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Dry powder treprostinil for the treatment of pulmonary hypertension

Abstract

A dry powder inhalation treatment for pulmonary arterial hypertension includes a dose of dry particles comprising greater than 25 micrograms of treprostinil enclosed in a capsule. The dry particles can include treprostinil, a wetting agent, a hydrophobicity modifying agent, a pH modifying agent and a buffer. A method of treating a patient having pulmonary arterial hypertension includes providing a patient a dry powder inhaler, providing the patient at least one capsule for use in the dry powder inhaler, the capsule including at least 25 micrograms of treprostinil.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application is a continuation of U.S. Patent Application Ser. No. 17/390,514, filed on Jul. 30, 2021, which is a continuation of U.S. Patent Application Ser. No. 17/104,348, filed Nov. 25, 2020, which is a continuation of U.S. patent application Ser. No. 16/099,135 (now U.S. Pat. No. 10,898,494), filed May 5, 2017, which is a U.S. national stage of International Application No. PCT/US2017/031301, filed May 5, 2017, which claims priority to and the benefit of U.S. Provisional Patent Application No. 62/332,013, filed May 5, 2016, U.S. Provisional Patent Application No. 62/404,960, filed Oct. 6, 2016, U.S. Provisional Patent Application No. 62/440,078, filed Dec. 29, 2016, and U.S. Provisional Patent Application No. 62/472,204, filed Mar. 16, 2017, all of which are incorporated herein by reference in their entireties.

TECHNICAL FIELD

(1) The present invention provides an improvement to the treatment of pulmonary hypertension, a condition that deteriorates the lives of many thousands of patients toward an untimely death. The present invention provides, for the first time, a stable, user friendly, uniform dry powder inhaled treprostinil formulation, methods of making, and use thereof in humans.

BACKGROUND

(2) Pulmonary arterial hypertension (PAH) is a complex, multifactorial, progressive, and life-threatening disease characterized by proliferative and obstructive changes in the pulmonary vasculature and involving numerous biochemical pathways and cell types. The disease is characterized by elevated pulmonary arterial pressure caused by narrowing of the blood vessels in the lungs and, ultimately, right ventricular failure. The disease carries a poor prognosis associated with significant morbidity and mortality, having a historical survival rate less than five years. PAH is a sub-group of pulmonary hypertension (PH), which is elevation of blood pressure in lungs. Endothelial dysfunction is thought to occur early on, leading to cell proliferation and structural changes in the pulmonary vasculature that lead to increased pulmonary arterial pressure (PAP) and resultant right ventricular enlargement and dysfunction. In addition, endothelial dysfunction results in chronically impaired production of vasoactive mediators, such as nitric oxide (NO) and prostacyclin, along with prolonged overexpression of vasoconstrictors, such as endothelin-1.

(3) PAH affects approximately 15 out of every one million individuals. There are approximately 1,000 new cases of PAH diagnosed in the United States each year. The mean age at diagnosis is between 50 and 65 years of age, although the disorder may present much earlier in childhood or even infancy. While gender-based prevalence estimates for PAH are variable, estimates for the overall prevalence of pulmonary hypertension (PH) in females is approximately twice that of males.

(4) PAH is part of a larger classification for pulmonary hypertension which is divided into five groups based on World Health Organization (WHO) criteria (designated as WHO Groups 1 through 5). PAH is used to describe exclusively WHO Group 1. Pulmonary hypertension is used to describe the remaining four groups (WHO Groups 2-5) and also when referring to all 5 groups collectively. WHO Group 1—PAH: Pulmonary arterial hypertension. WHO Group 2—PH: Pulmonary hypertension secondary to left heart disease. WHO Group 3—PH: Pulmonary hypertension secondary to lung diseases or hypoxemia. WHO Group 4—PH: Chronic thromboembolic pulmonary hypertension. WHO Group 5—PH: Pulmonary hypertension with unknown mechanisms.

(5) PAH initially presents as exertional dyspnea, lethargy, and fatigue and is often confused for other disease states. As PAH progresses and right ventricular failure develops, exertional chest pain (i.e., angina), exertional syncope, and peripheral edema may develop. Following confirmation of diagnosis based on hemodynamic parameters, treatment is recommended to lower pulmonary pressures and treat the symptoms of PAH. Although no cure exists for PAH, treatment of PAH is directed at improving hemodynamic measures, New York Heart Association (NYHA) functional class, the 6 minute walk distance (6MWD), quality of life, and, in some studies, survival.

(6) The severity of PAH may be classified according to the NYHA heart failure guidelines as follows: NYHA Class I: Patients with no limitation of activities; they suffer no symptoms from ordinary activities. NYHA Class II: Patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion. NYHA Class III: Patients with marked limitation of activity; they are only comfortable at rest. NYHA Class IV: Patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

(7) While the exact underlying cause of PAH is unclear, mutations in the bone morphogenic protein receptor type II (BMPR2) gene account for approximately 75% of familial PAH and up to 25% of apparently sporadic PAH cases. These mutations may promote cell division or prevent cell death, resulting in an overgrowth of cells in smaller pulmonary arteries. This overgrowth increases resistance to blood flow, triggering hypertension. Additional genetic abnormalities may also contribute to PAH.

(8) Currently Available Treatments

(9) There are five classes of drugs that have been approved to treat PAH, including endothelin receptor antagonists (ERAs), phosphodiesterase type 5 (PDES) inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonists, and prostacyclin analogs. Approved PAH therapies and their route of administration include: ERA: bosentan (oral) and ambrisentan (oral) PDES: sildenafil (oral, intravenous (IV) and tadalafil (oral) Soluble Guanylate Cyclase (sGC) Stimulator: riociguat (oral) Prostacyclin Receptor Agonist: selexipag (oral) Prostacyclin Analog: epoprostenol (IV) iloprost

(inhaled), and treprostinil (oral), (subcutaneous and IV), and (inhaled)

(10) Treprostinil is a chemically stable tricyclic benzidine prostanoid with vasodilator properties that is capable of reducing pulmonary vasoconstriction with minimal effects on systemic blood pressure. Treprostinil has been approved for the treatment of PAH under the trade names REMODULIN® (United Therapeutics Corporation; subcutaneous or IV infusion) and TYVASO® (United Therapeutics Corporation; inhaled via ultrasonic, pulsed nebulization delivery device). While both have proven effective for PAH, one advantage of TYVASO's inhaled route of administration is that it brings the drug very near the desired site of action (pulmonary arteries in the lungs).

(11) Despite the current treatment options for PAH patients, each option includes drawbacks, most notably for the inhaled route of administration Tyvaso requires use of a large, cumbersome nebulization device that requires power, water and user manipulation for cleaning and operating. Moreover, the nebulization device by its nature is not convenient to the patient as compared to carrying a small, concealable dry powder inhalation device such as those used for treating asthma and many other chronic and acute issues. Furthermore, nebulized treprostinil has shown clinical limitations on treprostinil dosing, which may limit the applicability of the inhaled route of administration to a smaller subsector of PAH patients than necessarily treatable via the inhaled route from a dry powder inhaled treprostinil product of the present invention.

SUMMARY OF THE INVENTION

(12) The present inventors have developed and reduced to practice an inhalation dry powder formulation of treprostinil that is produced using Liquidia's PRINT® Technology (Particle Replication in Nonwetting Templates), LIQUIDIA TECHNOLOGIES, INC. This PRINT particle formulation for dry powder delivery of treprostinil (otherwise referred to as LIQ861) is under clinical evaluation. The present applicants intend to use the same indication (i.e., treatment of pulmonary arterial hypertension [WHO Group 1] in patients with NYHA Class III symptoms, to improve exercise ability) dose and dose regimen (4×/day) as defined in the approved nebulized treatment label (TYVASO® UNITED THERAPEUTICS). In particular, the present invention provides for dosing levels that exceed the maximum tolerated dose delivered through a nebulizer. In some cases the present invention may also treat other indications under the pulmonary hypertension disease states.

(13) In some embodiments, a dry powder inhalation treatment for pulmonary arterial hypertension according to the present invention includes a dose of dry particles comprising greater than 25 micrograms of treprostinil enclosed in a capsule. In some embodiments, the dose of dry particles comprises from about 25 micrograms to about 400 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises from about 50 micrograms to about 350 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises from about 75 micrograms to about 300 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises from about 100 micrograms to about 300 micrograms of treprostinil. In some embodiments, the dose of dry particles includes greater than or equal to 100 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises greater than or equal to 150 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises greater than or equal to 200 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises greater than or equal to 250 micrograms of treprostinil. In some embodiments, the dose of dry particles comprises greater than or equal to 300 micrograms of treprostinil. In some embodiments, the dose of dry particles includes greater than or equal to 5 mg of the dry particles. In some embodiments, the dose of dry particles includes greater than or equal to 10 mg of the dry particles. In yet other embodiments, the dose of dry particles includes greater than or equal to 15 mg of the dry particles. In further embodiments, a dry powder treatment for pulmonary arterial hypertension, includes a single capsule enclosing 5 mg or more dry particles comprising 25 micrograms of treprostinil per each 5 mg of the dry particles.

(14) In some embodiments, a method of treating a patient having pulmonary arterial hypertension includes providing a patient a dry powder inhaler, providing the patient at least one capsule for use in the dry powder inhaler, wherein the capsule comprises at least 25 micrograms of treprostinil, and instructing the patient to utilize the dry powder inhaler to inhale the treprostinil. In some such embodiments, the capsule includes at least 50 micrograms of treprostinil. In some embodiments, the capsule includes at least 100 micrograms of treprostinil. In some embodiments, the capsule comprises at least 150 micrograms of treprostinil. In some embodiments, the capsule comprises greater than or equal to 200 micrograms of treprostinil. In some embodiments, the capsule comprises greater than or equal to 250 micrograms of treprostinil. In some embodiments, the capsule comprises greater than or equal to 300 micrograms of treprostinil. In some embodiments, the capsule comprises from about 25 micrograms to about 400 micrograms of treprostinil. In some embodiments, the capsule comprises from about 50 micrograms to about 350 micrograms of treprostinil. In some embodiments, the capsule comprises from about 75 micrograms to about 300 micrograms of treprostinil. In some embodiments, the capsule comprises from about 100 micrograms to about 300 micrograms of treprostinil. In further embodiments, the patient may be prescribed to use two capsules per dose cycle per day, generally with PAH requiring 4 times per day dosing. In some embodiments, the patient may be prescribed to use three capsules per day. In some embodiments, the patient may be prescribed to use four capsules per day. In some embodiments, a method of treating a patient having pulmonary arterial hypertension includes dosing the patient having pulmonary arterial hypertension with a dry powder dose of treprostinil, wherein the dose of treprostinil is greater than 85 micrograms (e.g., about 100 micrograms to about 350 micrograms). In some embodiments, the patient may be dosed one, two, three, four, or more times per day. A further method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than 12.5 micrograms of treprostinil to a patient per breath. In another embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than 25 micrograms of treprostinil to a patient per breath. In another embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, from about 12.5 to about 50 micrograms of treprostinil to a patient per breath. In yet another embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, about 25 to about 50 micrograms of treprostinil to a patient per breath. In a further embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than 50 micrograms of treprostinil to a patient per breath. In a further embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than or equal to 100 micrograms of treprostinil to a patient per breath. In a further embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than or equal to 150 micrograms of treprostinil to a patient per breath. In a further embodiment, a method of treating a patient having pulmonary arterial hypertension includes delivering, in dry powder, greater than or equal to 200 micrograms of treprostinil to a patient per breath.

(15) A dry powder inhalation composition for treating pulmonary arterial hypertension according to a further embodiment includes a plurality of dry powder particles comprising treprostinil, a non-reducing sugar, a wetting agent, a hydrophobicity modifying agent, a pH modifying agent and a buffer. In some such embodiments, the bulking agent comprises trehalose dihydrate. In some embodiments, the wetting agent comprises polysorbate 80. In some embodiments, the hydrophobicity modifying agent comprises L-leucine. In some embodiments, the pH modifying agent comprises sodium citrate dihydrate. In some embodiments, the buffer comprises sodium chloride. In certain embodiments, the composition comprises less than about 4 percent by weight water. In some embodiments, the composition comprises less than about 2 percent by weight water. In some embodiments, the composition comprises less than about 1 percent by weight water.

(16) In yet further embodiments, the dry powder particles include particles having a three dimensional shape including a width and length not less than 1 micrometer and not more than 2 micrometers and a depth not less than 0.3 micrometers and not more than 0.8 micrometers. In some embodiments, the dry powder particles comprise a dried solution comprising trehalose dihydrate, L-leucine, treprostinil sodium, polysorbate 80, sodium citrate dihydrate, sodium chloride and water. In some embodiments, the dry powder particles comprise by percent solids about 0.581 percent treprostinil sodium, about 92.32 percent trehelose, about 2.19 percent polysorbate 80, about 4.39 percent L-leucine, about 0.26 percent sodium citrate, and about 0.25 percent sodium chloride.

(17) A method of making a particle for dry powder delivery to the lung of a patient in need thereof, in some embodiments, includes molding a composition comprising about 12.30 weight percent trehelose dihydrate, about 0.53 weight percent L-leucine, about 0.07 weight percent treprostinil sodium, about 0.26 weight percent polysorbate 80, about 0.04 weight percent sodium citrate dihydrate, about 0.03 weight percent sodium chloride

and about 86.78 weight percent water into a particle. In some embodiments, the method of making the particle further includes drying the composition such that the particle comprises less than 4 percent by weight water.

Description

BRIEF DESCRIPTION OF THE FIGURES

(1) The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention can be embodied in different forms and thus should not be construed as being limited to the illustrated embodiments set forth herein.

(2) FIG. **1** shows a three-dimension rendering of a pollen particle according to an embodiment of the present invention.

(3) FIG. **2** shows an example NGI distribution for active particles (PAH-1R-0943-010). For each of the three data sets represented for each collection cup, the beginning of the run is the left hand bar (A**1**), the middle of the run is the center bar (B**1**), and the end of the run is the right hand bar (C**1**). Data was obtained using the Monodose Model 8 device (95 L/min, 2 sec).

(4) FIGS. **3**A and **3**B are tables including data for Cohort 1 of a clinical trial. The table shown in FIG. **3**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 1. Preliminary non-compartmental PK parameters for treprostinil are summarized in the table shown in FIG. **3**B.

