Protocol

A Phase 1, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of L606 for Inhalation in Healthy Subjects

Protocol Status: Final
Protocol Date: 14 November 2018

Investigational Product: L606

Protocol Number: PBI L606_2.0

IND Number: 137502

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Date

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INVESTIGATOR AGREEMENT

I have read the protocol and agree to conduct the study as described herein.

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SYNOPSIS

Title of study: A Phase 1, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of L606 for Inhalation in Healthy Subjects

Objectives:

The primary objective of the study is:

- To evaluate the safety and tolerability of single ascending doses of treprostinil after administration of L606 in healthy subjects.

The secondary objective of the study is:

- To assess the pharmacokinetic (PK) profile of single ascending doses of treprostinil after administration of L606 in healthy subjects.

Study design:

This will be a Phase 1, single-dose, randomized, double-blind, placebo-controlled, sequential-group study conducted at a single center.

Potential subjects will be screened (Days -28 to -2) to confirm that they fulfil the eligibility inclusion/exclusion criteria to enter the study. After verification of eligibility, subjects will be admitted to the Clinical Research Unit (CRU) on Day -1 (Check-in, the day before dosing). On Day 1, prior to dose administration, subjects will be randomized in a double-blinded fashion to L606 or placebo in a 6:2 randomization ratio.

The planned emitted dose escalation groups are:

- Group 1: 51 µg L606 or placebo volume equivalent to 51 µg
- Group 2: 102 µg L606 or placebo volume equivalent to 102 µg
- Group 3: 153 µg L606 or placebo volume equivalent to 153 µg
- Group 4: 204 µg L606 or placebo volume equivalent to 204 µg
- Group 5: 255 µg L606 or placebo volume equivalent to 255 µg
- Group 6: 306 µg L606 or placebo volume equivalent to 306 µg
- Group 7: 357 µg L606 or placebo volume equivalent to 357 µg
- Group 8: 408 µg L606 or placebo volume equivalent to 408 µg

L606 or placebo will be administered once in a solution for inhalation. The entire treatment procedure will be completed within 10 minutes.

On Day 3 (48 hours postdose), subjects will be discharged from the CRU after all protocol-specified assessments have been completed. Subjects will return for a Follow-up visit 7 to 10 days postdose. Based on the ongoing review of the safety, tolerability, and PK results, additional nonresidential visits may be required.

Safety and PK data will be reviewed following the completion of each group. Based on this data (as well as aggregate data from previous groups), a decision will be made to proceed with:

1) escalation to the next higher planned dose;
2) repeat evaluation of the same dose in an additional group;
3) evaluation of a lower intermediate dose before moving on to the next higher dose; or
4) discontinuation of dosing.

Number of subjects:

A total of approximately 64 subjects (8 subjects in each of 8 groups) will be enrolled in this study.

Diagnosis and main criteria for inclusion:
Healthy males and females 18 to 50 years of age, inclusive, with a body mass index between 18.5 and 32.0 kg/m^2, inclusive.

Investigational products, dose, and mode of administration:
Test product: L606 (liposomal treprostinil) solution, 1.5 mg treprostinil in 1.0 mL liposome solution
Administration route: Oral inhalation completed within 10 minutes

Reference product and mode of administration:
Reference product: placebo solution (L606 solution excluding the active pharmaceutical ingredient [treprostinil])
Administration route: Oral inhalation completed within 10 minutes

Duration of subject participation in the study:
Planned Screening duration: Approximately 4 weeks
Planned in-residence duration: Approximately 4 days
Planned total study duration (Screening to Follow-up): Approximately 6 weeks

Endpoints:
Safety:
Safety will be evaluated by the incidence and severity of adverse events (AEs), incidence of serious AEs, the monitoring of vital sign assessments, 12-lead electrocardiograms, clinical laboratory evaluations, and physical examination.

Pharmacokinetics:
The plasma concentrations of treprostinil after L606 inhalation dosing will be determined using a validated bioanalytical method (liquid chromatography by tandem mass spectrometric analysis), and analyzed using noncompartmental method. The PK parameters will include area under the plasma concentration-time curve (AUC) from time zero to 2 hours postdose ($AUC_{0\text{-}2hr}$), 4 hours postdose ($AUC_{0\text{-}4hr}$), 8 hours postdose ($AUC_{0\text{-}8hr}$), 12 hours postdose ($AUC_{0\text{-}12hr}$), and 24 hours postdose ($AUC_{0\text{-}24hr}$); AUC from time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$); AUC from time zero to infinity ($AUC_{0\text{-}\infty}$); maximum observed plasma concentration (C_{max}); time of the maximum observed plasma concentration (t_{max}); apparent plasma terminal elimination half-life ($t_{1/2}$); apparent total plasma clearance (CL/F); and apparent volume of distribution during terminal phase (V_z/F). Other PK parameters may be calculated as appropriate.

Statistical methods:
Safety:
All safety data will be listed. Descriptive statistics and changes from baseline will be calculated for safety parameters as appropriate. These summaries will be produced for each dose level of L606 and the pooled placebo subjects. No formal statistical analyses are planned for the safety endpoints.

Pharmacokinetics:
Plasma concentrations and PK parameters of treprostinil will be listed and summarized using descriptive statistics (arithmetic mean, standard deviation, minimum, median, and maximum, geometric mean, and geometric coefficient of variation, as appropriate). Individual and mean treprostinil concentration-time profiles will also be presented graphically.

The AUCs and C_{max} will be the primary endpoints for the evaluation of dose proportionality. Dose proportionality of treprostinil will be evaluated by assessing the slope from regression analysis of log(AUC or C_{max}) versus log(dose) from the power model for those subjects who receive L606.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{0-tlast}$	area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration
AUC_{0-2hr}	area under the plasma concentration-time curve from time zero to 2 hours postdose
AUC_{0-4hr}	area under the plasma concentration-time curve from time zero to 4 hours postdose
AUC_{0-8hr}	area under the plasma concentration-time curve from time zero to 8 hours postdose
AUC_{0-12hr}	area under the plasma concentration-time curve from time zero to 12 hours postdose
AUC_{0-24hr}	area under the plasma concentration-time curve from time zero to 24 hours postdose
BA	bioavailability
CFR	Code of Federal Regulations
CL/F	apparent total plasma clearance
C_{max}	maximum observed plasma concentration
COPD	chronic obstructive pulmonary disease
CRO	Contract Research Organization
CRU	Clinical Research Unit
CSA	clinical study agreement
EDC	electronic data capture
ECG	electrocardiogram
eCRF	electronic Case Report Form
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GCP	Good Clinical Practice
GLP	Good Laboratory Practice

HED	human equivalent dose
ICF	Informed Consent Form
ICH	International Council for/Conference on Harmonisation
IMP	investigational medicinal product
IRB	Institutional Review Board
IUD	intrauterine device
IV	intravenous
L606	Liposomal Treprostinil Inhalation Solution
MPC	mouthpiece cartridge
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
PAH	pulmonary arterial hypertension
PBI	Pharmosa Biopharm Inc
PK	pharmacokinetic(s)
QTcF	QT interval corrected for heart rate using Fridericia's method
SAE	serious adverse event
$t_{1/2}$	apparent plasma terminal elimination half-life
t_{max}	time of the maximum observed plasma concentration
TMF	Trial Master File
ULN	upper limit of normal
V_z/F	apparent volume of distribution during terminal phase

1. INTRODUCTION

1.1. Overview

Pharmosa Biopharm Inc (PBI) is developing L606 (Liposomal Treprostinil Inhalation Solution) (hereafter called L606), a new liposomal treprostinil formulation for inhalation use in the treatment of patients with World Health Organization (WHO) Group I pulmonary arterial hypertension (PAH) and New York Heart Association (NYHA) Class III symptoms, to increase exercise ability.

L606 is planned to be administered via the same inhaled route as Tyvaso®, which is USA Food and Drug Administration (FDA) approved for the indication of PAH in patients. L606 is composed of liposomes (>100 nm) in sodium citrate-bicarbonate buffer. The liposomes are composed of hydrogenated soybean phosphatidyl choline (HSPC), cholesterol, and sodium distearoyl phosphatidyl glycerol (DSPG-Na).

Liposomes as a pulmonary drug delivery system have been reported to enhance the therapeutic benefits of drugs and to reduce the potential for systemic adverse effects. Studies have established the high biocompatibility and biodegradability of liposomes as drug carriers in inhaled formulations.

The L606 solution uses PBI's proprietary liposomal formulation to encapsulate the treprostinil, which can be released slowly under a controlled manner/rate into the lung. This control enables modulation of drug release to achieve optimized drug exposure over an extended period of time and reducing local irritation on the respiratory tract. The L606 inhalation system consists of a mesh-vibrating nebulizer that has been approved for use in Europe.

