output at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Cardiac index [CI] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Mean pulmonary artery pressure [mPAP] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Mean pulmonary capillary wedge pressure [mPCWP] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline

Variable	Model	Population	Factors
The Change from baseline in Systolic pulmonary artery pressure [sPAP] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Diastolic pulmonary artery pressure [dPAP] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Pulmonary vascular resistance [PVR] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Pulmonary vascular resistance [PVR] Index at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Oxygen saturation by pulse oximeter [SpO2] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Mixed venous O2 at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Mixed venous O2 at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline
The Change from baseline in Right atrial pressure [RAP] at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline

7.6.3 Change in Echocardiogram Measurements Right Ventricular Function and Left Ventricular Function from Baseline

Analysis will be performed by cardiac imaging core laboratory at the Brigham and Women's Hospital.

7.6.4 Change in NT-proBNP from Screening to 18 Weeks

The change from screening for log transformed NT-proBNP at Week 18 will be analyzed using ANCOVA model at week 18, with the same factors/covariates as given for secondary parameter WHO functional class (Table 30).

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} \neq \mu_{Placebo}$ for at least one realization.

Table 30: Summary of NT-proBNP Analysis

[redacted]

Variable	Model	Population	Factors
The Change from baseline in NT-proBNP at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline

7.6.5 Change in Borg dyspnea Score Immediately Following 6MWT

The change from baseline for Borg Dyspnea at Week 18 will be analyzed using the ANCOVA model with same factors/covariates given for secondary parameter WHO functional class (Table 31).

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} > \mu_{Placebo}$ for at least one realization.

Table 31 Summary of Borg Dyspnea Analysis

Variable	Model	Population	Factors
The Change from baseline in Borg Dyspnea at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline

For open label period change in Borg Dyspenea at month 4, 8 and 12 from baseline and week 18 will be analysed using ANCOVA as defined above.

7.6.6 Change in 6MWD as Related to Degree of Drug Adherence from Baseline to 18 Weeks

The change from baseline in 6MWD related to degree of drug adherence at Week 18 will be analyzed using the same model as given for secondary parameter WHO functional class (Table 32).

The analysis will be replicated for following categories:

1) Average Compliance < 8 hours per day
2) Average Compliance ≥ 8 hours per day
3) Average compliance <12 hours per day
4) Average Compliance ≥ 12 hours per day
5) Average Compliance ≥ 16 hours per day

Hypothesis:

H_0: $\mu_{iNO\ 75} = \mu_{Placebo}$ versus H_1: $\mu_{iNO\ 75} > \mu_{Placebo}$ for at least one realization.

Table 32: Summary of 6MWD and Drug Adherence Analysis

Variable	Model	Population	Factors

Variable	Model	Population	Factors
The Change from baseline in 6MWD for drug adherence at Week 18	ANCOVA	ITT	Treatment Group, Prostanoid Usage and Baseline

7.6.7 The Number of Subjects with Unsatisfactory Clinical Response from Baseline to 18 Weeks

The proportions of patient with unsatisfactory clinical response from baseline at Week 18 will be analyzed using Cochrane Mantel Haenszel test (Table 33).

Hypothesis:

H0: P iNO 75 = P Placebo versus H1: P iNO 75 < P Placebo for at least one realization.

P: Proportions of patients with unsatisfactory clinical response.

SAS code:

SAS code as below:

PROC FREQ DATA = DATA;

TABLES TREAT*PROSSTATUS*RESP / CMH NOPERCENT NOCOL;

WEIGHT FREQ;

RUN;

Table 33: Summary of Clinical Response Analysis

Variable	Model	Population	Factors
Proportions of patients with unsatisfactory clinical response	CMH	ITT	Treatment Group and Prostanoid Usage

7.6.8 Subjects Undergoing Heart-Lung or Lung Transplantation, Number of Subjects Listed for Transplantation, and Deaths while Awaiting Transplantation, from Baseline to 18 Weeks

The proportions of patients undergoing heart-lung or lung transplantation, listed for transplantation and deaths while transplantation from baseline at Week 18 will be analyzed using Cochrane Mantel Haenszel test (Table 34).

Hypothesis:

H_0: $P_{iNO\ 75} = P_{Placebo}$ versus H_1: $P_{iNO\ 75} < P_{Placebo}$ for at least one realization.

P: Proportions of patients undergoing heart-lung or lung transplantation from baseline to Week 18.

SAS code:

SAS code as below:

[redacted]

PROC FREQ DATA = DATA;

TABLES TREAT*PROSTATUS*RESP / CMH NOPERCENT NOCOL;

WEIGHT FREQ;

RUN;

Table 34: Summary of Analysis

Variable	Model	Population	Factors
Proportions of patients undergoing heart or lung transplant	CMH	ITT	Treatment Group and Prostanoid Usage

7.6.9 Medical Resource Utilization from Baseline to 18 Weeks

The medical resource utilization will be summarized.