(5) FIGS. **4**A and **4**B are tables including data for Cohort 2 of a clinical trial. The table shown in FIG. **4**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 2. Preliminary non-compartmental PK parameters for treprostinil for Cohort 2 are summarized in the table shown in FIG. **4**B.

(6) FIGS. **5**A and **5**B are tables including data for Cohort 3 of a clinical trial. The table shown in FIG. **5**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 3. Preliminary non-compartmental PK parameters for treprostinil for Cohort 3 are summarized in the table shown in FIG. **5**B.

(7) FIGS. **6**A and **6**B are tables including data for Cohort 4 of a clinical trial. The table shown in FIG. **6**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 4. Preliminary non-compartmental PK parameters for treprostinil for Cohort 4 are summarized in FIG. **6**B.

(8) FIGS. **7**A and **7**B are tables including data for Cohort 5 for a clinical trial. The table shown in FIG. **7**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 5. Preliminary non-compartmental PK parameters for treprostinil for Cohort 5 are summarized in FIG. **7**B.

(9) FIGS. **8**A, **8**B, and **8**C are tables including data for Cohort 6 for a clinical trial. The table shown in FIG. **8**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 6-R. Preliminary non-compartmental PK parameters for treprostinil for Cohort 6-R are summarized in FIG. **8**B. Preliminary non-compartmental PK parameters for treprostinil for Cohort 6-Original are summarized in FIG. **8**C.

(10) FIGS. **8**D, **8**E, **8**F, and **8**G contain data for the clinical trial. Mean concentration-time data for each of the six cohorts is displayed on a linear scale in FIG. **8**D. Plots of the relationship between dose and Cmax and AUCinf are displayed in FIG. **8**E and FIG. **8**F, respectively. A plot of the relationship between dose and the oral clearance, CL/F, is shown in FIG. **8**G.

(11) FIG. **9** is an SEM image showing pollen-shaped particles according to an embodiment of the present invention.

(12) FIG. **10** is a flow diagram showing a process of manufacturing particles according to an embodiment of the present invention.

(13) FIG. **11** shows an example dry powder inhalation device which may be used to deliver particles to a patient in accordance with embodiments of the present invention.

DETAILED DESCRIPTION OF EMBODIMENTS

(14) Drug Substance

(15) The drug substance (DS) according to embodiments of the present invention is treprostinil, which is a synthetic analog of prostacyclin (PGI.sub.2). The IUPAC name for treprostinil is (2-[[(1R,2R,3aS,9aS)-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[g]naphthalen-5-yl]oxy]acetic acid).

(16) Inhalation Powder Drug Product

(17) The inhalation powder drug product according to certain aspects of the present invention provides a dry powder dosage form of treprostinil and excipients formed into a particle (drug product intermediate, or DP-intermediate) that is, in some embodiments, filled into a capsule, for example, a hydroxypropyl methylcellulose (HPMC) capsule (size 3) (LIQ861). In some embodiments, the DP-intermediate is a treprostinil/excipient matrix from which particles of precise size and shape are formed according to the methods herein. In one example, the particles of the DP-intermediate comprise a shape corresponding generally to a rounded triangular shape having a volume, where the inner portion of the rounded triangular shape, in size, fits a 1 micrometer equilateral triangle (otherwise referred to as being pollen-shaped). A three-dimensional rendering of such a particle shape is depicted in FIG. **1**. In another embodiment, the pollen-shape may be trefoil-shaped with an inscribed circle diameter of 1 micrometer, and a prescribed thickness of a value or range between 0.5 and 1 micrometer, or more preferred 0.7 micrometer. In addition, certain embodiments of the present drug product includes particles having 0.5% treprostinil used in a first clinical study to investigate dose levels of 25 mcg, 50 mcg, 75 mcg, 100 mcg, 125 mcg and 150 mcg treprostinil in LIQ861. In further embodiments, a drug product according to the present invention may provide dose levels of 175 mcg, 200 mcg, 225 mcg, 250 mcg, 275 mcg, 300 mcg, 325 mcg, or 350 mcg treprostinil. In further embodiments, a drug product according to the present invention may provide dose levels of 50 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil loaded into capsules for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide dose levels of 75 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil loaded into capsules for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide dose levels of 100 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil loaded into capsules for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide dose levels of 150 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% treprostinil loaded into capsules for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide dose levels of 200 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% mcg treprostinil loaded into capsules for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide dose levels of 300 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% treprostinil loaded into capsules for delivery to a patient in a dry powder.

(18) According to the present invention, due to the formulation of the present dry powder particles, the particles remain stable for long periods of time at relatively low humidity conditions. In some embodiments, the present invention provides dry powder particles packaged under sealed conditions that remain stable for more than 3 months at 40 degrees Celsius at 75 percent relative humidity. Therefore, the particles can be utilized to provide a patient with a dry powder inhaled drug form of treprostinil, not previously available until the present invention. This invention, in some embodiments, provides a user with a reduction in interaction with drug product by removing the requirements on the patient to reconstitute their drug product for use in a nebulizer device. The patient is also enabled to receive equal dosing with more than 50 percent reduction in breath treatments on a device, and in some embodiments more than 65 percent reduction in breath treatments.

(19) The present invention, in some embodiments, also provides a dry formulation of treprostinil, which upon delivery to a patient via the inhaled route, becomes soluble and pharmaceutically available in less than 10 seconds. In some embodiments, the dry formulation composition becomes soluble and pharmaceutically available in less than 5 seconds. In some embodiments, the dry formulation composition becomes soluble and pharmaceutically available in less than 2 seconds. In some embodiments, the dry formulation composition becomes soluble and pharmaceutically available in about 1 second. In some embodiments, the dry formulation composition becomes soluble and pharmaceutically available in less than 1 second. In some embodiments, the dry formulation composition becomes soluble and pharmaceutically available in less than about 0.5 seconds. Furthermore, the excipients in the dry particle formulation of the present invention maintain pH and salt gradient during processing such that the active agent remains in a state to become soluble in the lung conditions of a user.
(20) A detailed description of the LIQ861 formulation, particle composition, particle geometry, packaging, device, delivery, stability, dose, and a description of the use follows.
(21) In some embodiments, a formulation according to the present invention includes a drug substance (e.g., Treprostinil, Treprostinil Sodium) together with one or more excipients. In some embodiments, the one or more excipients may include a bulking agent, a wetting agent, a hydrophobicity modifier, a pH modifier, a buffer component, or combinations thereof. Examples of such formulations according to certain specific embodiments are provided in the tables below.
(22) TABLE-US-00001 LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (dihydrate form calculations) Quantity (mg/g) Percent Component Function (Active) Solids Treprostinil Drug Substance 5.3 0.53 Sodium (5.0 as treprostinil) Trehalose Dihydrate Bulking Agent 930 92.97 Polysorbate 80 Wetting Agent 20 2.00 L-Leucine Hydrophobicity 40 4.00 Modifier Sodium Citrate pH Modifier 2.7 0.27 Dihydrate Sodium Chloride Buffer Component 2.3 0.23
(23) TABLE-US-00002 LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (anhydrous form calculations) Quantity (mg/g) Percent Normalized Component Function (Active) Solids mg/g Treprostinil Drug 5.3 0.581 5.81 Sodium Substance (5.0 as treprostinil) Trehalose Bulking Agent 841 92.32 923.23 Polysorbate 80 Wetting Agent 20 2.19 21.94 L-Leucine Hydrophobicity 40 4.39 43.89 Modifier Sodium Citrate pH Modifier 2.4 0.26 2.60 Sodium Chloride Buffer 2.3 0.25 2.52 Component
Inhalation Device
(24) According to an embodiment of administering the present invention drug particle, LIQ861 is administered using an RS00 Model 8 dry powder inhalation device (Plastiape S.p.A.). The present invention provides for multi-day administration of LIQ861 according to some embodiments.
(25) Indication
(26) The present invention, according to an embodiment, is useful for the treatment of pulmonary arterial hypertension (WHO Group 1) in patients with NYHA Class III symptoms, to improve exercise ability.
(27) Chemistry, Manufacturing, and Controls (CMC)
(28) Drug Substance (DS)
(29) The drug substance according to embodiments of the present invention is treprostinil and the salt form used for LIQ861 is treprostinil sodium. Detailed information about treprostinil sodium, including physical and chemical properties, characterization, manufacturing and controls, container closure system, and stability attributes may be found in the Drug Master File (DMF) lodged with the FDA for treprostinil. General information on the DS is provided herein.
(30) Nomenclature
(31) The international non-proprietary name (INN) for LIQ861 is treprostinil sodium. The chemical name is 241R,2R,3aS,9aS)-2-hydroxy-1-((S)-3-hydroxyoctyl)-2,3,3a,4,9,9a-hexahydro-1H-cyclopenta[b]naphthalen-5-yloxy)acetic acid, sodium salt. The chemical abstracts service registration number is [289480-64-4].
(32) Structure
(33) The structure of treprostinil sodium is depicted herein below. The molecular formula is C.sub.23H.sub.33NaO.sub.5 and it has a molecular weight of 412.49 daltons.
(34) Chemical Structure of Treprostinil Sodium
(35) ##STR00001##
General Properties
(36) Treprostinil sodium appears as a white or pale yellowish powder. It is very soluble in water and ethanol, very slightly soluble in acetone, and practically insoluble in acetonitrile, n-hexane, and ethyl acetate. The specific optical rotation calculated with reference to the anhydrous and solvent free basis is [α].sub.D.sup.20=+38.0°˜+44.0°. It is hygroscopic. The pKa of treprostinil is 4.5, using aqueous titration with 20% ethanol as a co-solvent. The distribution coefficient of treprostinil in various buffer solutions at various pH levels indicates distribution into octanol layers at all pH levels.
(37) Inhalation Particle Drug Product—LIQ861
(38) Description and Composition of the Drug Product Particle
(39) The inhalation drug particle product, in some embodiments, includes or consists of a dry powder dosage form of treprostinil and excipients (drug product-intermediate; DP-intermediate; or drug particle) that may be filled into, for example, a HPMC capsule (size 3). The DP-intermediate, in some embodiments, is a treprostinil/excipient matrix from which particles of precise size (e.g., 1 μm) and shape (e.g., “pollen-shaped”) are created using Liquidia's PRINT Technology. The “pollen-shaped” particles may also be described as trefoil-shaped, with an inscribed circle diameter of 1 μm, and a thickness of 0.7 μm. A three-dimensional rendering of such a particle shape is depicted in FIG. **1**. LIQ861 comprised drug product capsule strengths of 25 mcg, 50 mcg, and 75 mcg treprostinil used in the first clinical study to investigate planned dose levels of 25 mcg, 50 mcg, 75 mcg, 100 mcg, 125 mcg and 150 mcg treprostinil. The 100 mcg, 125 mcg and 150 mcg doses may be made up of a combination of lower dose capsules. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 175 mcg, 200 mcg, 225 mcg, 250 mcg, 275 mcg, 300 mcg, 325 mcg, or 350 mcg treprostinil. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 50 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 75 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 100 mcg treprostinil plus or minus 10 mcg, 9 mcg, 8 mcg, 7 mcg, 6 mcg, 5 mcg, 4 mcg, 3 mcg, 2 mcg or 1 mcg treprostinil for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 150 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% treprostinil for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 200 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% mcg treprostinil for delivery to a patient in a dry powder. In further embodiments, a drug product according to the present invention may provide capsules with dose levels of 300 mcg treprostinil plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% treprostinil for delivery to a patient in a dry powder. A summary of the LIQ861 formulation, including powder composition, particle geometry, and a description of the dosing unit according to certain exemplary embodiments follows.
(40) TABLE-US-00003 LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (dihydrate) Quantity (mg/g) Percent Component Function (Active) Solids Treprostinil Drug 5.3 0.53 Sodium Substance (5.0 as treprostinil) Trehalose Dihydrate Bulking Agent 930 92.97 Polysorbate 80 Wetting Agent/ 20 20 Process Aide L-Leucine Hydrophobicity 40 40 Modifier Sodium Citrate pH Modifier 2.7 0.27

Dihydrate Sodium Chloride Buffer Component 2.3 0.23

(41) TABLE-US-00004 Inhalation Drug Product Dosing Unit Description Capsule Size 3 Opaque White HPMC Capsule Fill Description White to Off-White Powder Fill Particle Shape “pollen-shaped” Active Strength (μg) 0 (placebo)* 25 50 75 Formulation Powder 15 5 10 15 per Capsule (mg) *Excipients only (no treprostinil). Abbreviations: HPMC, hydroxypropyl methylcellulose