1.2. Inhaled Treprostinil Background

1.2.1. General Pharmacology

Treprostinil, 2-[[(1R,2R,3aS,9aS)-[[2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3hydroxyoctyl]-1Hbenz[f]inden-5-yl]oxy]acetic acid, is a chemically stable tricyclic analogue of prostacyclin, a strong vasodilator and potent inhibitor of platelet aggregation.[1] The pharmacology of treprostinil is well-characterized and approved for the treatment of PAH following either the subcutaneous[2], intravenous[2] (IV), inhaled[3] (as treprostinil sodium), or oral[4] (as treprostinil diolamine) routes of administration.

Treprostinil for inhalation (Tyvaso) is approved in the United States for the treatment of PAH (World Health Organization Group I) in patients with New York Heart Association functional classification III symptoms, to increase exercise ability.[3]

Tyvaso was developed for oral inhalation using the Tyvaso inhalation system, an ultrasonic, pulsed-delivery device.[5]

1.2.2. General Toxicology

A well-defined clinical safety profile exists for treprostinil sodium; acute toxicity studies and repeat-dose toxicity studies performed in both rats and dogs, and reproductive toxicity and genotoxicity studies support the chronic administration to patients.[3]

In addition, a 2-year rat carcinogenicity study was performed with treprostinil inhalation at target doses up to 5.26, 10.6, and 34.1 µg/kg/day which found no evidence for carcinogenic potential associated with inhaled treprostinil in rats at systemic exposure levels up to 35 times the clinical exposure at the target maintenance dose of 54 µg.[3]

1.2.3. Clinical Experience

Pilot clinical studies have elucidated the acute hemodynamic effects and relative pulmonary selectivity of Tyvaso, and have demonstrated clinical and hemodynamic improvement with chronically administered Tyvaso.[5] Both pilot studies confirmed a satisfactory safety profile in patients with PAH. The pivotal Phase 3 trial, TRIUMPH-I, demonstrated the efficacy and safety of Tyvaso (target dose of 54 µg four times daily) added to background therapies of bosentan or sildenafil in PAH patients, as assessed by improvements in exercise ability.[5]

The effects of Tyvaso diminish over the minimum recommended dosing interval of 4 hours. Overall, in clinical studies the most common adverse reactions (≥10%) to Tyvaso were cough, headache, nausea, dizziness, flushing, throat irritation, pharyngolaryngeal pain, and diarrhea.[3]

1.2.4. Pharmacokinetic Profile of Inhaled Treprostinil

Two studies characterized the pharmacokinetic (PK) profile of single doses of inhaled treprostinil in healthy volunteers. Eighteen subjects were enrolled in a 3 period crossover bioavailability (BA) study. The absolute BAs of the inhaled treprostinil 18 and 36 µg were compared to a 15-ng/kg/minute (60 minute) IV infusion of treprostinil. Forty subjects were enrolled in a maximum tolerated dose (MTD) study of 54, 72, 78, 84, and 90 µg of inhaled treprostinil. Maximum observed plasma concentration (C_{max}), time of the maximum observed plasma concentration (t_{max}), and area under the plasma concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) were determined in both studies.[6]

In the bioavailability (BA) study, C_{max} and $AUC_{0-\infty}$ were dose proportional following a single administration of inhaled treprostinil 18 or 36 µg and resulted in 64% and 72% absolute systemic BA, respectively. The mean t_{max} was 0.15 hours for both inhaled doses.[6]

In the MTD study, inhaled treprostinil was dose proportional for $AUC_{0-\infty}$ (mean range: 0.661 to 1.579 ng*hr/mL) and C_{max} (mean range: 790 to 1708 pg/mL). The mean t_{max} was 0.20 hours across all doses. Adverse events (AEs) of chest pain, chest discomfort, nausea, and vomiting in the 90 µg cohort were determined to be intolerable; thus the MTD for a single dose of inhaled treprostinil was 84 µg.[6]

Inhaled treprostinil remained detectable in the plasma approximately 4 hours after inhalation in both studies. Apparent plasma terminal elimination half-life ($t_{1/2}$), calculated using a noncompartmental model, ranged from 0.46 to 0.76 hours across both studies.[6]

1.3. Liposomal Treprostinil-related Potential Toxicity and Pharmacokinetics

Many studies have established the high biocompatibility and biodegradability of liposomes as drug carriers in inhaled formulations. The L606 solution is composed of liposomes encapsulating the treprostinil in sodium citrate-bicarbonate buffer. The liposomes are composed of hydrogenated soybean phosphatidyl choline, cholesterol, and distearoyl phosphatidyl glycerol. In addition to the treprostinil-related toxicity observed in the approved products, the liposomal treprostinil is speculated to be involved only with potential respiratory-related toxicity.

The L606 liposomes, with mean diameter >100 nm, are inhaled and reside in the lung. It is believed that treprostinil is released from inhaled liposomes in situ in the lung and adsorbed into the blood. Once released from liposomes in the pulmonary system, treprostinil is expected to follow the same absorption, distribution, metabolism, and excretion pathways as free drug, as previously demonstrated for Tyvaso, Remodulin, and Orenitram. Bioavailability of treprostinil in L606 was compared with treprostinil solution in terms of plasma AUC in rat. Intratracheal administration was used to reduce the effects of aerosol size distribution and nebulizer device. It was found that the 2 formulations are proportional to the applied doses. No saturation phenomena were observed within the tested dose range. The presence of liposome did not affect BA of released (free) treprostinil. Liposomal treprostinil demonstrated sustained release in the pulmonary system as compared to the immediate release of treprostinil solution. It was also indicated that release of treprostinil is triggered by simulated lung fluid and lung surfactant. In contrast, only 5% of treprostinil was released from liposome in the simulated nasal fluid within 8 hours.

1.4. Potential Benefits of L606 (Liposomal Treprostinil Inhalation Solution)

The actual concentration of drugs reaching resistance vessels may be greater with targeted delivery by aerosol.[7] As shown in rat toxicokinetic studies, inhaled treprostinil is rapidly absorbed by the lung. After crossing the pulmonary epithelium, the drug may diffuse into the blood or be collected by the pulmonary lymphatic fluid. Accordingly, treprostinil may reach resistance vessels without systemic circulation.[8]

The long duration of pulmonary vasodilation after a single inhalation of treprostinil may be partially explained by the stability of this prostanoid. It is theorized that L606 is deposited in the lung after inhalation, providing a slow release from the alveolar lining layer or the interstitial compartment to the pulmonary vascular smooth muscle cells.[9]

1.5. Study Rationale

This is the first-in-human study for L606. The principal aim of this study is to obtain safety and tolerability data when L606 is administered as an oral inhalation as single doses to healthy

subjects. This information, together with the PK data of treprostinil after administration of L606, will help establish the doses and dosage regimen suitable for administration to patients.

1.6. Benefit-risk Assessment

Tyvaso inhibits platelet aggregation and increases the risk of bleeding.[3] Subjects with increased bleeding risk will be excluded from this study.

Coadministration of a cytochrome P450 2C8 enzyme inhibitor (eg, gemfibrozil) may increase exposure (both C_{max} and AUC) to treprostinil. Increased exposure is likely to increase AEs associated with treprostinil administration. Overall, concomitant medications are not allowed during the study (Section 6.1). Also see the exclusion criteria for medication/product restrictions prior to Check-in (Section 4.2).

Healthy subjects in the current study will not receive any health benefit (beyond that of an assessment of their medical status) from participating in the study. The risks of participation are primarily those associated with adverse reactions to the investigational medicinal product (IMP), although there may also be some discomfort from collection of blood samples and other study procedures. More information about the known and expected benefits, risks, and reasonably anticipated AEs associated with L606 may be found in the Investigator's Brochure.[10]

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objective of the study is:

- To evaluate the safety and tolerability of single ascending doses of treprostinil after administration of L606 in healthy subjects.

The secondary objective of the study is:

- To assess the PK profile of single ascending doses of treprostinil after administration of L606 in healthy subjects.

2.2. Endpoints

2.2.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

- incidence and severity of AEs
- incidence of serious AEs (SAEs)
- vital sign assessments (supine blood pressure, supine pulse rate, and respiratory rate)
- 12-lead electrocardiogram (ECG) parameters
- incidence of laboratory abnormalities, based on hematology, clinical chemistry, and urinalysis test results
- physical examinations.

2.2.2. Secondary Endpoints

The single ascending dose PK endpoints of treprostinil are as follows:

- AUC from time zero to 2 hours postdose ($AUC_{0\text{-}2hr}$)
- AUC from time zero to 4 hours postdose ($AUC_{0\text{-}4hr}$)
- AUC from time zero to 8 hours postdose ($AUC_{0\text{-}8hr}$)
- AUC from time zero to 12 hours postdose ($AUC_{0\text{-}12hr}$)
- AUC from time zero to 24 hours postdose ($AUC_{0\text{-}24hr}$)
- AUC from time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$)
- $AUC_{0\text{-}\infty}$
- C_{max}
- t_{max}

- $t_{1/2}$
- apparent total plasma clearance (CL/F)
- apparent volume of distribution during terminal phase (V_z/F).