7.6.10 Hospitalization

The proportions of patients undergoing hospitalization will be analyzed using Cochrane-Mantel-Haenszel test.

Hypothesis:

H_0: $P_{iNO\ 75} = P_{Placebo}$ versus H_1: $P_{iNO\ 75} < P_{Placebo}$ for at least one realization.

P: Proportions of patients undergoing heart-lung or lung transplantation from baseline to Week 18.

SAS code:

SAS code as below:

PROC FREQ DATA = DATA;

TABLES TREAT*PROSTATUS*RESP / CMH NOPERCENT NOCOL;

WEIGHT FREQ;

RUN;

Total duration of hospitalization will be summarized by treatment arm and analysed using appropriate statistical test as part of exploratory analysis.

7.7 Safety Analysis

Adverse events (AEs and SAEs) and concomitant medication data will be analyzed and summarized based on Safety Analysis set.

7.7.1 Adverse Events:

The adverse events will be coded in accordance with Bellerophon standard procedures. The incidence of Treatment emerged adverse events (the percentage of subjects having experienced at least one AE) during a defined study period will be determined. Only adverse events with a start date on or after the date of randomization will be taken into account.

The following categories of adverse events will be compared between two treatment groups using Fisher's exact test.

From Date of Randomization through End of double blind period (Week 18):

- All events through end of blinded period (week 18),
- All events at Body System Organ class level through end of blinded period (week 18),
- All events at preferred term level through end of blinded period (week 18).

Comparison between groups of the incidence of serious adverse and the incidence of death (if any) will be done by Fisher's exact for the treatment period and the entire study through post treatment follow-up.

All AEs will be listed for each patient and summarized by formula groups according to the System Organ Class (SOC) and Preferred Term (PT) assigned to the event using MedDRA. All the summary tables will be presented from randomization through end of treatment for blinded phase of study and from randomization through end of follow-up period.

The following will be summarized for subjects in safety analysis set:

- Adverse events in any category - patient level
- Adverse events in any category - episode level
- Adverse events by system organ class and Preferred Term
- Adverse events by SOC and PT presented by maximum reported severity
- Adverse events related to study product, presented by SOC and PT
- Adverse events leading to withdrawal of study product, presented by PT, arranged by SOC
- Adverse events causality related to study product, presented by SOC and PT
- Adverse events leading to study product interruption, presented by PT, arranged by SOC
- Adverse events leading to dose reduction of study product, presented by PT, arranged by SOC
- Adverse events leading to increase in dose of study product, presented by PT, arranged by SOC
- Adverse events leading to discontinuation of study product, presented by PT, arranged by SOC
- Key information for serious adverse events
- Serious adverse events causality related to study product, presented by SOC and PT
- Serious adverse events leading to study product interruption, presented by PT, arranged by SOC
- AE's on special interest

AE/SAE data collected at screening will be presented for all enrolled patients separately.

7.7.2 Concomitant Medication:

All concomitant medications will be summarized by treatment arm and standard listings will be produced.

7.7.3 Laboratory Data and Physical examination:

All the laboratory parameters and physical examination data will be summarized by treatment arm.

7.7.4 Pulmonary Arterial Hypertension Specific Treatments:

A frequency table for all the PAH specific therapies will be summarized by treatment arm.

Change in PAH therapies at each visit will be summarized by sub categories like Phosphodiesterase Inhibitors (PDE 5 Inhibitors) and Endothelin Receptor Antagonists (ERAs).

7.7.5 Diuretic Therapy:

A frequency table for diuretic therapy usage between the treatment arms will be presented.

7.7.6 Acute Vasoreactivity Testing (AVT)

Comparison of the positive responders vs. non responders and change in 6MWD at 18 weeks for active and placebo separately.

8.0 Interim Analysis

A formal interim analysis is planned in this study as follows:

1. Stop for futility (null hypothesis is accepted; there is no difference between iNO and Placebo).
2. Stop for efficacy (null hypothesis is rejected; there is difference between iNO and Placebo favoring iNO).
3. Sample size determination.
4. Stop in case of safety concerns. Safety concerns may be some imbalances in adverse events frequency which is not in favor of iNO. No formal statistical guideline is provided for this purpose, the sponsor relies on the medical expertise of DMC members.
5. Continue the trial with the planned sample size until next stage if point 1, 2 or 4 not apply.

The following two designs will define the interim analysis strategy:

1) Design 1: Groups Sequential Design (for efficacy part)
2) Design 2: Sample Size Re-estimation (SSR) (for futility and sample size re-estimation)

The interim analysis schedule planned as follows:

Schedule	Requirement
1st	After 75 patients complete 18 Weeks of blinded treatment phase.
Final	After 150/188 (depending on CP and PP) patients complete 18 Weeks of blinded treatment phase.