(42) According to some embodiments of the present invention, drug particles are provided that include a composition having a target dose of 15-90 μg of delivered treprostinil to the patient (current TYVASO® label is 18-54 μg). In some embodiments of the present invention the dose of treprostinil provided to the patient can be, for example, 100 micrograms, 125 micrograms or 150 micrograms. In some embodiments of the present invention the dose of treprostinil provided to the patient, for example, can contain about 100 micrograms, about 125 micrograms or about 150 micrograms. In some embodiments, each dose contains greater than or equal to 200 micrograms of treprostinil. In some embodiments, each dose contains greater than or equal to 225 micrograms of treprostinil. In some embodiments, each dose contains greater than or equal to 250 micrograms of treprostinil. In some embodiments, each dose contains greater than or equal to 275 micrograms of treprostinil. In some embodiments, each dose contains greater than or equal to 300 micrograms of treprostinil. In some embodiments, each dose contains from about 10 micrograms to about 15 micrograms, 15 micrograms to about 20 micrograms, 20 micrograms to about 25 micrograms, 25 micrograms to about 30 micrograms, about 30 micrograms to about 35 micrograms, about 35 micrograms to about 40 micrograms, about 40 micrograms to about 45 micrograms, about 45 micrograms to about 50 micrograms, about 50 micrograms to about 55 micrograms, about 55 micrograms to about 60 micrograms, about 60 micrograms to about 65 micrograms, about 65 micrograms to about 70 micrograms, about 70 micrograms to about 75 micrograms, about 75 micrograms to about 80 micrograms, about 80 micrograms to about 85 micrograms, about 85 micrograms to about 90 micrograms, about 90 micrograms to about 95 micrograms, about 95 micrograms to about 100 micrograms, or about 100 micrograms to about 105 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 110 micrograms, 110 micrograms to about 120 micrograms, 120 micrograms to about 130 micrograms, 130 micrograms to about 140 micrograms, about 140 micrograms to about 150 micrograms, about 150 micrograms to about 160 micrograms, about 160 micrograms to about 170 micrograms, about 170 micrograms to about 180 micrograms, about 180 micrograms to about 190 micrograms, about 190 micrograms to about 200 micrograms, about 200 micrograms to about 210 micrograms, about 210 micrograms to about 220 micrograms, about 220 micrograms to about 230 micrograms, about 230 micrograms to about 240 micrograms, about 240 micrograms to about 250 micrograms, about 250 micrograms to about 260 micrograms, about 260 micrograms to about 270 micrograms, about 270 micrograms to about 280 micrograms, about 280 micrograms to about 290 micrograms, about 290 micrograms to about 300 micrograms, about 300 micrograms to about 310 micrograms, about 310 micrograms to about 320 micrograms, about 320 micrograms to about 330 micrograms, about 330 micrograms to about 340 micrograms, or about 340 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 25 micrograms to about 400 micrograms of treprostinil. In some embodiments, each dose contains from about 25 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 25 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 50 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 250 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 275 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 50 micrograms to about 75 micrograms of treprostinil. In some embodiments, each dose contains from about 50 micrograms to about 100 micrograms of treprostinil. In some embodiments, each dose contains from about 50 micrograms to about 150 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 100 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 125 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 150 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 175 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 200 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 75 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 125 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 150 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 175 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 200 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 100 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 150 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 175 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 200 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 125 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 175 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 200 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 150 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 200 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 175 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 225 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 275 micrograms of treprostinil. In some embodiments,

each dose contains from about 200 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 200 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 250 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 225 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 250 micrograms to about 275 micrograms of treprostinil. In some embodiments, each dose contains from about 250 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 250 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 250 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 275 micrograms to about 300 micrograms of treprostinil. In some embodiments, each dose contains from about 275 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 275 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 300 micrograms to about 325 micrograms of treprostinil. In some embodiments, each dose contains from about 300 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 325 micrograms to about 350 micrograms of treprostinil. In some embodiments, each dose contains from about 350 micrograms to about 375 micrograms of treprostinil. In some embodiments, each dose contains from about 375 micrograms to about 400 micrograms of treprostinil. In some embodiments, a patient may be provided with one, two, three, four, or more doses per day. In some embodiments, a patient may be provided up to one, two, three, or four doses per day. Each dose may be contained in a single capsule according to some embodiments, for example, a HPMC capsule (size 3). In other embodiments, a dose may be made up of a combination of lower dose capsules. In some embodiments, a patient may be provided with four doses per day to match the current treatment cycle (nebulized treprostinil) however the drug dose per treatment cycle under the present invention dry powder provides significantly higher dose levels to be safely administered, such as for example, up to 100 mcg of treprostinil per dosing, up to 125 mcg of treprostinil and up to 150 mcg of treprostinil per dosing as each were surprisingly demonstrated in the first clinical trial of LIQ861. In alternative embodiments, a patient may be provided with four doses per day to match the current treatment cycle (nebulized treprostinil) however the drug dose per treatment cycle under the present invention dry powder provides significantly higher dose levels to be achieved, such as for example, up to 200 mcg of treprostinil per dosing and up to 300 mcg of treprostinil per dosing as surprisingly demonstrated in pre-clinical toxicology studies using LIQ861.

(43) Treprostinil itself is poorly soluble in unbuffered water and low pH buffers. However, the solubility improves with increasing pH as the carboxylic acid is deprotonated. The sodium salt was selected for use in this product since it enhances dissolution in aqueous media and facilitates processing.

(44) Excipients

(45) According to some embodiments of the present invention, the DP-intermediate (anhydrous) is comprised of particles that include, for example, the following excipients: trehalose, polysorbate 80, L-leucine, sodium citrate, and sodium chloride. In some embodiments, the ratio of treprostinil sodium and excipients is 0.581:92.32:2.19:4.39:0.26:0.25 (wt:wt solids) treprostinil sodium:trehalose:polysorbate 80: leucine: sodium citrate:sodium chloride. A summary of the function, quantity, and compendial status of these excipients is provided herein.

(46) The excipients were selected based upon the following functional requirements for the formulation: Trehalose Dihydrate: Trehalose comprises the bulk of the particle and was selected because it is a non-reducing sugar with a high glass transition temperature. Trehalose is an example of a non-reducing sugar (as opposed to lactose, which is a reducing sugar) that can be used in the present invention. Trehalose is more chemically compatible with compounds containing primary amines, such as leucine. Ultra-Pure Polysorbate 80 (Ultra-Pure Tween 80): Polysorbate 80 is added as a processing aide/wetting agent to facilitate particle manufacturing. In some embodiments, Polysorbate 80 is a particle processing aide and enables film generation during particle manufacture by decreasing dewetting, leading to uniform particle morphology. L-leucine: Leucine is added as a hydrophobicity and surface modifier to reduce the hygroscopicity of the particle and improve aerosol efficiency. L-leucine is an example of a formulation additive to reduce hygroscopicity to improve stability of the final drug product powder. Sodium chloride and sodium citrate: Sodium citrate and sodium chloride are used to buffer the stock solution used in the PRINT Technology manufacturing process and to help control acidity in the particle. Sodium chloride and sodium citrate are examples of buffers that help maintain pH and control ionization/acidity of the formulation. In some embodiments of the present invention, pH is maintained between about pH 6.0 and 7.2.

(47) In addition to the active pharmaceutical ingredient the present drug particle comprises a bulking agent, wetting agent, hydrophobicity modifier, pH modifier and buffer. In some embodiments, the present drug particle comprises, along with the active ingredients, a bulking agent, hydrophobicity controlling agent, and a pH controlling agent.

(48) According to another embodiment of the present invention, LIQ861 contains five excipients as follows: treprostinil sodium:trehalose dihydrate:leucine:polysorbate 80:sodium citrate dihydrate: sodium chloride at ratios of 0.53:92.97:4:2:0.27:0.23. At an example treprostinil dose level of 100 µg/day of the present invention drug particles, a patient would receive the following daily excipient doses: 18.6 mg of trehalose dihydrate. Assuming a patient weighs 60 kg and has a lung mass of 1000 g, this is equivalent to 310 µg/kg and 18.6 µg/g of lung. 0.4 mg of polysorbate 80. Assuming a patient weighs 60 kg and has a lung mass of 1000 g, this is equivalent to 6.7 µg/kg and 0.4 µg/g of lung. 0.8 mg of leucine. Assuming a patient weighs 60 kg and has a lung mass of 1000 g, this is equivalent to 13.3 µg/kg and 0.8 µg/g of lung. 0.05 mg of sodium citrate and 0.05 mg of sodium chloride. Assuming a patient weighs 60 kg and has a lung mass of 1000 g, this is equivalent to 0.83 µg/kg for each compound and 0.05 µg/g of lung for each compound.

Formulation Development

(49) According to embodiments of the present invention, LIQ861 has been developed as a novel formulation of treprostinil for the treatment of PAH. Treprostinil is currently approved for use in the treatment of PAH by subcutaneous, IV, oral, and inhalation routes of administration. TYVASO is currently the only marketed inhaled formulation of treprostinil and is formulated as a liquid solution for administration using a nebulizer. The nebulized treprostinil is dosed, at maintenance dose, of 6 mcg drug per breath over 9 breaths for a dose of 54 mcg per dosing session. The nebulized treprostinil also has a maximum tolerated dose of 84 mcg over a dosing session with 14 breaths.

(50) LIQ861 is suitable for inhaled administration using a dry powder inhalation device. The physicochemical properties and performance characteristics, manufacturing process and packaging, and stability characteristics of the DP have been studied, and a suitable formulation has been identified for progression into human studies.

(51) Physiochemical and Biological Properties

(52) The “pollen-shaped” LIQ861 particles according to certain embodiments have an aerodynamic size to enable efficient delivery to the pulmonary arterioles (1≤MMAD≤5 µm) with a high FPF to limit oropharyngeal deposition. A scanning electron microscopy (SEM) image of the “pollen-shaped” feature is provided in FIG. **9**. The formulation of example particles shown in FIG. **9** is: treprostinil:trehalose:leucine:polysorbate 80:sodium citrate: sodium chloride (Batch LKI-1R-983-27). Example aerosol data for the active particles are also provided in the table below.

(53) During the development of the LIQ861 formulation, the applicants tested other possible particle shapes and sizes (e.g., 1.5 µm donut, 3.0 µm donut). Based upon these studies, the applicants observed that the “pollen-shaped” feature resulted in a greater FPF, reduced MMAD, acceptable ED, and dose uniformity characteristics when compared to other features both with and without treprostinil.

(54) TABLE-US-00005 Representative Aerosol Data (NGI) for Active Particles MMAD ED FPF Sample (µm) GSD (% nominal) (% ED) Treprostinil Sodium: 1.88 1.99 64 83 Trehalose: Leucine: Polysorbate 80: Sodium Citrate: Sodium Chloride (“pollen-shaped”) Abbreviations: NGI, Next Generation Impactor ™, MSP Corp.; MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation; ED, emitted dose; FPF,

fine particle fraction; wt, weight. Batch LKI-1R-0983-21.

Manufacture

(55) The manufacturing of LIQ861 particles according to some embodiments of the present invention is described below. A process flow diagram for the particles (also referred to as DP-intermediate) according to some embodiments is shown in FIG. **10**.

(56) In particular embodiments, the particles of the present disclosure are fabricated using PRINT® Technology (Liquidia Technologies, Inc., Morrisville, NC) particle fabrication. In particular, the particles are made by molding the materials intended to make up the particles in mold cavities.

(57) In some embodiments, the molds can be polymer-based molds and the mold cavities can be formed into any desired shape and dimension. Uniquely, as the particles are formed in the cavities of the mold, the particles are highly uniform with respect to shape, size, and composition. Due to the consistency among the physical and compositional makeup of the particles of the present compositions, the compositions of the present disclosure provide highly uniform release rates and dosing ranges. Methods and materials that may be used for fabricating the particles according to embodiments of the present disclosure are further described and disclosed in issued patents and co-pending patent applications, each of which are incorporated herein by reference in its entirety: U.S. Pat. Nos. 8,518,316; 8,444,907; 8,420,124; 8,268,446; 8,263,129; 8,158,728; 8,128,393; 7,976,759; U.S. Pat. Application Publications Nos. 2013-0249138, 2013-0241107, 2013-0228950, 2013-0202729, 2013-0011618, 2013-0256354, 2012-0189728, 2010-0003291, 2009-0165320, 2008-0131692; and pending U.S. Application Ser. No. 13/852,683 filed Mar. 28, 2013 and Ser. No. 13/950,447 filed Jul. 25, 2013.

(58) Particle Fabrication

(59) An aqueous stock solution is prepared at the desired total solids concentration. All other excipients are combined with treprostinil and then filtered prior to particle fabrication.

(60) The stock solution is applied in a thin layer to a continuous polyethylene terephthalate (PET) substrate backing layer. Forced air heat is used to drive off the water resulting in a dry film of treprostinil and excipients. The dried film is then brought into contact with a mold film, having cavities of the desired shape and size which the drug product particles will mimic, at an elevated temperature. The drug/excipient blend flows into the cavities of the mold, conforming to the shape defined by the cavity. The result is a uniform array of particles adhered to a PET backing layer. The particles are then allowed to cool to room temperature as the roll is wound up for later collection.

(61) In one example of the present drug particles, the following stock solution is used:

(62) Stock solution components used for manufacture of treprostinil particles, according to an embodiment:

(63) TABLE-US-00006 Target Solution Target Solution Concentration Concentration Stock component (Active) (Placebo) Target Trehalose .sup. 12% 12.7% Adjusted based on mass balance of other formulation components Leucine 0.52% 0.54% 0.52-0.54% (4% solids) Treprostinil Sodium 0.069% 0% 0.069% (0.53% solids) Polysorbate 80 0.26% 0.27% 0.26-0.28% (2% solids) Sodium Citrate 0.035% 0.037% Maintain pH stock solution for stability of treprostinil NaCl 0.030% 0.031% Maintain tonicity of stock solution Diluent (water) 87.0% 86.4% 86-91% evaluated; to coat appropriate formulation mass for processing and solubility of excipient component(s)

Dry Collection and Drying

(64) Next, the particles are dry collected, the process of removing the molded particles from the PET backing layer and thereby creating a bulk powder. The mold is first separated from the PET backing layer, exposing the particle array attached to the PET backing layer. The particle array is then passed across a blade, in some embodiments a plastic blade, to dislodge the particles from the backing layer. The particles can then be collected into a bulk powder for further processing.

(65) Humidity is controlled to less than 15% RH during collection, in some embodiments due to the hygroscopicity of the powder. Temperature is maintained at ambient, typically between 15 and 25° C.

(66) Drying and Bulk Packaging

(67) The drug particles are dried at less than or equal to 150 mTorr of nitrogen or dry air for at least 2 days in a benchtop lyophilizer at room temperature, according to some embodiments.

(68) In some embodiments, the particles of the present invention are dried to less than about 10 percent water content. In some embodiments, the particles of the present invention are dried to less than about 5 percent water content. In further embodiments, the particles of the present invention are dried to less than about 4 percent water content. In still further embodiments, the particles of the present invention are dried to less than about 2 percent water content. In a preferred embodiment, the product is dried to less than about 1 percent water content by Karl Fisher titration.

(69) Batch-to-Batch Uniformity of Drug Particles

(70) In some embodiments, the particle uniformity from batch-to-batch provides the present invention with an unexpected and exceptional advantage over the prior art. In certain embodiments, the uniformity within any given batch is unexpected and exceptionally advantageous over the prior art. The present invention includes highly conserved batch uniformity as shown in the following data. See the table below and also FIG. **2**.

(71) Uniformity: Sample aerosol data (NGI) for active particles (PAH-1R-0974-010)

(72) TABLE-US-00007 Uniformity: Sample aerosol data (NGI) for active particles (PAH-1R-0974-010) ED FPF F345 Sample MMAD GSD (% rec) (% ED) (fill) PAH-1R-0974-010-A1 First 1.74 1.88 81% 88% 42% PAH-1R-0974-010-B1 Middle 1.80 1.87 78% 87% 44% PAH-1R-0974-010-C1 Last 1.72 1.87 90% 89% 40%

In the example shown, fine particle fraction remained within plus/minus 1 percent within a single batch run.

Capsule Filling and Packaging

(73) In some embodiments, HPMC capsules are filled with the DP-intermediate in a humidity controlled ISO 8 environment using an XCELODOSE® (Capsugel) instrument. The filled HPMC capsules are packaged in a low humidity environment. Ten capsules are placed in a DESICAP® Vial and closed with a DESICAP® Cap. The closed vial is then placed into a foil bag with a desiccant canister prior to heat sealing the foil bag to form the packaged drug product.