Other PK parameters may be calculated as appropriate.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This will be a Phase 1, single-dose, randomized, double-blind, placebo-controlled, sequential-group study conducted at a single center. Overall, approximately 64 subjects are planned to be enrolled in 8 groups, with each group consisting of 8 subjects (Figure 2).

Potential subjects will be screened (Days -28 to -2) to confirm that they fulfil the eligibility inclusion/exclusion criteria to enter the study. After verification of eligibility, subjects will be admitted to the Clinical Research Unit (CRU) on Day -1 (Check-in; the day before dosing). Each subject will participate in 1 treatment period only and reside at the CRU from Day -1 to Day 3.

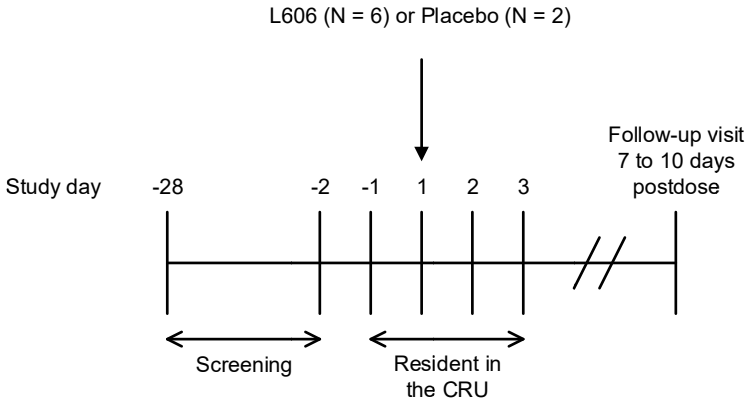
Sentinel dosing, where the first 2 subjects will be randomized 1:1 to L606 or placebo, will be used for the first 2 groups dosed. The remaining subjects in the group will be randomized 5:1 to L606 or placebo and administered study medication after the Investigator has reviewed the first 48 hours of safety data for the first 2 subjects. It is anticipated that any unanticipated safety and/or tolerability effects will be detected in the first 2 subjects dosed within a group. Sentinel dosing will not be required for subsequent groups.

All subjects will return for a Follow-up visit 7 to 10 days postdose.

Based on the ongoing review of the safety, tolerability, and PK results, additional nonresidential visits may be required. The number of additional visits per subject will not exceed 3 and will not extend beyond 28 days after dosing.

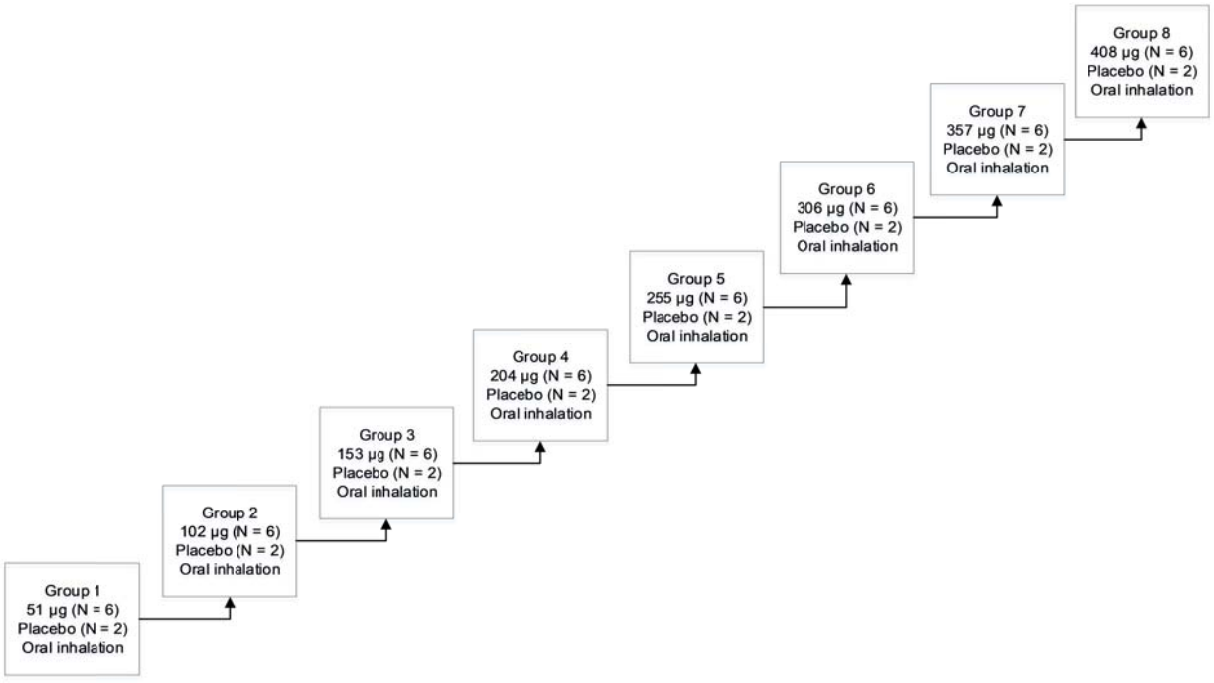
An overview of the study design is shown in Figure 1 and the planned dose levels are shown in Figure 2.

Figure 1: Study Schematic



The total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 6 weeks.

Figure 2: Planned L606 Emitted Dose Levels



Clinical laboratory evaluations, physical examination, vital signs, safety 12-lead ECGs, the incidence and severity of AEs, and the incidence of SAEs will be monitored to assess safety and tolerability. A Schedule of Assessments is presented in Appendix 5.

Safety data (in particular those assessments which are considered for dose escalation stopping/dosing stopping criteria; Section 3.7) and PK data (as available) will be reviewed following the completion of each group. Based on this data (as well as aggregate data from previous groups), a decision will be made to proceed with:

1) escalation to the next higher planned dose;
2) repeat evaluation of the same dose in an additional group;
3) evaluation of a lower intermediate dose before moving on to the next higher dose; or
4) discontinuation of dosing.

3.2. Study Start and End of Study Definitions

The start of the study is defined as the date the first enrolled subject signs an Informed Consent Form (ICF). The point of enrollment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

3.3. Additional Groups

Following review of the safety, tolerability, and PK data, additional dose groups may be added to the study in a protocol amendment. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included. The requirement for additional groups will be agreed with the Sponsor, documented in the Trial Master File (TMF), and the Institutional Review Board will be notified of the changes.

3.4. Discussion of Study Design, Including the Choice of Control Groups

A sequential-group, ascending-dose design has been chosen for safety reasons as this will be the L606 first-in-human study. Oral inhalation doses have been chosen for the study as this is the intended clinical route of administration.

Based upon the nonclinical data and clinical safety and efficacy information on the active ingredient, the duration of each treatment period is considered adequate to achieve the study objectives.

This study will be double-blind and placebo-controlled in order to avoid bias in the collection and evaluation of data during its conduct. Placebo has been chosen as the control treatment to assess whether any observed effects are treatment-related or simply reflect the study conditions.

The L606 data, along with clinical experience with treprostinil, suggest that single doses of L606 are unlikely to result in significant adverse effects in healthy subjects. Conducting the study in healthy subjects mitigates the potential confounding effects of the disease state and concomitant medications.

Sentinel dosing will be used in this study. To minimize risk, there will be an interval of at least 48 hours for evaluation of safety after the first 2 subjects have been dosed (1 with L606 and 1 with placebo) in each of the first 2 groups.

3.4.1. Dose Interval

The pharmacology of treprostinil is well-characterized and approved for the treatment of PAH in 3 other formulations. L606 is being developed to accomplish high drug loading efficiency and sustained release properties, enabling a drug therapeutic effect over a prolonged time period and reducing local irritation on the respiratory tract. Because the treprostinil exposure will consequently be different from the exposure of other formulations, the safety profile cannot be entirely extrapolated to L606 (Liposomal Treprostinil Inhalation Solution). Therefore, dosing for the first 2 groups will be such that 2 subjects (1 L606 and 1 placebo) will be dosed 48 hours before the remaining 6 subjects. Dose administration of the remaining 6 subjects in the group will be at the Investigator's discretion.

3.5. Selection of Doses in the Study

Dose administration using an inhalation system needs to account for differences between the dose loaded into the nebulizer (loaded dose), the dose emitted from the nebulizer (emitted dose), and the dose delivered to the lung (respirable dose). For the L606 inhalation system, approximately 85% of the loaded dose is emitted and approximately 60% of the emitted dose is respirable. For comparison, approximately 80% of the dose emitted by the Tyvaso inhalation system is respirable.