Traditional group sequential design (GSD) alpha spending function Gamma=-3 (Hwang, Shih, deCani, 1990) is used to control the overall type 1 error at 0.025, 1-sided. The interim analysis rule (Table 35) is as follows.

Table 35: Interim and Final Analysis Rules:

Category	Rule
Efficacy	At interim analysis (after 75 patients), if the one-sided p<0.0046 then the study will stop for efficacy (Wald Statistics)
Efficacy	At final analysis (after 150 patients or 188 patients depending on CP and PP), if the one-sided p < 0.0229 then the study will yield an efficacy conclusion. (based on CHW Statistics)
Futility	At interim analysis, if conditional power (CP) or predictive power (PP) ≤ 10% then the study stops for futility.
Continue	At interim analysis, if 10% < CP and PP < 57.2% then trial continues to 150 patients.
SSR	At interim analysis, if 57.2% ≤ CP or PP < 80% the sample size is increased to N=188. The lower bound of the promising zone interval was calculated based on the minimum treatment effect needed to achieve 70% or higher CP.
Continue	At interim analysis, if CP and PP ≥ 80% and 1-sided p>0.0046, then the trial continues to N = 150 patients.

This is the "promising zone" sample size re-estimation (SSR) design of Mehta & Pocock (2011), which uses the Cui, Hung, Wang (1999) method of analysis to combine the test statistics for the separate analyses of the IA and post-IA parts of the trial, if the trial does not stop at IA.

The final analysis is via weighted Z statistic using equal weights for the IA and post IA test statistics. It requires p<0.0229 1-sided for statistical significance to control the overall type 1 error level at 0.025 1-sided with gamma=-3 error spending function (Hwang, Shih, deCani 1990).

9.0 Part II Analysis:

The primary objective of part II open label is incidence of AE and SAE. All the tables for Part I AE's will be replicated for Part II to assess long term safety and tolerability.

The secondary objective is to evaluate the change from baseline in 6MWDfor subjects who change from Placebo to active therapy. Standard descriptive statistics will be presented. No formal statistical analysis is planned.

10.0 Statistical Software

SAS 9.4 or above and EAST 6.4 will be used for statistical analysis of the study.

11.0 Changes of analysis from Protocol

Changes	Reason

12.0 References

1. Anderson JR, Nawarskas JJ. Pharmacotherapeutic management of pulmonary arterial hypertension. Cardiol Rev. 2010 May-Jun;18(3):148-62.

2. McGoon MD, Garvan CK. Pulmonary Hypertension: Diagnosis and Management. Mayo Clin Proc. 2009;84(2):191-207.

3. McLaughlin W, et al. Randomized study of adding inhaled iloprost to existing bosentan in pulmonary arterial hypertension. Am J Respir Crit Care Med 2006;174(11):1257-1263.

4. Ghofrani HA, Galiè N, Grimminger F, Grünig E, Humbert M, Jing ZC, et al. Riociguat for the Treatment of Pulmonary Arterial Hypertension. N Engl J Med 2013;369:330-40.

5. Barst RJ, Gibbs JS, Ghofrani HA, Hoeper MM, McLaughlin VV, Rubin LJ, et al. Updated evidence-based treatment algorithm in pulmonary arterial hypertension. J Am Coll Cardiol. 2009 Jun 30;54(1 Suppl):S78-84.

6. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, et al. Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). Circulation. 2010 Jul 13;122(2):164-72. Epub 2010 Jun 28.

7. Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest. 2007 Jun;131(6):1917-28.

8. Hwang IK, Shih WJ, and DeCani JS (1990). Group sequential designs using a family of type I error probability spending functions. Statistics in Medicine, 9, 1439-1445.

9. Mehta CR and Pocock SJ (2011). Adaptive Increase in Sample Size when Interim Results are Promising: A Practical Guide with Examples. Statistics in Medicine30 (28): 3267-8

10. Cui L, Hung HM, Wang SJ. Modification of sample size in group sequential clinical trials. Biometrics. 1999 Sep;55(3):853–857. PMID: 11315017

11. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. Galiè N1, Rubin Lj, Hoeper M, Jansa P, Al-Hiti H, Meyer G, Chiossi E, Kusic-Pajic A, Simonneau G.

12. Modern Approaches to Clinical Trials Using SAS®: Classical, Adaptive, and Bayesian Methods. Sandeep Menon, Richard C. Zink

13. Galiè N, Rubin L, Hoeper M, Jansa P, Al-Hiti H, Meyer G, et al. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. Lancet. 2008 Jun 21;371(9630):2093-100.