(74) Stability studies

(75) According to the formulation of the present drug particle, it was desired to minimize uncontrolled exposure to ambient humidity. The drug particles according to embodiments of the present invention are shown to be stable for at least 9 months when stored under controlled humidity conditions at 25° C./60% RH. In some embodiments, the drug particles are shown to be stable for at least 6 months when stored under controlled humidity conditions at 40° C./75% RH. In some embodiments, the drug particles are shown to be stable for at least 9 months when stored under desiccated conditions at 25° C./60% RH. In some embodiments, the drug particles are shown to be stable for at least 6 months when stored under desiccated conditions at 40° C./75% RH. Studies were conducted to determine the stability of the drug particles at 25° C./60% RH and 40° C./75% RH.

(76) Prototype Stability Study

(77) The purpose of the Prototype Stability Study was to evaluate the stability of drug particles in capsules. Both the 25 and 75 μg strengths were evaluated when stored at 25° C./60% RH and 40° C./75% RH. For the study, drug particles were placed into size 3 HPMC opaque capsules (Capsugel Vcaps). Ten filled capsules were placed into HDPE vials (Desicap) which were sealed with a stopper. The stoppered vial was placed into a foil overwrap with desiccant sachets.

(78) Data for the 25 μg dose drug particles stored at 25° C./60% RH is shown in the table below.

(79) TABLE-US-00008 Time Points Test Specifications Initial 1 Month 3 Months 6 Months 9 Months Assay 0.450-0.550% w/w 0.489 0.520 0.493 0.494 0.486 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 1.92 2.11 2.2 2.1 2.1 Particle Size Results GSD (μm) 1.72 1.67 1.6 1.6 1.7 Distribution FPF (%) 87 84 82.9 83.6 83.6 Delivered Dose Report Average 19.9 21.6 19.65 19.47 18.86 Uniformity Results (μg)

(80) Data for the 25 μg dose drug particles stored at 40° C./75% RH is shown in the table below.
(81) TABLE-US-00009 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.489 0.512 0.502 0.492 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 1.92 2.09 2.1 2.1 Particle Size Results GSD (μm) 1.72 1.65 1.6 1.6 Distribution FPF (%) 87 85 84.8 84.7 Delivered Dose Report Average 19.9 21.2 18.86 19.28 Uniformity Results (μg)
(82) Data for the 75 μg dose drug particles stored at 25° C./60% RH is shown in the table below.
(83) TABLE-US-00010 Time Points Test Specifications Initial 1 Month 3 Months 6 Months 9 Months Assay 0.450-0.550% w/w 0.489 0.500 0.496 0.494 0.487 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.13 2.17 2.2 2.2 2.2 Particle Size Results GSD (μm) 1.60 1.61 1.6 1.6 1.6 Distribution FPF (%) 85 84 84.2 83.7 82.3 Delivered Dose Report Average 63.6 63.0 60.76 59.62 60.01 Uniformity Results (μg)
(84) Data for the 75 μg dose drug particles stored at 40° C./75% RH is shown in the table below.
(85) TABLE-US-00011 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.489 0.509 0.506 0.491 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.13 2.14 2.2 2.1 Particle Size Results GSD (μm) 1.60 1.61 1.6 1.6 Distribution FPF (%) 85 85 84.8 85.3 Delivered Dose Report Average 63.6 61.0 59.86 59.42 Uniformity Results (μg)

Clinical Trial Material Stability Study

(86) The purpose of the Clinical Trial Material Stability Study was to evaluate the stability of drug particles in capsules. Three strengths were evaluated: 25, 50, and 75 μg active agent doses within capsules. As in the previous study, two storage conditions were evaluated: 25° C./60% RH and 40° C./75% RH. For the study, drug particles were placed into size 3 HPMC opaque capsules (Capsugel Vcaps). Ten filled capsules were placed into HDPE vials (DESICAP) which were sealed with a stopper. The stoppered vial was placed into a foil overwrap with desiccant sachets.
(87) Data for the 25 μg dose drug particles stored at 25° C./60% RH is shown in the table below.
(88) TABLE-US-00012 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.520 0.505 0.504 0.504 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.3 2.3 2.2 2.1 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 84.1 82.7 83.6 85.0 Delivered Dose Report Average 19.784 20.48 19.24 19.47 Uniformity Results (μg) (μg)
(89) Data for the 25 μg dose drug particles stored at 40° C./75% RH is shown in the table below.
(90) TABLE-US-00013 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.520 0.518 0.501 0.506 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.3 2.2 2.2 2.2 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 84.1 84.1 85.8 84.7 Delivered Dose Report Average (μg) 19.784 20.73 18.89 18.80 Uniformity Results
(91) Data for the 50 μg dose drug particles stored at 25° C./60% RH is shown in the table below.
(92) TABLE-US-00014 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.515 0.509 0.509 0.506 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.2 2.2 2.2 2.2 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 86.2 85.6 85.7 85.3 Delivered Dose Report Average (μg) 40.417 40.75 39.14 40.05 Uniformity Results (μg)
(93) Data for the 50 μg dose drug particles stored at 40° C./75% RH is shown in the table below.
(94) TABLE-US-00015 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.515 0.520 0.505 0.501 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.2 2.2 2.2 2.2 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 86.2 86.5 86.2 84.1 Delivered Dose Report Average (μg) 40.417 39.55 38.96 37.50 Uniformity Results
(95) Data for the 75 μg dose drug particles stored at 25° C./60% RH is shown in the table below.
(96) TABLE-US-00016 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.517 0.512 0.509 0.508 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.3 2.3 2.3 2.2 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 84.8 84.1 84.5 85.1 Delivered Dose Report Average (μg) 61.851 63.91 59.88 60.25 Uniformity Results (μg)
(97) Data for the 75 μg dose drug particles stored at 40° C./75% RH is shown in the table below.
(98) TABLE-US-00017 Time Points Test Specifications Initial 1 Month 3 Months 6 Months Assay 0.450-0.550% w/w 0.517 0.513 0.503 0.495 Treprostinil as free acid (%) Aerodynamic Report MMAD (μm) 2.3 2.3 2.2 2.2 Particle Size Results GSD (μm) 1.6 1.6 1.6 1.6 Distribution FPF (%) 84.8 85.1 85.8 84.9 Delivered Dose Report Average (μg) 61.851 61.94 58.61 58.17 Uniformity Results

Dry Powder Inhalation Device

(99) The RS00 Model 8 is a commercially available monodose dry powder inhalation device that is manufactured by Plastiape S.p.A (Italy) in accordance with ISO and FDA standards. The overall design of RS00 Model 8 device is shown in FIG. **11**.
(100) A cap, which is retained on the mouthpiece, is designed to prevent ingress of dirt and other foreign material into the inhaler when not in use. The plastic side portions cover the air inlet holes, but the cap does not provide a hermetic seal to the device. The cap does not form part of the actuation process.
(101) When assembled, the mouthpiece is mounted on the inhaler body, but is removable for cleaning purposes. To assemble the mouthpiece, the off-set peg at the base of the mouthpiece is placed into the corresponding hole in the inhaler body and the mouthpiece is rotated until it snaps closed. The snap closure ensures that the mouthpiece and inhaler body are properly aligned and that no spurious airflow occurs. The mouthpiece contains a mesh that aids particle size reduction and prevent capsule ingestion during inhalation.
(102) The inhaler body component contains two side buttons, each housing four pins for piercing a capsule. The pins are inserted in the corresponding housing of the pushbuttons and the heads of the pins are retained in their position by a back-plate that is ultrasonically welded to the pushbutton. The buttons and pins are each maintained in their outward position by four small steel springs in each button. A three-component snap-lock system on the inhaler body ensures correct alignment of the mouthpiece when closed.
(103) A capsule piercing area is located internally, adjacent to the pins. When a capsule is inserted in this area, depressing the buttons causes the button pins to pierce the capsule ends, thereby preparing the capsule for emptying. Above the capsule piercing area, there are 2 tangential air inlets and a circular chamber. These allow the capsule to spin when the patient inhales through the device. Capsule spinning creates a centrifugal effect on the powder that promotes efficient emptying.
(104) The performance of the premetered dry powder inhaler is a combination of the characteristics of LIQ861 (including the powder and capsule) and the inhalation device itself.
(105) Nonclinical Studies
(106) Treprostinil is a tricyclic benzidine analogue of endogenous PGI.sub.2. The major pharmacologic actions of treprostinil are direct vasodilation of pulmonary and systemic arterial vascular beds and inhibition of platelet aggregation. It was developed for chronic administration as a continuous subcutaneous infusion as a treatment for patients with PAH. PGI.sub.2, an endothelial cell derived substance, is a potent vasodilator and inhibitor of platelet aggregation. The hemodynamic properties of treprostinil are similar to those of PGI.sub.2 but, unlike PGI.sub.2, treprostinil is chemically stable.
(107) A series of in vivo studies were conducted to evaluate pharmokinetics (PK) and toxicology of the present invention dry powder treprostinil formulation.
(108) Pilot, non-GLP, single-dose, inhalation PK study of treprostinil in dogs (Study 19073)
(109) This study compared the single dose PK of LIQ861 (administered via DPI) to Tyvaso (administered via nebulizer) at a target lung deposition of 3 μg/kg in 3 beagle dogs. Results showed generally similar treprostinil PK profiles following dosing with LIQ861 compared with Tyvaso. In this pilot single administration PK study, treprostinil (dry powder formulation; estimated lung deposition of 3.0 to 3.4 μg/kg) and treprostinil (nebulized liquid; target lung deposition of 3 μg/kg) were compared in 3 beagle dogs. The results showed generally similar treprostinil PK profiles following dosing with treprostinil (dry powder formulation) compared with treprostinil (nebulized liquid). The study design and results are discussed in more

detail herein.
(110) The applicants conducted a study comparing plasma concentrations and pharmacodynamics (PD) following administration of treprostinil sodium (nebulized liquid versus a dry powder formulation similar to the LIQ861 formulation of the present invention) as a single inhalation exposure (via controlled ventilation) to anesthetized beagle dogs. Treprostinil sodium was prepared as a nebulized liquid from the same DS used to prepare the dry powder formulation. The dry powder formulation was manufactured using PRINT Technology and utilized the same drug substance, treprostinil sodium, but was different in excipient concentrations compared to LIQ861. Importantly, the excipient concentrations of the present invention provide highly consistent and reproducible batch to batch manufacturing of the LIQ861 product. The formulations used in this study will be referred to as treprostinil (nebulized liquid) and treprostinil (dry powder formulation), respectively, in description of this study. The study design, results, and conclusions are described below.
(111) In study 19073, 3 dogs received a single inhalation administration of nebulized treprostinil (nebulized liquid; estimated lung deposition of 3.4 µg/kg). After a 2-day washout, the dogs received a single inhalation administration of treprostinil (dry powder formulation; estimated lung deposition of 3.0 to 3.4 µg/kg). Blood was collected for plasma analysis of treprostinil concentrations prior to each administration and at 2, 5, 10, 20, 30, 60, 120, and 180 minutes after the completion of each administration. In addition, 2 different dogs (one assigned to each treprostinil formulation) were used to monitor the following PD endpoints (hemodynamic changes): systemic arterial blood pressures [mean arterial pressure (MAP, mmHg), systolic arterial pressure (mmHg), diastolic arterial pressure (mmHg)], pulmonary artery pressure (PAP, mmHg), right atrial pressure (RAP, mmHg), pulmonary capillary wedge pressure (PCWP, mmHg) or left atrial pressure (mmHg), cardiac output (CO, L/min the average of 3), total peripheral resistance (TPR), pulmonary vascular resistance (PVR), and heart rate (HR). The PD effects were assessed prior to initiation of dose administration and at target times of 5, 10, 20, 30, 60, 120, and 180 minutes after the completion of the administration. In contrast to the first three dogs, the dogs assigned to monitor the PD effects were anesthetized for the duration of data collection. The dog assigned treprostinil (nebulized liquid) received an estimated lung deposition of 4.0 µg/kg and the dog assigned to treprostinil (dry powder formulation) received an estimated lung deposition of 2.5 µg/kg. Blood was collected at the same time points as first 3 dogs.
(112) Treprostinil (nebulized liquid and dry powder formulation) had no effect on HR, PAP, RAP, PCWP, or CO, but had a slight effect on decreasing and then increasing arterial blood pressure. Treprostinil (nebulized liquid) appeared to decrease stroke volume, increase TPR, and decrease PVR. Treprostinil (dry powder formulation) appeared to increase stroke volume, decrease TPR, and decrease PVR. The Study Director concluded that the pilot data were inconclusive for comparing the potential PD effects of treprostinil (nebulized liquid) to the treprostinil (dry powder formulation) formulation; however, there appeared to be no important differences in PD effects associated with administration of treprostinil in either formulation.
(113) Pilot, Non-GLP, Single-Dose, Inhalation PK Study of LIQ861 in Male Rats (Study 75670)
(114) This study evaluated the PK of treprostinil in male rats following single inhalation of a range of LIQ861 doses up to a feasible dose. Systemic exposure data from this study was used to determine appropriate doses and blood sampling times for a definitive, comparative PK bridging study of LIQ861 and nebulized treprostinil. Results from this study were used to select dose levels and an optimal blood sampling paradigm for a definitive PK bridging study.
SUMMARY: STUDY 75670
(115) The objective of the study was to determine the pharmacokinetic profile of treprostinil in male Sprague Dawley rats when administered as the test item, PRINT Treprostinil dry powder (PRINT-Tre), as a single 4 hour inhalation at targeted dose levels of 0.15, 0.75, and 1.5 mg/kg. Results from this study will be used to determine appropriate dose levels and sampling time points for a definitive PK bridging study.
(116) The test item was administered once by inhalation to 3 male rats per group as described in the table below:
(117) TABLE-US-00018 Achieved Mean Achieved Aerosol Achieved Aerosol Total Inhaled Dose Concentration Concentration Group Group Level of Treprostinil of Treprostinil of Trehalose No. Designation (mg/kg/day) (µg/L) (µg/L) 1 Low Dose 0.158 1.06 150.46 2 Mid Dose 0.707 4.72 664.85 3 High Dose 1.409 9.39 1298.81
(118) Assessments of mortality, clinical signs and body weights were performed. Blood samples were collected and analyzed for treprostinil content.
(119) No mortality occurred. No clinical signs were observed and body weights were unaffected.
(120) The overall achieved gravimetric and analytical aerosol concentrations for all groups were within 16% of the targeted concentrations. Corresponding average treprostinil dose levels for all groups were within 7% of the targeted dose levels and a clear dose differentiation between groups for each sex was achieved. The gravimetric particle size MMADs from all groups were between 1.2 and 1.6 µm (GSD 2.06 to 2.56). For both treprostinil and trehalose, the chemical determination of particle size distribution ranged from 1.3 to 1.8 µm with the corresponding GSDs between 1.65 and 2.15. The particle size distribution was considered respirable gravimetrically and chemically.
(121) Mean PK parameters for PRINT-Tre treatment groups obtained by non-compartmental analysis of the mean treprostinil plasma concentration data sets are summarized as follows:
(122) TABLE-US-00019 T.sub.1/2 T.sub.max C.sub.max AUC.sub.0-Tlast AUC.sub.INF Group (hr) (hr) (ng/mL) (hr*ng/mL) (hr*ng/mL) 1 Mean 1.01 3.75 6.800 17.320 18.335 SD 0.521 0.00 0.951 2.281 1.806 N 3 3 3 3 3 2 Mean 1.68 3.75 31.933 81.289 93.369 SD 0.967 0.00 9.500 19.478 19.372 N 3 3 3 3 3 3 Mean 1.48 3.75 46.130 121.285 137.512 SD 0.619 0.00 20.580 53.331 53.418 N 3 3 3 3 3
(123) In conclusion, single inhalation administration for 4 hours of PRINT-Tre at a high average treprostinil dose of 1.409 mg/kg/day by Sprague-Dawley rats was well tolerated as there were no significant test item related findings. The exposure to treprostinil generally increased in a dose proportional manner between the low dose and the mid dose. The exposure between the mid and high dose increased in a slightly less than dose proportional manner. However, animals in the high dose group were exposed to aerosol concentrations far below target for the last 16 to 26 minutes of inhalation, which may account for the less than dose proportional increase in exposure. Based on these results, similar dose levels are recommended for the following definitive PK study. Blood sampling time points during the test item inhalation period may be adjusted so as to better characterize exposure during test item administration.
INTRODUCTION
(124) The objective of the study was to determine the pharmacokinetic profile of treprostinil in male Sprague Dawley rats when administered as the test item, PRINT Treprostinil dry powder (PRINT-Tre), as a single 4 hour inhalation at targeted dose levels of 0.15, 0.75, and 1.5 mg/kg. Results from this study will be used to determine appropriate dose levels and sampling time points for a definitive PK bridging study.
(125) The study was not performed in compliance with GLP regulations but followed appropriate Standard Operating Procedures (SOPs).
(126) Experimental Design
(127) The test item was administered to groups of rats by inhalation administration for one day as described in the table below:
(128) TABLE-US-00020 Targeted Total Targeted Aerosol Targeted Aerosol Inhaled Dose Level Concentration Concentration No. of Group Group of Treprostinil of Treprostinil of Trehalose Animals No. Designation (mg/kg/day).sup.a (µg/L) (µg/L) Males 1 Low Dose 0.15 1 130.7 3 2 Mid Dose 0.75 5 653.5 3 3 High Dose 1.5 10 1306.9 3 .sup.a= Targeted aerosol concentrations were calculated based on an estimated body weight of 0.250 kg
(129) Following dosing, a series of 6 blood samples for pharmacokinetic evaluation were taken.
(130) Characterization of Test Item
(131) TABLE-US-00021 Test item*: Identity: PRINT Treprostinil Content: 92.75% of Trehalose, 4% of Leucine, 2% of Tween80, 0.26% of NA Citrate Dihydrate, 0.25% of NaCl: 0.74% of Treprostinil sodium (0.67% treprostinil) Storage Conditions: Cool (2 to 8° C.), protect from moisture (e.g., dessicant) Handling Precautions: Standard laboratory precautions. Handle under dry conditions (Relative Humidity ≤ 23%) Supplier: Liquidia Technologies Inc.
Treatment