The planned L606 doses were selected based on current nonclinical pharmacology and toxicology data, along with clinical data for Tyvaso (Treprostinil Inhalation Solution). The initial, recommended, and maximum tolerated emitted doses for Tyvaso, with corresponding respirable doses, are listed in Table 2. Additional clinical information for Tyvaso is summarized in Section 1.2.3.

In a Good Laboratory Practice (GLP) 28-day inhalation toxicology study, the L606 no observed adverse effect level (NOAEL) in Sprague Dawley rats was 20.0 µg/kg (respirable dose). This corresponds to a human equivalent dose (HED) of 3.23 µg/kg (193.55 µg dose for a 60 kg human) as calculated from Food and Drug Administration (FDA) guidance (FDA 2005). The proposed starting emitted dose is 51 µg L606, giving an approximately 3.8-fold safety margin compared to the HED for the NOAEL in the rat. The proposed emitted dose is expected to yield a respirable dose of 30.6 µg, which provides an approximately 6.3-fold safety margin compared with the HED for the NOAEL in rat.

The estimated AUC ratio of NOAEL in rat to L606 starting dose in human is approximately 97-fold, which was calculated from the AUC for NOAEL in rat (55.4 ng•h/mL) divided by estimated L606 AUC at starting dose (approximately 0.57 ng•h/mL). The estimated L606 AUC at starting dose (approximately 0.57 ng•h/mL) was derived from the equality of two ratios; 1) AUC ratio of Tyvaso to L606 at starting dose in healthy volunteers (0.8 ng•h/mL) to

2) respiratory dose ratio of Tyvaso 43.2 µg/kg (80% of emitted dose 54 µg/kg) to L606 30.6 µg/kg (60% of emitted dose 51 µg/kg).

Starting dose calculations are summarized in Table 1.

Table 1: Summary of Starting Dose Calculations

	Respirable Dose	**Emitted Dose**	**AUC (ng•hr/mL)**
Rat NOAEL (28-day inhalation toxicology study)	20.0 µg/kg	-	55.4
HED	3.23 µg/kg (193.55 µg/60 kg)	-	-
Proposed Starting Dose in Humans	30.6 µg (60% of emitted dose)	51 µg	-
Estimated AUC at Starting Dose	-	-	0.57 0.8 ng•h/mL / (43.2 µg/kg / 30.6 µg/kg)
Safety Margin to Respirable HED or AUC	6.3-fold (193.55 µg / 30.6 µg)	3.8-fold (193.55 µg / 51 µg)	~97-fold (55.4 ng•h/mL / 0.57 ng•h/mL)

Abbreviations: AUC = area under the plasma concentration-time curve; HED = human equivalent dose; NOAEL = no observed adverse effect level.

A non-GLP 10-day inhalation toxicity study in rats showed L606 had reduced clinical incidence during inhalation exposure compared to Tyvaso, suggesting L606 may be better tolerated. Treprostinil C_{max} is considered related to patient adverse effects both locally and systemically. Encapsulation of treprostinil in liposome improved the PK profile in terms of reducing C_{max} and exerting a stable plasma level for an extended duration (up to 8 hours). In contrast, the Tyvaso exhibited an immediate release in lung and rapid adsorption, distribution, and metabolism in plasma, resulting in high peak-trough fluctuations during a treatment session of 4 hours. L606 formulation exhibited a preferential PK profile with a comparable C_{max} value at the target dose of 48 µg/kg to that of Tyvaso at the target dose of 6 µg/kg. These results suggest the MTD for L606 could be 8 times higher than the MTD for Tyvaso. The planned maximum L606 emitted dose is 480 µg, which is expected to yield a respirable dose that is less than 4 times the Tyvaso maximum tolerated respirable dose.

Details of all doses administered will be documented in the TMF.

Table 2: Tyvaso Reference Doses and Planned L606 Doses

Treprostinil Formulation	Reference/Cohort	Loaded Dose (µg)	Loaded Volume (µL)	Emitted Dose (µg)	Respirable Dose (µg)
Tyvaso	Initial Dose	--	--	18	14.4
	Recommended Dose	--	--	54	43.2
	Maximum Tolerated Dose	--	--	84	67.2
L606[a]	Cohort 1	60	40	51	30.6
	Cohort 2	120	80	102	61.2
	Cohort 3	180	120	153	91.8
	Cohort 4	240	160	204	122.4
	Cohort 5	300	200	255	153.0
	Cohort 6	360	240	306	183.6
	Cohort 7	420	280	357	214.2
	Cohort 8	480	320	408	244.8

Tyvaso emitted dose × 80% = respirable dose (80% is the fine particle fraction of the Tyvaso inhalation system)
L606 loaded dose × 85% = emitted dose (85% is the emission efficiency of the L606 nebulizer)
L606 emitted dose × 60% = respirable dose (60% is the fine particle fraction of the L606 nebulizer)
[a] 1.5 mg active pharmaceutical ingredient (treprostinil) in 1.0 mL liposome solution

3.6. Dose Escalation

Doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety, tolerability, and PK from the lower dose levels. Doses may be reduced and may be lower than the starting dose. There will be a minimum of 7 days between dose escalations to allow sufficient time for an adequate safety review.

Between each dose escalation, the safety data review committee, consisting of the Principal Investigator, Medical Monitor, and Sponsor's Representative, will review all available blinded data to ensure it is safe to proceed with the planned dose escalation. An interim safety report, summarizing results from all available safety assessments, will be sent to the Sponsor prior to the start of each successive dose escalation group. Any clinically significant results will be discussed with the Sponsor before dose escalation continues. Interim PK data will also be reviewed in terms of dose escalation and to confirm that the study design remains appropriate. In the event of a disagreement between Sponsor and Investigator on the dose escalation decision, the decision of the Investigator will be upheld.

3.7. Dose Escalation Stopping/Dosing Stopping Criteria

If any of the following scenarios occur within a group, dose escalation will be stopped, dose levels will not be repeated, and higher dose levels will not be tested:

- Clinically relevant signs or severe symptoms of similar nature occur in ≥2 subjects within a group, which in the opinion of the safety review team warrant stopping of dose escalation.

- A symptomatic drop in blood pressure from supine to standing of >20 mmHg systolic and/or >10 mmHg diastolic is seen in ≥2 subjects within a group.
- Occurrence of any SAE, which in the opinion of the Principal Investigator, is deemed to be related to study drug.
- Evidence of clinically significant increases in liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], bilirubin, and gamma glutamyl transferase, defined as ALT or AST >5 × the upper limit of normal [ULN]; or ALT or AST >3 × ULN with a total bilirubin of >2 × ULN with symptoms [ie, monitor for drug-induced liver injury] compared with Day -1) in ≥2 subjects within a group (confirmed with repeat testing).
- Moderate nausea or vomiting that prevents a subject from eating a meal on 3 or more occasions on 2 successive days in ≥2 subjects within a group.
- Symptomatic decrease in supine systolic or diastolic blood pressure of ≥30 or ≥20 mmHg, respectively, from predose baseline in ≥2 subjects within a group.
- Symptomatic decrease in orthostatic systolic or diastolic blood pressure of ≥30 or ≥20 mmHg, respectively, from supine value in ≥2 subjects within a group.
- Forced expiratory volume in 1 second (FEV1) or forced vital capacity (FVC) <65% of predose baseline in ≥2 subjects within a group.
- Symptomatic bronchospasm requiring therapy in ≥2 subjects within a group.
- Incapacitating dyspnea or coughing, hypoxemia (oxygen saturation ≤90%) in ≥2 subjects within a group.
- Methemoglobin ≥7% in ≥2 subjects within a group.

4. SELECTION OF STUDY POPULATION

4.1. Inclusion Criteria

Subjects must satisfy all of the following criteria at the Screening visit unless otherwise stated:

1. Males or females, of any race, 18 to 50 years of age, inclusive, at Screening.
2. Body mass index between 18.5 and 32.0 kg/m^2, inclusive, at Screening.
3. In good health, determined by no clinically significant findings from medical history, physical examination, 12-lead ECG, vital sign measurements, and clinical laboratory evaluations (congenital nonhemolytic hyperbilirubinemia [eg, Gilbert's syndrome] is not acceptable) at Screening or Check-in as assessed by the Investigator (or designee).
4. Ability of the Subject to generate spirometry according to minimum ATS/ERS guidance criteria.
5. Females will not be pregnant or lactating, and females of childbearing potential and males will agree to use contraception as detailed in Section 6.6.

6. Able to comprehend and willing to sign an ICF and to abide by the study restrictions.
7. Agree to abstain from consuming alcohol from 72 hours prior to Check-in.
8. Agree to refrain from strenuous exercise from 7 days prior to Check-in.
9. Agree to abstain from consuming foods and beverages containing poppy seeds, grapefruit, or Seville oranges from 7 days prior to Check-in.
10. Agree to abstain from consuming caffeine-containing foods and beverages from 48 hours prior to Check-in.
11. Agree to abstain from consuming carbonated drinks (including sparkling water and soda) from 48 hours prior to Check-in.