(132) Acclimatization to Exposure System
(133) Before the animals were exposed to the aerosol of the test item, rats were accustomed to the restraint procedure over a period of 3 days. The animals were gradually accustomed to restraint in the dosing tubes used during the exposures up to the duration that was used for aerosol administrations.
(134) Animal Exposure
(135) Exposure system used: Flow-past rodent inhalation exposure system Exposure method: Inhalation by nose-only exposure Test Item type: Dry-Powder formulation Generation method: Piston feed/rotating brush generator Duration of exposure: 240 minutes
(136) The target aerosol concentrations and dose levels were as follows:
(137) TABLE-US-00022 Targeted Aerosol Targeted Aerosol Targeted Dose Level Concentration Concentration Group Group of Treprostinil of Treprostinil of Trehalose No. Designation (mg/kg/day).sup.a (μg/L) (μg/L) 1 Low Dose 0.15 1 130.7 2 Mid Dose 0.75 5 653.5 3 High Dose 1.5 10 1306.9 .sup.a= Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg.
Estimation of Achieved Dose Levels
The target dose levels were estimated using the following formula:
(138) $D_T = \frac{E_c \times RMV \times T}{BW}$ D.sub.L=Achieved dose levels (mg/kg/day)= E.sub.c=Actual concentration delivered to the animals (mg/L air) RMV=Respiratory Minute Volume (L/min) according to the method of Bide, Armour and Yee J. App. Toxicol., Vol. 20, 2000: RMV(L/min)=0.499×BW (kg).sup.0.809 T=Time, duration of daily exposure (min.) BW=Mean body weight (kg) during exposure period. This estimation of total inhaled dose assumed 100% deposition within the respiratory tract.
Inhalation Exposure System
(139) The powder aerosol was produced using a piston feed/rotating brush generator. The aerosol produced was diluted as necessary to achieve the target aerosol concentration and discharged through a 40-mm diameter tube into a flow-past inhalation exposure system. The airflow rate through the exposure system was monitored and recorded manually during each aerosol generation period. Airflow to the exposure system was controlled by the absolute volume of air supplying the generation apparatus using variable area flowmeters. Control of the aerosol exhaust flow from the animal exposure system was achieved using an exhaust valve, and the overall balance of airflows in the exposure system was monitored using pressure gauges. The system provided a minimum of 1.0 L/min to each animal exposure port and was balanced to ensure a slight positive pressure at the site of the proposed animal exposure. This ensured that there was no dilution of the generated aerosol. An equal delivery of aerosol to each proposed exposure position was achieved by employing a distribution network that was identical for each individual exposure position attached to the system.
(140) Inhalation System Monitoring
(141) Determinations of aerosol concentration, particle size distribution, oxygen concentration, relative humidity and temperature were measured on samples collected from a representative port of the exposure chamber. The sample flow rates were precisely controlled using variable area flow meters that were calibrated before use using a primary airflow calibrator. The absolute volume of each aerosol concentration sample was measured with a wet type gas meter.
(142) Oxygen Concentration
(143) The oxygen concentration of the generated atmosphere was measured once during each aerosol exposure. Oxygen concentrations of the exposure atmospheres were maintained between 19-23%.
(144) Relative Humidity/Temperature
(145) The temperature and relative humidity of the generated atmosphere were measured once during each aerosol exposure. Temperatures of the exposure atmospheres were maintained between 19-24° C.
(146) Determination of Aerosol Concentration
(147) At least one aerosol concentration filter sample was collected on glass fiber filter and weighed on each day in order to measure the gravimetric concentration of the test item in the generated aerosol. The filter samples were transferred to the analytical chemistry laboratory for chemical determination of Treprostinil and Trehalose concentrations using an analytical method (Study No. 41609 and Study No. 41635).
(148) Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)
(149) The distribution of particle size in the generated aerosols was measured once during each exposure by collecting samples into a 7-Stage Mercer Cascade Impactor. The MMAD and the Geometric Standard Deviation (GSD) were calculated based on the results obtained from the impactor using a log-probit transformation.
(150) In-Life Observations
(151) Mortality
(152) Mortality checks were performed at least once a day during all phases of the study.
(153) Clinical Observations
(154) Cage-side clinical signs (ill health, behavioral changes etc.) were recorded at least once daily during all phases of the study, except on detailed clinical examination days, where the cage-side clinical signs were replaced by a DCE.
(155) A detailed clinical examination of each rat was performed on arrival as part of the health status, as well as on Day 1, prior to dosing.
(156) Animal whose health status was judged to warrant additional evaluation was examined by a Clinical Veterinarian.
(157) Body Weights
(158) Body weights were recorded for all animals once at arrival as per health status, once prior to group assignment and on Day 1 (prior to dosing).
(159) Pharmacokinetics
(160) A series of 6 blood samples (approximately 0.3 mL each) was collected from each rat on Day 1 at −15, 5, 15, 30, 75 and 105 minutes after treatment. Thus a total blood volume of 1.8 mL was taken from each rat during the course of the study. For this purpose, each rat (unanesthetized) was bled by jugular venipuncture and the samples were collected into tubes containing the anticoagulant, K2EDTA. Tubes were placed on wet ice pending processing.
(161) Following collection, the samples were centrifuged (2500 rpm for 10 minutes at approximately 4° C.) and the resulting plasma was recovered and stored frozen (≤−60° C.) in labeled tubes.
(162) Deviations to the pharmacokinetic time points were noted in the raw data and were made available with the samples. The location of blood withdrawal was noted in the raw data.
(163) Non-compartmental analysis of treprostinil concentrations in plasma were performed by using the Phoenix WinNonlin 6.3 software.
(164) The following configuration was used for the analysis:
(165) Sampling Method: Sparse
(166) AUC Calculation Method: Linear Trapezoidal with Linear Interpolation
(167) Lambda Z (λ.sub.z) Method: Best fit for λ.sub.z, Log regression
(168) Weighting (λ.sub.z calculation): Uniform
(169) Pharmacokinetic parameters (including abbreviation and description for each parameter) are described in the following table:
(170) TABLE-US-00023 Parameters Abbreviation Unit* Area under the plasma drug concentration- AUC.sub.0-Tlast μg*hr/mL time curve from the time of dosing to the last quantifiable concentration Area under the plasma drug concentration- AUC.sub.INF μg*hr/mL time curve from the time of dosing extrapolated to infinity Terminal elimination half-life T.sub.1/2 hr The maximum plasma concentration C.sub.max μg/mL Time to maximum plasma concentration T.sub.max hr *Different units may be presented in the study report

Data Evaluation and Statistics
(171) Numeric and non-numeric data obtained during the study were reported only as individual values.
RESULTS
(172) Aerosol Concentrations
(173) Achieved gravimetric test atmosphere concentrations were as follows:
(174) TABLE-US-00024 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (mg\L) (mg\L) (%) Target 1 0.156 0.165 17.2 105.9 2 0.781 0.728 14.0 93.2 3 1.563 1.439 43.5 92.0
(175) Achieved analytical test atmosphere concentrations for treprostinil were as follows:
(176) TABLE-US-00025 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (µg\L) (µg\L) (%) Target 1 1 1.06 17.6 105.9 2 5 4.72 13.9 94.4 3 10 9.39 43.6 93.9
(177) Achieved analytical test atmosphere concentrations for trehalose were as follows:
(178) TABLE-US-00026 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (µg\L) (µg\L) (%) Target 1 130.7 150.46 18.8 115.1 2 653.5 664.85 15.0 101.7 3 1306.9 1298.81* 44.1 99.4 *Last 2 aerosol concentrations samples for trehalose were estimated with a 92.79% difference from gravimetric data as analytical results were BLQ
(179) The overall achieved gravimetric and analytical aerosol concentrations for all groups were within 16% of the targeted concentrations. The generated atmospheres were considered stable over the treatment period as % CV were all below 20%, except for Group 3. The increased % CV for Group 3 was caused by the stoppage of the Rotating Brush Generator (RBG) due to lack of test item remaining in the canister with 26 minutes left in the generation (16 minutes of dosing left for animal **3001**A, 21 minutes left for animal **3002**A and 26 minutes left for animal **3003**A). Though a new test item canister was installed on the RBG apparatus, the aerosol concentrations were much lower than targeted for the last 26 minutes. However, the overall aerosol concentrations were still considered acceptable for the study as there was a significant difference in aerosol concentration between groups.
(180) Dose Levels
(181) Overall achieved doses for treprostinil are presented below:
(182) TABLE-US-00027 Targeted Duration of Body Estimated % from Group Dose Levels Exposure Weight Achieved Doses Targeted Dose No. (mg/kg/day) (min) Animal (kg) (mg/kg/day) Level 1 0.15 240 1001A 0.326 0.157 104.7 1002A 0.309 0.159 106.0 1003A 0.314 0.158 105.3 Average 0.158 105.3 2 0.75 240 2001A 0.319 0.703 93.7 2002A 0.308 0.708 94.4 2003A 0.304 0.709 94.5 Average 0.707 94.3 3 1.5 240 3001A 0.322 1.396 93.1 3002A 0.321 1.397 93.1 3003A 0.281 1.433 95.5 Average 1.409 93.9
(183) Average achieved dose levels for all groups were within 7% of the targeted dose levels therefore the dose levels were considered acceptable for the study as a clear dose differentiation between groups for each sex was achieved.
(184) Particle Size Distribution
(185) The average gravimetric particle size distribution measurement data were as follows:
(186) TABLE-US-00028 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (µm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (µm) GSD µm 1 95.8 92.4 82.4 45.4 32.4 21.5 12.3 0.0 1.2 2.28 93 2 86.8 83.3 76.0 39.4 28.0 15.4 8.9 0.0 1.5 2.56 85 3 94.0 90.9 77.3 30.3 13.4 8.9 5.3 0.0 1.6 2.06 90 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(187) The average chemical determination of particle size distribution for treprostinil were as follows:
(188) TABLE-US-00029 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (µm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (µm) GSD µm 1 96.4 93.9 83.3 42.8 28.1 16.5 6.9 0.0 1.3 2.06 94 2 91.7 88.5 81.1 37.9 25.8 11.8 4.9 0.0 1.5 2.15 90 3 95.2 91.5 76.6 25.7 8.8 5.1 1.8 0.0 1.7 1.86 91 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(189) The average chemical determination of particle size distribution for trehalose were as follows:
(190) TABLE-US-00030 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (µm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (µm) GSD µm 1 95.8 91.6 81.5 38.4 24.1 13.0 4.2 0.0 1.4 2.01 93 2 94.4 88.9 83.3 31.5 26.0 11.1 5.6 0.0 1.4 2.11 91 3 95.2 90.4 77.0 25.5 9.6 4.8 0.0 0.0 1.8 1.65 94 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(191) The particle size distribution was considered respirable for this study as the MMADs were below 4 µm and the GSD were within 1.5 and 3.
(192) Exposure Chamber Conditions
(193) Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.
(194) TABLE-US-00031 Oxygen Group Humidity Temperature Concentration No. (% RH) (° C.) (%) 1 31.4 21.2 20.9 2 34.2 21.7 20.9 3 26.2 20.7 20.9
(195) Exposure atmosphere oxygen concentrations, temperature and relative humidity ranges were considered acceptable on all occasions.
(196) Mortality
(197) There were no mortalities during the study.
(198) Clinical Signs
(199) There were no adverse clinical signs observed during the study.
(200) Slight decreased activity, piloerection and partially closed eyes were seen in animal **3001**A right before the 15 minute time point. However these were not observed afterwards and were not observed in any other animal therefore were not deemed test item related.
(201) Body Weight
(202) Body weights were performed for dose level calculation purposes.
(203) Pharmacokinetics
(204) Following the administration of PRINT-Tre at all achieved dose levels, mean C.sub.max ranged from 6.800 to 46.133 ng/mL. The mean maximum plasma concentration (T.sub.max) was reached at 3.75 hour (15 minutes before end of dosing) for all groups. The mean AUC.sub.0-Tlast (AUCINF) ranged from 17.320 (18.335) to 121.258 (137.512) hr*ng/mL. Following T.sub.max, the treprostinil plasma concentrations declined gradually with an estimated mean T.sub.1/2 ranging from 1.01 to 1.68 hours.
(205) Over the dose range, exposure to treprostinil (based on C., AUC.sub.0-Tlast and AUCINF) generally increased in dose proportional manner between the low dose (0.158 mg/kg) and the mid dose (0.707 mg/kg). When dose level increased 4.5-fold from low to mid dose, C.sub.max and AUC.sub.0-Tlast increased 4.7-fold. Treprostinil exposure between the mid dose (0.707 mg/kg) and high dose (1.409 mg/kg) increased in a slightly less than dose proportional manner (2-fold increase in dose with a 1.4-(C.sub.max) to 1.5-fold (AUC.sub.0-Tlast) increase in exposure). However, because animals in the high dose group were exposed to aerosol concentrations far below target for the last 16 to 26 minutes of the exposure period, exposure levels may have been effected and could account for the less than dose proportional increase in exposure.
CONCLUSION
(206) Single inhalation administration for 4 hours of PRINT-Tre at a high average treprostinil dose of 1.409 mg/kg/day to Sprague-Dawley rats was well tolerated as there were no significant test item related findings. The exposure to treprostinil generally increased in a dose proportional manner between the low dose and the mid dose. The exposure between the mid and high dose increased in a slightly less than dose proportional manner. However, animals in the high dose group were exposed to aerosol concentrations far below target for the last 16 to 26 minutes of inhalation, which may account for the less than dose proportional increase in exposure. Based on these results, similar dose levels are recommended for the following definitive PK study. Blood sampling time points during the test item inhalation period may be adjusted so as to better characterize exposure during test item administration.

(207) Non-GLP, Single-Dose, Inhalation, Comparative PK Study of LIQ861 and Nebulized Treprostinil in Rats (Study 75658)
(208) This study evaluated and compared the PK profile of LIQ861 to treprostinil (nebulized) to establish a bridge between the two formulations.
(209) The non-GLP, single administration by inhalation, PK study of treprostinil in rats (Study 75658) has been completed by Liquidia (referred to as the definitive PK bridging study). This study compared the systemic exposure of LIQ861 versus nebulized liquid treprostinil sodium. The observed systemic exposures revealed no meaningful differences between formulations, providing a bridge between the LIQ861 formulation and the marketed Tyvaso formulation and thereby permitting use of Tyvaso nonclinical toxicology studies to support the LIQ861 formulation per the 505(b)(2) pathway.
(210) In Study 75658, systemic exposure of LIQ861 versus nebulized treprostinil sodium was compared in rats. LIQ861 was delivered over a 4-hour exposure period at total delivered dose levels of 0.273, 0.762, and 1.50 mg/kg body weight. Nebulized treprostinil sodium was delivered at a single dose level (0.785 mg/kg total delivered dose) for the same exposure period (4 hours) as LIQ861. Blood was collected for plasma analysis of treprostinil concentrations at 30 and 60 minutes following the start of administration, immediately post-administration (240 min), and at 5, 15, 30, 75, and 105 minutes following the end of administration.
(211) Pharmacokinetic parameters from Study 75658. Individual plasma concentrations of treprostinil ranged from 0.345 to 67.4 ng/mL. Maximum plasma concentration was reached 0.5 to 4 hours after the start of the 4-hour exposure period. Maximum concentration (Cmax) and area under the curve (AUC) values were similar between males and females within treatment groups. Dose-related increases in Cmax and AUC values were observed for the three LIQ861 dose groups. Relative bioavailability of LIQ861 compared to nebulized treprostinil based on dose normalized AUC-time curve extrapolated to time infinity (AUCinf) ranged from 1.2 to 2.2.
(212) TABLE-US-00032 Summary of Mean Noncompartmental PK Parameters by Treatment and Sex for Study 75658 Achieved Mean Dose Type of Level t.sub.1/2 T.sub.max C.sub.max AUC.sub.0-Tlast AUC.sub.INF inhalation Group (mg/kg) Sex R.sup.2 (hr) (hr) (ng/mL) (hr*ng/mL) (hr*ng/mL) Treprostinil 1 0.785 Female 0.99 0.59 4.00 16.2 62.4 63.5 Sodium Male 0.97 0.77 0.50 16.5 59.3 60.6 (Nebulized) Dry Powder 2 0.273 Female 0.92 1.77 4.00 5.38 22.2 24.2 (PRINT Male 1.00 0.73 1.00 7.18 26.9 27.4 treprostinil) 3 0.762 Female 0.97 0.66 4.00 32.8 107 110 Male 0.90 0.95 4.00 54.4 144 149 4 1.498 Female 0.84 0.67 0.50 44.5 174 182 Male 1.00 0.90 4.00 44.1 143 148 Abbreviations: Cmax, maximal concentration; Tmax, time of maximal concentration; AUClast, area under the concentration-time curve to the last measured timepoint; t½, half-life; AUCinf, area under the concentration-time curve extrapolated to time infinity. PRINT treprostinil = LIQ861 DP-intermediate.
(213) TABLE-US-00033 Calculated Relative Bioavailability (Combined Genders) Based on AUCinf (Dose Corrected) from Study 75658 Frel Dose Level Mean AUCinf (PRINT/ Group Treatment (mg/kg) (hr * ng/mL) Treprostinil) 1 Treprostinil 0.785 62.1 NA 2 PRINT- 0.273 25.8 1.20 treprostinil Low 3 PRINT- 0.762 129 2.15 treprostinil Mid 4 PRINT- 1.498 165 1.39 treprostinil High Abbreviations: AUCinf, area under the concentration-time curve extrapolated to time infinity; Frel, relative bioavailability; NA, not applicable. PRINT treprostinil = LIQ861 DP-intermediate.
Summary: Study 75658
(214) The objectives of the study were to determine the pharmacokinetic (PK) profile of Treprostinil in Sprague-Dawley rats when administered as PRINT-Treprostinil (PRINT-Tre) by 4-hour inhalation at 0.15, 0.75, and 1.5 mg/kg, to determine the PK profile of Treprostinil in Sprague-Dawley rats when administered as nebulized Treprostinil sodium in solution (Tre solution) by 4-hour inhalation at 0.75 mg/kg and to compare the PK profiles of Treprostinil when administered as PRINT-Tre and Tre solution.
(215) The test item was administered once to 6 male and 6 female rats per group by nose-only inhalation for 4 hours as described in the table below:
(216) TABLE-US-00034 Achieved Mean Achieved Achieved Total Inhaled Aerosol Aerosol Dose Level of Concentration Concentration Group Group Treprostinil of Treprostinil of Trehalose No. Designation (mg/kg/day) (µg/L) (µg/L) 1 Tre Solution 0.785 5.07 0 2 PRINT-Tre 0.273 1.76 254.84 (Low Dose) 3 PRINT-Tre 0.762 4.95 719.53 (Mid Dose) 4 PRINT-Tre 1.498 9.73 1394.33 (High Dose)
(217) Assessments of mortality, clinical signs and body weights were performed. Pharmacokinetic samples were collected and the analysis of these samples was performed.
(218) No mortality occurred and no clinical signs were observed.
(219) The overall achieved aerosol concentrations for all groups were within 10% of the targeted concentrations gravimetrically and for both treprostinil and trehalose, except for Group 2 which were significantly above the targeted concentrations (76 to 95%). Corresponding average achieved dose levels for all groups were within 5% of the targeted dose levels, except for Group 2 which was 82% above the targeted dose level. However, the dose levels were considered acceptable for the study as a clear dose differentiation between groups for each sex was achieved.
(220) The particle size MMADs from Groups 2 to 4 were between 1.7 and 2.0 µm gravimetrically (GSD 1.90 to 2.67); for both treprostinil and trehalose, chemical particle size distribution ranged from 1.6 to 1.8 µm with the corresponding GSDs between 1.89 and 2.24. The particle size MMAD for Group 1 was 0.5 µm with a corresponding GSD of 2.60. The particle size distribution was considered respirable.
(221) With administration of PRINT-Tre at an achieved dose level of 0.273 mg/kg, 0.762 mg/kg or 1.498 mg/kg, plasma exposure to treprostinil was generally similar in both sexes; however, exposure was slightly lower in females than males at the mid-dose level and slightly higher in females than males at the high-dose level.
(222) Based on AUC.sub.0-Tlast, AUCINF and C.sub.max, values for both sexes, plasma exposure increased more than proportionally between the low- and mid-dose levels. But between the mid- and high-dose levels, plasma exposure increased less than proportionally for females and there was no increase in the exposure for males. The maximum mean treprostinil plasma concentration (T.sub.max) was at the end of inhalation for both sexes, except for low-dose males and high-dose females, where mean T. was at 1 and 0.5 hours after inhalation began, respectively.
(223) At the low-dose level, mean treprostinil plasma concentration was similar after 0.5, 1, or 4 hours of inhalation exposure to PRINT-Tre, suggesting that steady state was achieved within the first 30 minutes of exposure. The same was true for females at the high-dose level; however, for males at the high-dose level and for both sexes at the mid-dose level, mean treprostinil plasma concentration was greater at the end of inhalation than after one hour of inhalation. When inhalation ended, treprostinil plasma concentrations declined gradually. Given the degree of individual variation, the estimated mean T.sub.1/2 values were similar at all dose levels and ranged from 0.7 to 1.8 hours in males and 0.7 to 1.0 hours in females.
(224) For Tre solution, with an administration at 0.785 mg/kg, plasma exposure to treprostinil was generally similar in both sexes. The maximum mean treprostinil plasma concentration (T.sub.max) was at the end of inhalation. Mean treprostinil plasma concentration was similar after 0.5, 1, or 4 hours of inhalation exposure to Tre solution, suggesting that steady state was achieved within the first 30 minutes of exposure. When inhalation ended, treprostinil plasma concentrations declined gradually, with estimated mean T.sub.1/2 values of 0.6 hours in males and 0.8 hours in females.
(225) Administration of PRINT-Tre and Tre solution at nearly equivalent dose levels (0.76 and 0.79 mg/kg, respectively) resulted in plasma exposures to treprostinil that were greater with PRINT-Tre than with Tre solution. Specifically, mean AUC.sub.0-Tlast was approximately twice as high (126 versus 61 h*ng/mL, respectively) and mean C.sub.max was three times as high (44 versus 16 ng/mL, respectively). As would be expected, once treprostinil entered the systemic circulation, it was cleared from plasma at a similar rate, with mean T.sub.1/2 values of 0.7 to 1.0 hours for PRINT-Tre and 0.6 to 0.8 hours for Tre solution.
(226) In conclusion, single inhalation administration for 4 hours of PRINT Treprostinil at a high average dose of 1.498 mg/kg/day to Sprague-Dawley rats was well tolerated as there were no test item related findings. At an equivalent dose level, plasma exposures to treprostinil was greater with PRINT-Tre than with Tre solution; specifically, mean AUC.sub.0-Tlast was approximately twice as great and mean C.sub.max was three times as great. As would be expected, once treprostinil entered the systemic circulation, it was cleared from plasma at a similar rate regardless of how it was administered.
(227) The objectives of the study were to: 1. Determine the pharmacokinetic (PK) profile of Treprostinil in Sprague-Dawley rats when administered

as PRINT-Treprostinil (PRINT-Tre) by 4-hour inhalation at 0.15, 0.75, and 1.5 mg/kg. 2. Determine the PK profile of Treprostinil in Sprague-Dawley rats when administered as nebulized Treprostinil sodium in solution (Tre solution) by 4-hour inhalation at 0.75 mg/kg. 3. Compare the PK profiles of Treprostinil when administered as PRINT-Tre and Tre solution.

Experimental Design

(228) The test items were administered to groups of rats by a 4-hour inhalation administration as described in the table below:

(229) TABLE-US-00035 Targeted Total Targeted Inhaled Dose Aerosol Targeted Aerosol Level of Concentration of Concentration of Group Group Treprostinil Treprostinil Trehalose No. of Animals No. Designation (mg/kg/day) (μg/L) .sup.a (μg/L) .sup.a Males Females 1 Tre Solution 0.75 5 0 6 6 2 PRINT-Tre 0.15 1 130.7 6 6 3 PRINT-Tre 0.75 5 653.5 6 6 4 PRINT-Tre 1.5 10 1306.9 6 6 .sup.a = Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg

(230) During and after the inhalation period, a series of 8 blood samples for pharmacokinetic evaluation were taken.

(231) Justification for Selection of Route of Administration, Species and Dose Levels

(232) The route of administration was chosen because it is the intended human therapeutic route.

(233) The rat was selected because it is a rodent species recommended by various regulatory authorities. Background data are available. Also, rats were used as the test system for previous toxicity studies with Treprostinil sodium solution that supported development and approval of that product. Using rats in the current study allowed comparison with the previous studies.

(234) The high-dose level for PRINT-Tre was the feasible dose attainable based on technical aerosol trials with the test item (Study No. 41610).