4.2. Exclusion Criteria

Subjects will be excluded from the study in the instance of any of the following criteria at the Screening visit unless otherwise stated:

1. Clinically relevant abnormalities identified during Screening, physical examination, 12-lead ECG, or laboratory examinations.
2. Clinically significant history of hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neurological, respiratory, endocrine, genitourinary, and/or musculoskeletal disease, glaucoma, a psychiatric disorder, or any other chronic disease, whether controlled by medication or not.
3. History of anaphylaxis, significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless deemed not clinically significant by the Investigator (or designee).
4. History of postural hypotension, unexplained syncope, or hypertension.
5. History of asthma, chronic obstructive pulmonary disease (COPD), or reactive airways conditions or findings consistent with asthma or COPD on spirometry testing.
6. Blood pressure <90 mmHg systolic or <60 mmHg diastolic after supine at Screening or Check-in upon repeat testing.
7. Blood pressure >150 mmHg systolic or >90 mmHg diastolic after supine at Screening or Check-in upon repeat testing.
8. Pulse rate >100 bpm after supine for 5 minutes at Screening or Check-in upon repeat testing.
9. Have a predisposing condition that could interfere with the absorption, distribution, metabolism, or excretion of drugs.
10. Use tobacco- or nicotine-containing products within 6 months prior to Check-in, or have a history of >1 pack cigarettes daily use over multiple years of smoking.
11. History of alcoholism or drug/chemical abuse within 2 years prior to Check-in.

12. Have a history of alcohol abuse or a history of or current impairment of organ function reasonably related to alcohol abuse.
13. Have a history of or current evidence of abuse of licit or illicit drugs or a positive urine screen for drugs of abuse.
14. Alcohol consumption of >21 units per week. One unit of alcohol equals 12 oz (360 mL) beer, 1½ oz (45 mL) liquor, or 5 oz (150 mL) wine.
15. Positive urine drug screen (including cotinine) at Screening and Check-in or positive alcohol breath or urine test result at Check-in.
16. Positive hepatitis panel and/or positive human immunodeficiency virus test at Screening.
17. Participation in a clinical study involving administration of an investigational drug (new chemical entity) within 30 days prior to Check-in.
18. Use or intend to use any medications/products known to alter drug absorption, metabolism, or elimination processes, including St. John's Wort, within 30 days prior to Check-in, unless deemed acceptable by the Investigator (or designee).
19. Use or intend to use any prescription medications/products within 14 days prior to Check-in with the exception of hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives, unless deemed acceptable by the Investigator (or designee).
20. Use or intend to use slow-release medications/products considered to still be active within 14 days prior to Check-in, unless deemed acceptable by the Investigator (or designee).
21. Use or intend to use any nonprescription medications/products or herbal supplements within 7 days prior to Check-in, unless deemed acceptable by the Investigator (or designee). Use of nonsteroidal anti-inflammatory drugs or aspirin is prohibited within 14 days prior to Check-in.
22. Receipt of blood products within 2 months prior to Check-in.
23. Donation of blood or plasma, or the loss of a significant volume of blood (>450 mL) within 6 weeks prior to Screening.
24. Poor peripheral venous access.
25. Have a history of bleeding problems or abnormal bleeding tendencies.
26. Platelet or coagulation factor levels below the lower limit of normal, unless considered not clinically significant by the Investigator.
27. Have previously completed or withdrawn from this study or any other study investigating treprostinil, and have previously received the investigational product.
28. History of any recent infection within 2 weeks of Check-in.
29. In the opinion of the Investigator (or designee), should not participate in this study.

4.3. Subject Number and Identification

Subjects will have a unique identification number used at Screening. Subjects will be assigned a subject number prior to the first dosing occasion at the time of their randomization. Assignment of subject numbers will be in ascending order and no numbers will be omitted (eg, Subjects 101, 102, etc.). Replacement subjects (Section 4.4) will be assigned a subject number corresponding to the number of the subject he/she is replacing plus 1000 (eg, Subject 1101 replaces Subject 101).

Subjects will be identified by subject number only on all study documentation. A list identifying the subjects by subject number will be kept in the Site Master File.

4.4. Subject Withdrawal and Replacement

A subject is free to withdraw from the study at any time. In addition, a subject will be withdrawn if any of the following criteria are met:

- change in compliance with any inclusion/exclusion criterion that is clinically relevant and affects subject safety as determined by the Investigator (or designee)
- noncompliance with the study restrictions that might affect subject safety or study assessments/objectives, as considered applicable by the Investigator (or designee)
- any clinically relevant sign or symptom that, in the opinion of the Investigator (or designee), warrants subject withdrawal.

If a subject is withdrawn, the Sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the subject's electronic Case Report Form. If a subject is withdrawn, efforts will be made to perform all follow-up assessments, if possible (Appendix 5). Other procedures may be performed at the Investigator's (or designee's) and/or Sponsor's discretion. If the subject is in-house, these procedures should be performed before the subject is discharged from the clinic. The Investigator (or designee) may also request that the subject return for an additional Follow-up visit. All withdrawn subjects will be followed until resolution of all their AEs or until the unresolved AEs are judged by the Investigator (or designee) to have stabilized.

Subjects who are withdrawn for reasons not related to study drug may be replaced following discussion between the Investigator and the Sponsor. Subjects withdrawn as a result of AEs thought to be related to the study drug will generally not be replaced.

4.5. Study Termination

The study may be discontinued at the discretion of the Investigator (or designee), Sponsor, or Sponsor's Medical Monitor if any of the following criteria are met:

- AEs unknown to date (ie, not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)

- increased frequency, severity, and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at Check-in as baseline signs and symptoms)
- medical or ethical reasons affecting the continued performance of the study
- difficulties in the recruitment of subjects
- cancellation of drug development.

5. STUDY TREATMENTS

5.1. Description, Storage, Packaging, and Labeling

L606 is an oral inhalation product being developed to accomplish high drug loading efficiency and sustained release properties, enabling a drug therapeutic effect over a prolonged time period and reducing local irritation on the respiratory tract. The L606 inhalation system consists of a mesh-vibrating nebulizer that has been approved in Europe.

L606 inhalation system is an electronic, lightweight, and virtually silent drug delivery device. The vibrating mesh technology generates a fine-particle aerosol. L606 inhalation device includes a main unit, 1 medicup (or a mouthpiece cartridge [MPC]), and/or 1 mouth piece. The device should be stored at room temperature.

The IMP (1.5 mg active pharmaceutical ingredient [API; treprostinil] in 1.0 mL liposome solution and placebo [L606 solution excluding the API]) will be supplied by the Sponsor (or designee), along with the batch/lot numbers and Certificates of Analysis. The IMP will be provided in a type-1 glass ampule and stored according to the instructions on the label.

All IMPs will be stored at the CRU in a location that is locked with restricted access, under conditions according to the instructions on the label.

The dose for administration will be measured in the CRU. Prior to dosing, subjects will be trained to adopt a specific breath pattern to be used during dose administration. Administration instructions and training will be provided and included in separate documents. Subjects will self-administer the dose using the L606 oral inhalation system under the supervision of a trained professional in the CRU.

5.2. Study Treatment Administration

Each dose of L606 and placebo solution will be self-administered by the subject via oral inhalation. The entire treatment procedure will be completed within 10 minutes.

Subjects will be administered the IMP in numerical order while seated and may be semi-recumbent but will not be permitted to lie supine for 2 hours after dosing, except as necessitated by the occurrence of an AE(s) and/or study procedures.

The planned emitted dose escalation groups are:

- Cohort 1: 51 µg L606 or placebo volume equivalent to 51 µg
- Cohort 2: 102 µg L606 or placebo volume equivalent to 102 µg
- Cohort 3: 153 µg L606 or placebo volume equivalent to 153 µg
- Cohort 4: 204 µg L606 or placebo volume equivalent to 204 µg
- Cohort 5: 255 µg L606 or placebo volume equivalent to 255 µg
- Cohort 6: 306 µg L606 or placebo volume equivalent to 306 µg
- Cohort 7: 357 µg L606 or placebo volume equivalent to 357 µg
- Cohort 8: 408 µg L606 or placebo volume equivalent to 408 µg

5.3. Randomization

The randomization code will be produced by PPD biostatistics (or a qualified designee) using a computer-generated pseudo-random permutation procedure.

Prior to the start of the study, a copy of the master randomization code will be supplied in sealed envelopes to the PPD CRU pharmacy staff and the biopharmaceutical analyst at the bioanalytical laboratory.