(235) The low- and mid-dose levels for PRINT-Tre were selected on the basis of a previous pilot PK study in rats (Study No. 75670).

(236) The dose level for Tre solution was selected to match the mid-dose level of PRINT-Tre to allow direct comparison. Characterization of Test Items Content: 92.79% of Trehalose, 4% of Leucine, 2% of Tween80, 0.26% of NA Citrate, 0.24% of NaCl: 0.71% of Treprostinil Storage Conditions: Cool (2 to 8° C.), protected from moisture (e.g., dessicant) Handling Precautions: Standard laboratory precautions. Handle under dry conditions (Relative Humidity≤23%) Supplier: Liquidia Technologies Inc.

(237) TABLE-US-00036 Test item 2*: Identity: Treprostinil Sodium Decription: White or pale yellowish powder Batch No.: TN115E010 Expiry Date: May 28, 2017 Purity: 101.49% Storage Conditions: Cool (2 to 8° C.) Handling Precautions: Standard laboratory precautions Supplier: Yonsung Fine Chemicals Co., LTD

Preparation of Test Item

(238) PRINT-Tre was used as provided by the Sponsor. A glove box under nitrogen was used for handling, aliquoting or packing of the canisters. Relative humidity (RH) inside the glove box was monitored and recorded using a hygrometer and was kept below 23% RH.

(239) For Group 1, the treprostinil sodium was dissolved in purified water to achieve the desired formulation concentration. A representative sample (0.5 mL in duplicate) was collected to verify the formulation concentration of Treprostinil in the formulation.

(240) Treatment

(241) Acclimatization to Exposure System

(242) Before the animals were exposed to the aerosol of the test item, rats were accustomed to the restraint procedure over a period of 3 days. The animals were gradually accustomed to restraint in the dosing tubes used during the exposures up to the duration that was used for aerosol administrations.

(243) Animal Exposure

(244) Exposure system used: Flow-past rodent inhalation exposure system Exposure method: Inhalation by nose-only exposure Test Item type: Solution (Group 1), Dry Powder (Groups 2 to 4) Generation method: Nebulization (Group 1) and Piston feed/rotating brush generator (Group 2 to 4) Duration of exposure: 240 minutes

(245) The target aerosol concentrations and dose levels were as follows:

(246) TABLE-US-00037 Targeted Targeted Targeted Dose Aerosol Aerosol Level of Concentration Concentration Group Group Treprostinil of Treprostinil of Trehalose No. Designation (mg/kg/day) (μg/L) .sup.a (μg/L) 1 Tre solution 0.75 5 0 2 PRINT-Tre 0.15 1 130.7 (Low Dose) 3 PRINT-Tre 0.75 5 653.5 (Mid Dose) 4 PRINT-Tre 1.5 10 1306.9 (High Dose) .sup.a = Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg.

(247) Estimation of Achieved Dose Levels The target dose levels were estimated using the following formula:

(248) $D_T = \frac{E_c \times RMV \times T}{BW}$ D.sub.L=Achieved dose levels (mg/kg/day) E.sub.c=Actual concentration delivered to the animals (mg/L air) RMV=Respiratory Minute Volume (L/min) according to the method of Bide, Armour and Yee 2000 J. App. Toxicol., Vol. 20: kW (L/min)=0.499×BW (kg)° 0.809 T=Time, duration of daily exposure (min.) BW=Mean body weight (kg) during exposure period. This estimation of total inhaled dose assumed 100% deposition within the respiratory tract.

Inhalation Exposure System

(249) The powder aerosol for Groups 2 to 4 was produced using a piston feed/rotating brush generator while the liquid aerosol for Group 1 was produced by metering the flow of the formulation to a clinical nebulizer (Sidestream). The aerosol produced was diluted as necessary to achieve the target aerosol concentration and discharged through a 40-mm diameter tube into a flow-past inhalation exposure system. The airflow rate through the exposure system was monitored and recorded manually during each aerosol generation period. Airflow to the exposure system was controlled by the absolute volume of air supplying the generation apparatus using variable area flowmeters. Control of the aerosol exhaust flow from the animal exposure system was achieved using an exhaust valve, and the overall balance of airflows in the exposure system was monitored using pressure gauges. The system provided a minimum of 1.0 L/min to each animal exposure port and was balanced to ensure a slight positive pressure at the site of the animal exposure. This ensured that there was no dilution of the generated aerosol. An equal delivery of aerosol to each exposure position was achieved by employing a distribution network that was identical for each individual exposure position attached to the system.

(250) Inhalation System Monitoring

(251) Determinations of aerosol concentration, particle size distribution, oxygen concentration, relative humidity and temperature were measured on samples collected from a representative port of the exposure chamber. The sample flow rates were precisely controlled using variable area flow meters that were calibrated before use using a primary airflow calibrator. The absolute volume of each aerosol concentration sample was measured with a wet type gas meter.

(252) Oxygen Concentration

(253) The oxygen concentration of the generated atmosphere was measured once during each aerosol exposure. Oxygen concentrations of the exposure atmospheres were maintained between 19-23%.

(254) Relative Humidity/Temperature

(255) The temperature and relative humidity of the generated atmosphere were measured once during each aerosol exposure. Temperatures of the exposure atmospheres were maintained between 19-24° C.

(256) Determination of Aerosol Concentration

(257) At least one aerosol concentration filter sample was collected for all groups on each aerosol generation. The filter samples from Groups 2 to 4 were weighed in order to measure the gravimetric concentration of the test item in the generated aerosol. The filter samples were transferred to the analytical chemistry laboratory for chemical determination of Treprostinil and Trehalose concentrations. The filter samples for Group 1 were not weighed gravimetrically and were only transferred to the analytical laboratory for determination of Treprostinil concentrations. The analysis in the analytical laboratory was performed using an analytical method (Study No. 41609).

(258) Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)
(259) The distribution of particle size in the generated aerosols was measured once for Groups 1 to 4 by collecting samples into a 7-Stage Mercer Cascade Impactor. All sample substrates obtained from Groups 2 to 4 were weighed gravimetrically and then transferred to the analytical chemistry laboratory for chemical determination of particle size of aerosolized Treprostinil and Trehalose. All sample substrates obtained from Group 1 were only transferred to the analytical laboratory for chemical determination of particle size of aerosolized Treprostinil. The analysis in the analytical laboratory was performed using an analytical method (Study No. 41609).
(260) The MMAD and the Geometric Standard Deviation (GSD) were calculated based on the results obtained from the impactor using a log-probit transformation.
(261) Reporting of Analytical Results
(262) The analytical report containing the results from the filter and particle size distribution sample analyses were prepared. Any samples not employed in the primary analysis or any remaining sample from the primary analysis were retained until it was determined by the analyst and Study Director that it was not be required for confirmatory analysis. These samples were discarded and their disposition recorded in the raw data.
(263) In-Life Observations
(264) Mortality
(265) Mortality checks were performed at least once a day during all phases of the study.
(266) Clinical Observations
(267) Cage-side clinical signs (ill health, behavioral changes etc.) were recorded at least once daily during all phases of the study, except on detailed clinical examination days, where the cage-side clinical signs were replaced by a DCE.
(268) A detailed clinical examination of each rat was performed on arrival as part of the health status, as well as on Day 1, prior to dosing.
(269) Body Weights
(270) Body weights were recorded for all animals once at arrival as per health status, once prior to group assignment and on Day 1 (prior to dosing).
(271) Pharmacokinetics
(272) A series of 8 blood samples (approximately 0.3 mL each) was collected 30 minutes and 1 hour after exposure began, immediately after exposure ended (IPE), and again at 5, 15, 30, 75 and 105 minutes post-dosing as per the table below. Thus a total blood volume of 1.2 mL was taken from each rat during the course of the study. For this purpose, each rat (unanesthetized) was bled by jugular venipuncture and the samples were collected into tubes containing the anticoagulant, K2EDTA. Tubes were placed on wet ice pending processing.
(273) TABLE-US-00038 Toxicokinetic time point 30 min 1 hour Group Number of post post 5 min 15 min 30 min 75 min 105 min Number animals/sex start start IPE post end post end post end post end post end 1 3 + ✓ ✓ ✓ ✓ 3 # ✓ ✓ ✓ ✓ ✓ 2 3 + ✓ ✓ ✓ ✓ ✓ 3 # ✓ ✓ ✓ ✓ ✓ 3 3 + ✓ ✓ ✓ ✓ 3 # ✓ ✓ ✓ ✓ 4 3 + ✓ ✓ ✓ ✓ 3 # ✓ ✓ ✓ ✓ + animals with the lowest identification numbers # animals with the highest identification numbers
(274) Following collection, the samples were centrifuged (2500 rpm for 10 minutes at approximately 4° C.) and the resulting plasma was recovered and stored frozen (≤−60° C.) in labeled tubes.
(275) Deviations to the pharmacokinetic time points were noted in the raw data and were made available with the samples. The location of blood withdrawal was noted in the raw data.
(276) The plasma analysis was performed and the bioanalytical data was prepared for inclusion in the final report.
(277) The pharmacokinetic parameters were calculated and the non-compartmental analysis of PRINT-Tre and Tre solution treprostinil concentrations in plasma was performed by using the Phoenix WinNonlin 6.3 software.
(278) The following configuration was used for the analysis: Sampling Method: Sparse AUC Calculation Method: Linear Trapezoidal with Linear Interpolation Lambda Z (λ.sub.z) Method: Best fit for λ.sub.z, Log regression Weighting (λ.sub.z calculation): Uniform
(279) Pharmacokinetic parameters (including abbreviation and description for each parameter) were described in the following table:
(280) TABLE-US-00039 Parameters Abbreviation Unit* Area under the plasma drug concentration- AUC.sub.0-Tlast μg*hr/mL time curve from the time of dosing to the last quantifiable concentration Area under the plasma drug concentration- AUC.sub.INF μg*hr/mL time curve from the time of dosing extrapolated to infinity Terminal elimination half-life T.sub.1/2 hr The maximum plasma concentration C.sub.max μg/mL Time to maximum plasma concentration T.sub.max hr
Data Evaluation and Statistics
(281) Numeric and non-numeric data obtained during the study were reported only as individual values.
RESULTS
(282) Formulation Analysis
(283) Formulation concentration for Group 1 was as follows:
(284) TABLE-US-00040 Average Targeted Average Measured Group Concentration Concentration % of Targeted No. (mg/mL) (mg/mL) Concentration 1 0.50 0.492 98.4
(285) The formulation concentration for Group 1 was within 2% of the targeted concentration therefore the formulation concentration was considered acceptable for the study.
(286) Aerosol Concentrations
(287) Achieved gravimetric test atmosphere concentrations were as follows:
(288) TABLE-US-00041 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (mg\L) (mg\L) (%) Target 2 0.156 0.283 55.5 181.4 3 0.781 0.814 16.3 104.2 4 1.563 1.548 21.9 99.0
(289) Achieved chemical test atmosphere concentrations for treprostinil were as follows:
(290) TABLE-US-00042 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (μg\L) (μg\L) (%) Target 1 5 5.07 3.6 101.4 2 1 1.76 55.2 176.3 3 5 4.95 19.8 99.1 4 10 9.73 22.3 97.3
(291) Achieved chemical test atmosphere concentrations for trehalose were as follows:
(292) TABLE-US-00043 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (μg\L) (μg\L) (%) Target 2 130.7 254.84 54.4 195.0 3 653.5 719.53 20.5 110.1 4 1306.9 1394.33 23.2 106.7
(293) The overall achieved aerosol concentrations for all groups were within 10% of the targeted concentrations gravimetrically and for both treprostinil and trehalose, except for Group 2 which were significantly above the targeted concentrations (76% and 95% for treprostinil and trehalose, respectively). The generated atmospheres were considered stable over the treatment period except for Group 2 (CV %˜54%). However, the overall aerosol concentrations were still considered acceptable for the study as there was a significant difference in aerosol concentration between groups.
(294) Achieved Dose Levels
(295) Overall achieved doses for treprostinil are presented below:
(296) TABLE-US-00044 Targeted Duration of Body Estimated % from Group Dose Level Exposure Weight Achieved Doses Targeted Dose No. (mg/kg/day) (min) Sex (kg) (mg/kg/day) Level 1 0.75 240 Male 0.308 0.760 101.4 Female 0.212 0.817 108.9 Combined 0.260 0.785 104.7 2 0.15 240 Male 0.301 0.265 176.7 Female 0.211 0.284 189.1 Combined 0.256 0.273 182.3 3 0.75 240 Male 0.321 0.737 98.2 Female 0.215 0.795 106.0 Combined 0.268 0.762 101.6 4 1.5 240 Male 0.317 1.451 96.7 Female 0.219 1.557 103.8 Combined 0.268 1.498 99.9
(297) Average achieved dose levels for all groups were within 5% of the targeted dose levels, except for Group 2 which was 82% above the targeted dose level. However, the dose levels were considered acceptable for the study as a clear dose differentiation between groups for each sex was achieved.