5.4. Blinding

The following controls will be employed to maintain the double-blind status of the study:

- The placebo solution will be identical in appearance to L606.
- The Investigator and other members of staff involved with the study will remain blinded to the treatment randomization code during the assembly procedure.
- Interim bioanalytical data will be provided to PPD in a blinded manner.

To maintain the blind, the Investigator will be provided with a sealed randomization code for each subject, containing details of their treatment. These individual sealed envelopes will be kept in a limited access area that is accessible 24 hours a day. In order to manage subject safety or to support dose escalation decisions (in the event of possibly treatment-related SAEs or severe AEs), the decision to unblind resides solely with the Investigator. If it becomes necessary to break the code during the study, the date, time, and reason will be recorded in the subject's source data and on the individual envelope and will be witnessed by a second person.

5.5. Treatment Compliance

The following measures will be employed to ensure treatment compliance:

- All doses will be self-administered under the supervision of a trained professional in the CRU. Administration instructions and training will be provided and included in separate documents.
- At each dose preparation, a predose and postdose inventory will be performed on the dose containers.

5.6. Drug Accountability

The Investigator (or designee) will maintain an accurate record of the receipt of the study supplies received. In addition, an accurate drug disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensing. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

For each batch of unit doses, the empty used unit dose containers will be discarded upon satisfactory completion of the compliance and accountability procedures. Any unused assembled unit doses will be retained until completion of the study.

At the completion of the study, all unused supplies will be returned to the Sponsor or disposed of by the study site, per the Sponsor's written instructions.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Subjects will refrain from use of any prescription or nonprescription medications/products (as indicated in the exclusion criteria) during the study until the Follow-up visit, unless the Investigator (or designee) and/or Sponsor have given their prior consent.

Paracetamol/acetaminophen (2 g/day for up to 3 consecutive days); hormone replacement therapy; oral, implantable, transdermal, injectable, or intrauterine contraceptives are acceptable concomitant medications. The administration of any other concomitant medications during the study is prohibited without prior approval of the Investigator (or designee), unless its use is deemed necessary in a medical emergency. Any medication taken by a subject during the course of the study and the reason for its use will be documented in the source data.

6.2. Diet

While confined at the study site, subjects will receive a standardized diet at scheduled times that do not conflict with other study-related activities. Subjects will be fasted overnight (at least 8 hours) before collection of blood samples for safety laboratory tests.

On the days with intensive PK assessments (Day 1), meals will be identical for each group.

On Day 1, subjects will be fasted for at least 8 hours prior to dosing until approximately 0.5 to 1 hour after dosing, when a snack will be provided. With the exception of water rinses given as

needed with the dose, subjects will not be allowed fluids from 1 hour prior to until 1 hour after dosing, unless necessary per the Investigator (or designee). Meals will be provided as appropriate at other times. Other than the fluid restrictions on dosing days, water will be freely available at all times.

Foods and beverages containing poppy seeds, grapefruit, or Seville oranges will not be allowed from 7 days prior to Check-in until the Follow-up visit.

Caffeine-containing foods and beverages will not be allowed from 48 hours before Check-in until the Follow-up visit.

Carbonated drinks (including sparkling water and soda) will not be allowed from 48 hours before Check-in until Discharge from the CRU.

Consumption of alcohol will not be permitted from 72 hours prior to Check-in until Discharge from the CRU.

6.3. Smoking

Subjects will not be permitted to use tobacco- or nicotine-containing products within 6 months prior to Check-in until the Follow-up visit.

6.4. Exercise

Subjects are required to refrain from strenuous exercise from 7 days prior to Check-in until the Follow-up visit and will otherwise maintain their normal level of physical activity during this time (ie, will not begin a new exercise program or participate in any unusually strenuous physical exertion).

6.5. Blood Donation

Subjects are required to refrain from donation of blood within 6 weeks prior to Screening, plasma within 6 weeks prior to Screening, and platelets within 6 weeks prior to Screening until 56 days after the Follow-up visit.

6.6. Contraception

Female subjects who are of nonchildbearing potential will not be required to use contraception. Females of nonchildbearing potential are defined as permanently sterile (ie, due to hysterectomy or bilateral oophorectomy) confirmed by history, or postmenopausal (defined as at least 12 months postcessation of menses without an alternative medical cause). Postmenopausal status will be confirmed with a screening serum follicle-stimulating hormone level >40 mIU/mL.

Female subjects of childbearing potential must be willing to use a highly effective method of birth control (ie, contraceptive measure with a failure rate of <1% per year if used properly) in conjunction with a barrier contraception (ie, male or female condom with spermicide) from the

time of signing the ICF until 90 days after the dose of study drug. Highly effective methods of contraception include:

- intrauterine device (IUD; eg, Mirena®). Steel or copper IUDs are not acceptable
- established use of oral, implantable, transdermal, or injectable hormonal method of contraception associated with inhibition of ovulation
- ensure male partner is sterilized (performed at least 90 days prior to the Screening visit), with verbal confirmation of surgical success (for female subjects on the study, the vasectomized male partner should be the sole partner for that subject)
- bilateral tubal ligation or occlusion (performed at least 90 days prior to the Screening visit)
- bilateral salpingectomy.

Male subjects will be surgically sterile for at least 90 days or, when sexually active with female partners of childbearing potential, will be required to use a male condom with spermicide from Check-in until 90 days after the dose of study drug. Sexual intercourse with female partners who are pregnant or breastfeeding should be avoided unless condoms are used from the time of the first dose until 90 days after the dose of study drug. Male subjects are required to refrain from donation of sperm from Check-in until 90 days after the dose of study drug.

Subjects who practice true abstinence, because of the subject's lifestyle choice (ie, the subject should not become abstinent just for the purpose of study participation), are exempt from contraceptive requirements. Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception. Subjects who practice true abstinence as a lifestyle choice agree to remain abstinent for 90 days after the last dose of study drug.

For subjects who are exclusively in same-sex relationships, contraceptive requirements do not apply. However, subjects who are exclusively in same-sex relationships agree to refrain from heterosexual contact for 90 days from the last dose.

7. STUDY ASSESSMENTS AND PROCEDURES

Every effort will be made to schedule and perform the procedures as closely as possible to the nominal time, giving considerations to appropriate posture conditions, practical restrictions, and the other procedures to be performed at the same timepoint.

When multiple assessments (ie, ECGs, vital signs, and blood draws for PK or clinical laboratories) are scheduled to be performed at the same timepoint, the blood draws will be obtained at the scheduled timepoint, and ECGs and vital signs will be performed prior to the scheduled timepoint. The order of events should always be 1) resting for ECG, 2) vital signs, 3) blood collection, and 4) other assessments.

7.1. Pharmacokinetic Assessments

7.1.1. Sample Collection and Processing

Blood samples (approximately 1 × 4 mL) will be collected by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 5. Furthermore, up to 3 additional blood samples may be taken from each subject per treatment period, with the maximum volume of blood withdrawn per subject not exceeding the limit detailed in Appendix 3. Any changes to the scheduled times of PK assessments will be agreed with the Sponsor and documented in the TMF. Samples taken from subjects who received placebo will not be analyzed.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

7.1.2. Analytical Methodology

Plasma concentrations of treprostinil will be determined using a validated liquid chromatography by tandem mass spectrometric analysis method. Specifics of the analytical method will be provided in separate documents.

7.2. Safety and Tolerability Assessments

7.2.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting SAEs are detailed in Appendix 1.

Between the time of signing the ICF and drug administration, only SAEs will be recorded. All AEs (serious and nonserious) will be recorded as of the time of dose administration until final discharge from the study. Subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as “How have you been feeling since you were last asked?”, at least once each day while resident at the study site and at each study visit. Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the study.

All nonserious AEs, whether reported by the subject voluntarily or upon questioning, or noted on physical examination, will be recorded from initiation of study drug until study completion. Nonserious AE information should also be collected from the start of a washout period or other observational period intended to establish a baseline status for the subjects. Serious AEs will be recorded from the time the subject signs the ICF until study completion. The nature, time of onset, duration, and severity will be documented, together with an Investigator’s (or designee’s) opinion of the relationship to study drug.

Adverse events recorded during the course of the study will be followed up, where possible, until resolution. This will be completed at the Investigator’s (or designee’s) discretion.

7.2.2. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations (including clinical chemistry, hematology, urinalysis, serology, and coagulation assessments) at the times indicated in the Schedule of Assessments in Appendix 5. Clinical laboratory evaluations are listed in Appendix 2. Additional clinical laboratory evaluations will be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of clinical laboratory safety evaluations is required.

Subjects will be asked to provide urine samples for drugs of abuse screen and cotinine test, and will undergo an alcohol breath or urine test at the times indicated in the Schedule of Assessments in Appendix 5. For all female subjects, a pregnancy test will be performed at the times indicated in the Schedule of Assessments in Appendix 5.

An Investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

7.2.3. Vital Signs

Supine blood pressure, supine pulse rate, respiratory rate, and oral body temperature will be assessed at the times indicated in the Schedule of Assessments in Appendix 5. Vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

Day 1 predose blood pressure, pulse rate, and respiratory rate will be measured in triplicate at approximately 2-minute intervals. The median value will be used as the baseline value in the data analysis. All subsequent measurements will be performed singly. Oral body temperature will be measured singly.

Subjects must be supine for at least 5 minutes before blood pressure and pulse rate measurements.

For orthostatic vital sign measurements, the supine blood pressure and pulse rate will be measured after the subject has been supine for at least 5 minutes. The subject will then stand for at least 2 minutes and the standing blood pressure and pulse rate will be measured.

7.2.4. 12-Lead Electrocardiogram

Resting 12-lead ECGs will be recorded after the subject has been supine and at rest for at least 5 minutes at the times indicated in the Schedule of Assessments in Appendix 5. Single 12-lead ECGs will be repeated twice and the results averaged if either of the following criteria apply:

- QT interval corrected for heart rate using Fridericia's method (QTcF) value >500 msec
- QTcF change from the baseline (predose) is >60 msec.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of ECGs is required. The Investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

7.2.5. Continuous 12-Lead Holter Monitoring

Electrocardiogram parameters will be measured using a 24-hour continuous 12-lead Holter monitor beginning the night of Day -1 in order to obtain 8 to 12 hours of predose baseline data, and through 24 hours postdose.

Subjects will be connected to a Holter ECG recording device and the ECG recording will be started. Subjects will be instructed to avoid strenuous physical exercise or any other activity that could interfere with the quality of the Holter ECG recording or lead to detachment of ECG skin electrodes during the continuous Holter recording period.

During the Holter recording period, the Holter leads should be checked prior to each scheduled PK blood collection. Subjects should be instructed to inform appropriate study site personnel immediately upon discovery of a device or lead failure. Any failures of the Holter device or accessory hardware (electrodes, leads, batteries, etc.) will be recorded in the source documents.

All data will be sent for central ECG evaluation. A data file containing central ECG evaluation will be provided for inclusion in study analysis. Any Holter ECG reports provided to the site will be archived as source documentation only. Holter ECG start and end times should be recorded on the CRF.

Details of the Holter ECG recording will be provided in the ECG manual provided by the central reader.

7.2.6. Spirometry

Spirometry (forced expiratory volume in 1 second and forced vital capacity) will be measured in a sitting position, if possible, at the times indicated in the Schedule of Assessments in Appendix 5.

A spirometer that meets the 2005 American Thoracic Society/European Respiratory Society recommendations will be used. Spirometry should be performed in accordance with the American Thoracic Society/European Respiratory Society guidelines and per details provided separately.[11]

Pulmonary function tests (FEV1 and FVC) will be measured in a sitting position if possible. Subjects who have a history of asthma or show findings consistent with asthma on spirometry testing will be excluded per exclusion criterion number 5. Pre- and post-bronchodilator spirometry testing will be completed at Screening using albuterol; all other timepoints will not require post-bronchodilator testing.

7.2.7. Physical Examination

A full physical examination or symptom-directed physical examination will be performed at the timepoints specified in the Schedule of Assessments in Appendix 5.

7.2.8. Body Weight and Height

Body weight (in underclothes) and height will be recorded at the times indicated in the Schedule of Assessments in Appendix 5. Body mass index will be calculated.

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time L606 is being administered to humans. However, the number of subjects in the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study.

8.2. Analysis Populations

8.2.1. Pharmacokinetic Population

The PK population will include all subjects who received 1 dose of L606 and have evaluable PK data.

8.2.2. Safety Population

The safety population will include all subjects who received 1 dose of study treatment (L606 or placebo) and have at least 1 postdose safety assessment.

8.3. Pharmacokinetic Analyses

Noncompartmental PK analysis will be performed on individual plasma concentration data, using commercial software such as Phoenix® WinNonlin®. Plasma concentrations of treprostinil and PK parameters will be listed and summarized using descriptive statistics (arithmetic mean, standard deviation, minimum, median, and maximum, geometric mean, and geometric coefficient of variation, as appropriate). Individual and mean treprostinil concentration-time profiles will also be presented graphically.

The AUCs and C_{max} will be the primary endpoints for the evaluation of dose proportionality. Dose proportionality of treprostinil will be evaluated by assessing the slope from regression analysis of log(AUC or C_{max}) versus log(dose) from the power model for those subjects who receive L606.

Further details of the statistical analysis of the data will be addressed in the Statistical Analysis Plan.

8.4. Safety Analysis

All safety data will be listed. Descriptive statistics and changes from baseline will be calculated for safety parameters as appropriate. These summaries will be produced for each dose level of

L606 and the pooled placebo subjects. No formal statistical analyses are planned for the safety endpoints. Each AE will be coded using the Medical Dictionary for Regulatory Activities.

8.5. Interim Analysis

Between each dose escalation the safety data review committee, consisting of the Principal Investigator, Medical Monitor, and Sponsor's Representative, will review all available blinded data to ensure it is safe to proceed with the planned dose escalation. An interim safety report, summarizing results from all available safety assessments, will be sent to the Sponsor prior to the start of each successive treatment period. Any clinically significant results will be discussed with the Sponsor before dose escalation continues. Interim PK data will also be reviewed in terms of dose escalation and to confirm that the study design remains appropriate.

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10. APPENDICES

Appendix 1: Adverse Event Reporting

Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

Assessment of Severity

The Investigator will be asked to provide an assessment of the severity of the AE using the following categories:

- **Mild**: Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate**: Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- **Severe**: Interrupts usual activities of daily living, or significant affects clinical status, or may require intensive therapeutic intervention.

Relationship to Study Treatment

The Investigator will make a determination of the relationship of the AE to the study drug using a 4-category system according to the following guidelines:

- **Not Related**: The AE is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Unlikely Related**: The temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly Related**: The AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the subject's clinical state or the study procedures/conditions.
- **Related**: The AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesized cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

Action Taken for Adverse Events

The Investigator or designee will record the action taken for the AE in the electronic Case Report Form (eCRF). Actions taken will include:

- **Dose increased**: The medication schedule was modified by addition; either by changing the frequency, strength, or amount.
- **Dose not changed**: The medication schedule was not changed.
- **Dose reduced**: The medication schedule was modified by subtraction; either by changing the frequency, strength, or amount.
- **Drug interrupted**: The medication schedule was modified by temporarily terminating the prescribed regimen of medication.
- **Drug withdrawn**: The medication schedule was modified through termination of the prescribed regimen of medication.
- **Not applicable**
- **Unknown**

Follow-up of Adverse Events

Every reasonable effort will be made to follow up with subjects who have AEs. Any subject who has an ongoing AE that is possibly related or related to the investigational medicinal product (IMP) or study procedures at the Follow-up visit will be followed up, where possible, until resolution. This will be completed at the Investigator's (or designee's) discretion. Any subject who has an ongoing AE that is not related or unlikely related to the IMP or study procedures at the Follow-up visit can be closed out as ongoing at the Investigator's discretion.

Adverse Drug Reactions

All noxious and unintended responses to an IMP (ie, where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg, Investigator's Brochure for an unapproved IMP).

Serious Adverse Events

A serious AE (SAE) is defined as any untoward medical occurrence that at any dose either:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- results in a congenital anomaly/birth defect
- results in an important medical event (see below).

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Instances of death or congenital abnormality, if brought to the attention of the Investigator at any time after cessation of the study treatment and considered by the Investigator to be possibly related to the study treatment, will be reported to the Sponsor.

Definition of Life-threatening

An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred (ie, does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

Definition of Hospitalization

Adverse events requiring hospitalization should be considered serious. In general, hospitalization signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the Clinical Research Unit. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered as serious.

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AEs and should be recorded on a Clinical Assessment Form and added to the eCRF. If anything untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'nonserious' attributed according to the usual criteria.

Serious Adverse Event Reporting

Food and Drug Administration (FDA)-reportable AEs are AEs that are associated with the use of the drug and are serious and unexpected. Food and Drug Administration-reportable AEs will be reported by the study site to the Sponsor, Medical Monitor assigned by the Sponsor, and the responsible Institutional Review Board (IRB).

The Sponsor and Medical Monitor will be notified in writing (eg, facsimile) within 24 hours of when an AE that is potentially FDA-reportable is first recognized or reported.

Subsequently, a written confirmation or summary of the AE (using FDA Form 3500A or equivalent) will be sent to the Sponsor within 3 working days of the original notification.

The IRB will be notified of any FDA-reportable AEs within the timeframe required by the IRB. The IRB Serious and Unexpected Adverse Experience Submission Form will be completed and submitted with the copy of the written confirmation or summary of the AE.

The responsibility for reporting SAEs will be transferred to the Sponsor 30 days after the end of the study.

Appendix 2: Clinical Laboratory Evaluations

Clinical chemistry:	Hematology:	Urinalysis:
Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Cholesterol Creatinine Gamma-glutamyl transferase Glucose Potassium Sodium Total bilirubin[a] Total protein Uric acid	Hematocrit Hemoglobin Mean cell hemoglobin Mean cell hemoglobin concentration Mean cell volume Platelet count Red blood cell (RBC) count RBC distribution width White blood cell (WBC) count WBC differential: Basophils Eosinophils Lymphocytes Monocytes Neutrophils Prothrombin time[b] Activated partial thromboplastin time[b] International normalized ratio[b]	Bilirubin Blood Color and appearance Glucose Ketones Leukocyte esterase Nitrite pH Protein Specific gravity Urobilinogen Microscopic examination (if protein, leukocyte esterase, nitrite, or blood is positive)
Serology[b]:	**Drug screen[c]:**	**Hormone panel - females only:**
Anti-hepatitis B surface antibody Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency virus (HIV-1 and HIV-2) antibodies	Including but not limited to: Amphetamines/methamphetamines Barbiturates Benzodiazepines Cocaine (metabolite) Methadone Phencyclidine Opiates Tetrahydrocannabinol/ Cannabinoids Cotinine Alcohol breath or urine test	Follicle-stimulating hormone[b] (postmenopausal females only) Serum pregnancy test (human chorionic gonadotropin)[d]

[a] Direct bilirubin will be analyzed if total bilirubin is elevated.
[b] Only analyzed at Screening.
[c] Only analyzed at Screening and Check-in, with the exception of alcohol testing, which is only analyzed at Check-in.
[d] Performed at both Screening and Check-in for all females.

Appendix 3: Total Blood Volume

The maximum number of samples will include the optional 3 additional blood samples for pharmacokinetics.

The following blood volumes will be withdrawn for each subject.

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Safety laboratory tests	9-12	5	48
Serology	3.5	1	3.5
Treprostinil pharmacokinetics	4	17	68
Total:			119.5

Appendix 4: Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations.

The protocol, protocol amendments, Informed Consent Form (ICF), Investigator's Brochure, and other relevant documents (eg, advertisements) must be submitted to an Institutional Review Board (IRB) by the Investigator and reviewed and approved by the IRB before the study is initiated.

Any amendments to the protocol will require IRB and regulatory authority (as locally required) approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
- Notifying the IRB of serious adverse events or other significant safety findings as required by IRB procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each subject will be provided with a study-specific ICF giving details of the study drugs, procedures, and potential risks of the study. Subjects will be instructed that they are free to obtain further information from the Investigator (or designee) and that their participation is voluntary and they are free to withdraw from the study at any time.

Subjects will be given an opportunity to ask questions about the study prior to providing consent for participation.

Following discussion of the study with Clinical Research Unit personnel, subjects will sign 2 copies of the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. One copy will be given to the subject, and the other will be maintained in the subject's records.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

Subject Data Protection

Subjects will be assigned a unique identifier and will not be identified by name in electronic Case Report Forms (eCRFs), study-related forms, study reports, or any related publications. Subject and Investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual subjects or Investigators will be redacted according to applicable laws and regulations.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must also be informed that his/her study-related data may be examined by Sponsor or Contract Research Organization (CRO) auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the Sponsor.

Data Quality Assurance

The following data quality steps will be implemented:

- All subject data relating to the study will be recorded on eCRFs unless directly transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.
- PPD is responsible for the data management of this study including quality checking of the data. Predefined, agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a Data Management Plan.
- A Study Monitor will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator in accordance with 21 CFR 312.62(c) unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Investigator Documentation Responsibilities

All individual, subject-specific study data will also be entered into a 21 CFR Part A1-compliant electronic data capture (EDC) system on an eCRF in a timely fashion.

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to PPD electronically, will be integrated with the subject's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each subject who signs an ICF and undergoes any screening procedures, according to the eCRF completion instructions. The Sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, data queries, and site notifications.

Publications

If on completion of the study the data warrant publication, the Investigator may publish the results in recognized (referred) scientific journals subject to the provisions of the clinical study agreement (CSA). Unless otherwise specified in the Master Services Agreement, the following process shall occur:

If the Investigator expects to participate in the publication of data generated from this site, the institution and Investigator shall submit reports, abstracts, manuscripts, and/or other presentation materials to the Sponsor for review before submission for publication or presentation. The Sponsor shall have 60 days to respond with any requested revisions, including without limitation, the deletion of confidential information. The Investigator shall act in good faith upon requested revisions, except the Investigator shall delete any confidential information from such proposed publications. The Investigator shall delay submission of such publication or presentation materials for up to an additional 90 days in order to have a patent application(s) filed.

Appendix 5: Schedule of Assessments

Table 3: Schedule of Assessments

	Screening (Day -28 to Day -2)		Treatment Period			Follow-up Visit
		Check-in (Day -1)	Day 1	Day 2	Discharge (Day 3)	Day 8 to Day 11 (7 to 10 days postdose)
Informed consent	X					
Demographic data	X					
Inclusion/exclusion criteria	X	X				
Medical history	X	X[a]				X
Serology	X					
Urine drugs of abuse screen (including cotinine)	X	X				
Alcohol breath or urine test	X	X				
Pregnancy test	X	X				X
Study residency						
Check-in		X				
Discharge					X	
Nonresidential visit	X					X
Dosing with L606 or placebo			X			
Inhalation administration training[b]		X				
Safety and tolerability						
Adverse events[c]	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X
Physical examination[d]	X	X			X	X
Body weight, height, and BMI[e]	X	X				
Safety 12-lead ECG[f]	X	X	X	X	X	X
12-Lead Holter monitor recording[g]		X	X	X		
Vital signs[h]	X	X	X	X	X	X
Oxygen saturation[i]		X		X	X	X
Spirometry[j]	X	X		X	X	X
Clinical laboratory evaluations[k]	X	X		X	X	X
PT, aPTT, INR	X					
Methemoglobin[l]	X			X		
Pharmacokinetics						
Blood sampling for PK analysis of treprostinil [m]			X	X		

Abbreviations: AE = adverse event; aPTT = activated partial thromboplastin time; BMI = body mass index; ECG = electrocardiogram; ICF = Informed Consent Form; INR = international normalized ratio; PK = pharmacokinetic; PT = prothrombin time; SAE = serious adverse event.

[a] Updated medical history (from Screening).

[b] All doses will be self-administered under the supervision of a trained professional in the CRU. Administration instructions and training will be provided and included in separate documents.

[c] Between the time of signing the ICF and drug administration, only SAEs will be recorded. All AEs (serious and nonserious) will be recorded as of the time of dose administration until final discharge from the study.

[d] Full physical examination at Screening and symptom-directed physical examinations at Check-in, Discharge, and Follow-up.

[e] Weight is measured at Screening and Check-in. Height and BMI is measured only at Screening.

[f] Twelve-lead safety ECGs will be collected at Screening; Check-in; Day 1 predose, 4, and 24 hours postdose; Discharge; and Follow-up. Subjects will be required to rest in a supine position for at least 5 minutes prior to the ECG extraction timepoints. Assessment times postdose are relative to the end of the inhalation treatment.

[g] Electrocardiogram parameters will be measured using a 24-hour continuous 12-lead Holter monitor beginning the night of Day -1 in order to obtain 8 to 12 hours of predose baseline data, and through 24 hours postdose.

[h] Supine blood pressure, supine pulse rate, and respiratory rate will be taken at Screening; Check-in; Day 1 predose and at 0.25, 30, 1, 1.5, 2, 4, 8, 12, and 24 hours postdose; Discharge; and Follow-up. Oral body temperature will be taken at Screening; Check-in; Day 1 predose; Discharge; and Follow-up. Orthostatic vital signs will be assessed at Check-in; Day 1 predose; and at 1, 4, and 8 hours postdose. Assessment times postdose are relative to the end of the inhalation treatment.

[i] Oxygen saturation will be measured at rest by standard pulse oximetry Check-in, Day 2, and Discharge.

[j] Spirometry (forced expiratory volume in 1 second and forced vital capacity) will be measured in a sitting position, if possible, at Screening, Check-in, Day 2, Discharge, and Follow-up. Pre- and post-bronchodilator spirometry testing will be completed at Screening using albuterol; all other timepoints will not require post-bronchodilator testing.

[k] Clinical laboratory evaluations including clinical chemistry, hematology, and urinalysis.

[l] Methemoglobin will be measured noninvasively using a finger probe.

[m] Blood sampling for PK analysis of treprostinil at Day 1 predose and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 14, 16, and 24 hours postdose. Sampling times postdose are relative to the end of the inhalation treatment.