(298) Particle Size Distribution
(299) The average gravimetric particle size distribution measurement data were as follows:
(300) TABLE-US-00045 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (μm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (μm) GSD μm 2 87.9 80.0 61.0 31.2 21.8 16.2 9.1 0.0 1.7 2.67 80 3 90.7 85.0 66.9 24.9 14.8 9.0 3.0 0.0 1.8 2.12 85 4 91.7 84.0 61.6 23.5 8.1 4.3 0.8 0.0 2.0 1.90 86 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(301) The average chemical determination of particle size distribution for treprostinil were as follows:
(302) TABLE-US-00046 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (μm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (μm) GSD μm 1 95.9 95.7 95.4 95.1 85.1 50.1 22.0 0.0 0.5 2.60 98 2 94.0 86.3 64.4 29.3 19.8 14.1 6.1 0.0 1.6 2.24 87 3 95.3 90.3 71.5 25.9 15.2 9.3 2.7 0.0 1.6 1.97 90 4 94.1 88.4 64.3 23.9 8.2 4.4 1.5 0.0 1.8 1.89 88 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(303) The average chemical determination of particle size distribution for trehalose were as follows:
(304) TABLE-US-00047 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (μm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (μm) GSD μm 2 94.3 86.7 63.8 26.4 16.8 14.1 6.1 0.0 1.6 2.22 87 3 96.0 92.0 72.3 22.2 12.0 8.0 4.0 0.0 1.6 1.97 90 4 95.7 91.4 68.0 27.9 12.8 8.6 4.3 0.0 1.6 2.00 90 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(305) The particle size distribution was considered respirable for this study as the MMADs were below 4 μm and the GSD were within 1.5 and 3.
(306) Exposure Chamber Conditions
(307) Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.
(308) TABLE-US-00048 Oxygen Group Humidity Temperature Concentration No. (% RH) (° C.) (%) 1 58.5 21.0 20.9 2 35.1 21.6 20.9 3 39.0 21.5 20.9 4 39.4 21.2 20.9
(309) Exposure atmosphere oxygen concentrations, temperature and relative humidity ranges were considered acceptable on all occasions.
(310) Mortality
(311) There were no mortalities during the study.
(312) Clinical Signs
(313) There were no clinical signs observed during the study.
(314) Body Weight
(315) Body weights were performed for dose level calculation purposes.
(316) Pharmacokinetics
(317) With administration of PRINT-Tre at an achieved dose level of 0.273 mg/kg, 0.762 mg/kg or 1.498 mg/kg, plasma exposure to treprostinil was generally similar in both sexes; however, exposure was slightly lower in females than males at the mid-dose level and slightly higher in females than males at the high-dose level.
(318) Based on AUC.sub.0-Tlast, AUCINF and C.sub.max, values for both sexes, plasma exposure increased more than proportionally between the low- and mid-dose levels. But between the mid- and high-dose levels, plasma exposure increased less than proportionally for females and there was no increase in the exposure for males. The maximum mean treprostinil plasma concentration (T.sub.max) was at the end of inhalation for both sexes, except for low-dose males and high-dose females, where mean T.sub.max was at 1 and 0.5 hours after inhalation began, respectively.
(319) At the low-dose level, mean treprostinil plasma concentration was similar after 0.5, 1, or 4 hours of inhalation exposure to PRINT-Tre, suggesting that steady state was achieved within the first 30 minutes of exposure. The same was true for females at the high-dose level; however, for males at the high-dose level and for both sexes at the mid-dose level, mean treprostinil plasma concentration was greater at the end of inhalation than after one hour of inhalation. These data are summarized below.
(320) TABLE-US-00049 Males Females Dose (mg/kg) = 0.273 0.762 1.498 0.273 0.762 1.498 0.5 hours of inhalation 6.1 22 26 4.2 18 44 1 hour of inhalation 7.2 21 27 5.1 19 35 4 hours of inhalation 5.4 54* 44{circumflex over ( )} 5.4 33** 44 *Individual values were 33, 63, and 67 ng/mL {circumflex over ( )}Individual values were 34, 48, and 50 ng/mL **Individual values were 22, 27, and 49 ng/mL
(321) When inhalation ended, treprostinil plasma concentrations declined gradually. Given the degree of individual variation, the estimated mean T.sub.1/2 values were similar at all dose levels and ranged from 0.7 to 1.8 hours in males and 0.7 to 1.0 hours in females.
(322) For Tre solution, with an administration at 0.785 mg/kg, plasma exposure to treprostinil was generally similar in both sexes.
(323) The maximum mean treprostinil plasma concentration (T.sub.max) was at the end of inhalation. Mean treprostinil plasma concentration was similar after 0.5, 1, or 4 hours of inhalation exposure to Tre solution, suggesting that steady state was achieved within the first 30 minutes of exposure. These data are summarized below.
(324) TABLE-US-00050 Males Females 0.5 hours of inhalation 17 12 1 hour of inhalation 11 14 4 hours of inhalation 16 16
(325) When inhalation ended, treprostinil plasma concentrations declined gradually, with estimated mean T.sub.1/2 values of 0.6 hours in males and 0.8 hours in females.
(326) Administration of PRINT-Tre and Tre solution at nearly equivalent dose levels (0.76 and 0.79 mg/kg, respectively) resulted in plasma exposures to treprostinil that were greater with PRINT-Tre than with Tre solution. Specifically, mean AUC.sub.0-Tlast was approximately twice as high (126 versus 61 h*ng/mL, respectively) and mean C. was three times as high (44 versus 16 ng/mL, respectively). As would be expected, once treprostinil entered the systemic circulation, it was cleared from plasma at a similar rate, with mean T.sub.1/2 values of 0.7 to 1.0 hours for PRINT-Tre and 0.6 to 0.8 hours for Tre solution.
CONCLUSION
(327) Single inhalation administration for 4 hours of PRINT Treprostinil at a high average dose of 1.498 mg/kg/day to Sprague-Dawley rats was well tolerated as there were no test item related findings. At an equivalent dose level, plasma exposures to treprostinil was greater with PRINT-Tre than with Tre solution; specifically, mean AUC.sub.0-Tlast was approximately twice as great and mean C.sub.max was three times as great. As would be expected, once treprostinil entered the systemic circulation, it was cleared from plasma at a similar rate regardless of how it was administered.
(328) Non-GLP, 7-Day, Repeat-Dose, Range-Finding (DRF), Inhalation Study with LIQ861 in Rats (Study 75654)
(329) Results from the completed comparative PK study will be used to select dose levels to be tested in this DRF study, which will evaluate local toxicity in the respiratory tract as well as systemic treprostinil toxicity. Results will be used to select appropriate dose levels for a 2-week GLP repeat-dose toxicology study in rats.
(330) Summary: Study 75654
(331) The objectives of the study were to evaluate the toxicity of the test item, PRINT Treprostinil, and the excipients that make up the control item, PRINT Placebo, when administered to Sprague-Dawley rats by nose-only inhalation for 4 hours a day for 7 days. Results were used to help select dose levels for a subsequent 14-day GLP inhalation toxicology study.
(332) Groups of 6 rats (3/sex) were exposed by 4-hour inhalation daily for 7 days to air, PRINT Placebo, or PRINT Treprostinil at treprostinil dose levels of approximately 170, 680, or 1370 μg/kg, as described in the table below:
(333) TABLE-US-00051 Mean Dose Levels and Concentrations.sup.a Treprostinil Trehalose Leucine Group Group Dose Level Aerosol Conc. Dose Level Aerosol Conc. Dose Level No. Designation (μg/kg/day) (μg/L) (mg/kg/day) (μg/L) (mg/kg/day).sup.b 1 Air Control 0 0 0 0 0 2 Placebo Control.sup.b 0 0 281.2 1832.13 12.0 3 PRINT-Tre 170 1.10 33.1 216.30 1.3 (Low Dose) 4 PRINT-Tre 680 4.44 133.5 869.99 5.1 (Mid Dose) 5 PRINT-Tre 1370 8.94 266.6 1735.84 10.3 (High Dose) .sup.a= Based on the mean body weight of each group during the dosing period. .sup.b= Calculated with a content of 4% of Leucine in PRINT Treprostinil and PRINT Placebo and using Trehalose percentage of 93.5% in PRINT Placebo

for Group 2 and Treprostinil percentage of 0.53% in PRINT Treprostinil for Groups 3 to 5.
(334) The particle size MMADs from Groups 2 to 5 were between 1.3 and 2.0 µm gravimetrically (GSD 1.96 to 2.46); for both treprostinil and trehalose, chemical particle size distribution ranged from 1.3 to 2.1 µm with the corresponding GSDs between 1.87 and 1.95. No mortality occurred. No clinical signs were observed while coagulation, clinical chemistry and urinalysis parameters were unaffected and no test item-related findings were seen macroscopically.
(335) Rats tolerated daily administration of PRINT Placebo or PRINT-Tre at up to 1.37 mg/kg/day by 4-hour inhalation for 7 days.
INTRODUCTION
(336) The objectives of the study were to: 1. Evaluate the toxicity of the test item, PRINT Treprostinil, when administered to Sprague-Dawley rats by nose-only inhalation for 4 hours a day for 7 days. 2. Evaluate the toxicity of the excipients that make up the control item, PRINT Placebo, when administered to Sprague-Dawley rats by nose-only inhalation for 4 hours a day for 7 days. 3. Determine the dose levels of PRINT Treprostinil for the following 14-day GLP inhalation toxicology study from the results of this dose range-finding study.
Experimental Design
(337) Synopsis
(338) The test and control items were administered to groups of 6 rats (3/sex) by 4-hour inhalation daily for 7 days, as described in the table below. The first day of dosing was designated as Day 1.
(339) TABLE-US-00052 Targeted Aerosol Targeted Dose Level Leucine Group Concentration (µg/L) (mg/kg/day) Dose Level No. Test Material Treprostinil Trehalose Treprostinil.sup.a Trehalose.sup.b (mg/kg/day).sup.c 1 Air Control 0 0 0 0 0 2 PRINT Placebo.sup.b 0 1684.6 0 262.9 11.2 3 PRINT-Tre 1 175.2 0.15 26.3 1.1 (Low Dose) 4 PRINT-Tre 5 876.2 0.75 131.4 5.7 (Mid Dose) 5 PRINT-Tre 10 1752.5 1.5 262.9 11.3 (High Dose) .sup.a= Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg .sup.b= The target dose level for the placebo control was the same dose level as the high dose group (Group 5) .sup.c= Calculated with a content of 4% of Leucine in PRINT Treprostinil and PRINT Placebo (using Trehalose percentage of 93.5% in PRINT Placebo for Group 2 and Treprostinil percentage of 0.53% in PRINT Treprostinil for Groups 3 to 5)
(340) The high-dose level for PRINT-Tre was the feasible dose attainable based on technical aerosol trials with the test item. (Study No. 41610).
(341) The low- and mid-dose levels for PRINT-Tre were selected on the basis of a previous PK study in rats (Study No. 75658).
(342) Test and Control Item Information
(343) Test Item Action
(344) Treprostinil, the active ingredient in PRINT-Tre, is a prostacyclin compound approved for treatment of pulmonary arterial hypertension.
(345) Characterization of Test Item
(346) Content: 92.97% of Trehalose, 4% of Leucine, 2% of Tween80, 0.27% of Sodium Citrate Dihydrate, 0.23% of Sodium Chloride: 0.53% of Treprostinil sodium Storage Conditions: Cool (2 to 8° C.), protected from moisture (e.g., desiccant) Handling Precautions: Standard laboratory precautions. Handled under dry conditions (relative humidity ≤23%) Supplier: Liquidia Technologies Inc.
Characterization of Placebo Control Item Content: -LKI-1R-983-3: 93.53% of Trehalose, 4% of Leucine, 2% of Tween80, 0.24% of Sodium Citrate Dihydrate, 0.23% of Sodium Chloride LKI-1R-983-27: 93.5% of Trehalose, 4% of Leucine, 2% of Tween80, 0.27% of Sodium Citrate Dihydrate, 0.23% of Sodium Chloride Storage Conditions: Cool (2 to 8° C.), protected from moisture (e.g., desiccant) Handling Precautions: Standard laboratory precautions. Handled under dry conditions (relative humidity ≤23%) Supplier: Liquidia Technologies Inc.
Characterization of Air Control Description: Medical Grade Air (NQ 5710-500/2000) Supplied By: Kaeser SM-11 Air Compressor
Preparation of Test and Control Items
(347) PRINT-Tre and PRINT Placebo were used as provided by the Sponsor. A glove box under nitrogen was used for handling, aliquoting or packing of the canisters. Relative humidity (RH) inside the glove box was monitored and recorded using a hygrometer and was kept below 23% RH.
(348) Treatment
(349) Acclimatization to Exposure System
(350) Before the rats were presented to exposure atmosphere, rats were accustomed to the restraint procedure over a period of 3 days. The animals were gradually accustomed to restraint in the dosing tubes used during the exposures up to the duration that was used for aerosol administrations.
(351) Animal Exposure
(352) Exposure system used: Flow-past rodent inhalation exposure system
(353) Exposure method: Inhalation by nose-only exposure
(354) Test and Control Item type: Air (Group 1), Dry Powder (Groups 2 to 5)
(355) Generation method: Piston feed/rotating brush generator (Groups 2 to 5)
(356) Duration of exposure: 240 minutes
(357) The target aerosol concentrations and dose levels were as follows:
(358) TABLE-US-00053 Targeted Targeted Targeted Targeted Estimated Total Inhaled Aerosol Total Inhaled Aerosol Total Inhaled Dose Level of Concentration Dose Level of Concentration Dose Level of Group Group Treprostinil of Treprostinil Trehalose of Trehalose Leucine No. Designation (mg/kg/day).sup.a (µg/L) (mg/kg/day).sup.a (µg/L) (mg/kg/day).sup.c 1 Air Control 0 0 0 0 0 2 Placebo Control.sup.b 0 0 262.9 1684.6 11.2 3 PRINT-Tre 0.15 1 26.3 175.2 1.1 (Low Dose) 4 PRINT-Tre 0.75 5 131.4 876.2 5.7 (Mid Dose) 5 PRINT-Tre 1.5 10 262.9 1752.5 11.3 (High Dose) .sup.a= Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg .sup.b= The target dose level for the placebo control was the dose level as the high dose group (Group 5) .sup.c= Calculated with a content of 4% of Leucine in PRINT Treprostinil and PRINT Placebo (using Trehalose percentage of 93.5% in PRINT Placebo for Group 2 and Treprostinil percentage of 0.53% in PRINT Treprostinil for Groups 3 to 5)
(359) Estimation of Achieved Dose Levels The target dose levels were estimated using the following formula:
(360) $D_T = \frac{E_c \times RMV \times T}{BW}$ D.sub.L=Achieved dose levels (mg/kg/day)= E.sub.c=Actual concentration delivered to the animals (mg/L air) RMV=Respiratory Minute Volume (L/min) according to the method of Bide, Armour and Yee. J. App. Toxicol., Vol. 20, 2000: kW (L/min)=0.499×BW (kg).sup.0.809 T=Time, duration of daily exposure (min.) BW=Mean body weight (kg) during exposure period. This estimation of total inhaled dose assumed 100% deposition within the respiratory tract.
Inhalation Exposure System
(361) The powder aerosol for Groups 2 to 5 was produced using a piston feed/rotating brush generator. The aerosol produced was diluted as necessary to achieve the target aerosol concentration and discharged through a 40-mm diameter tube into a flow-past inhalation exposure system. The airflow rate through the exposure system was monitored and recorded manually during each aerosol generation period. Airflow to the exposure system was controlled by the absolute volume of air supplying the generation apparatus using variable area flowmeters. Control of the aerosol exhaust flow from the animal exposure system was achieved using an exhaust valve, and the overall balance of airflows in the exposure system was monitored using pressure gauges. The system provided a minimum of 1.0 L/min to each animal exposure port and was balanced to ensure a slight positive pressure at the site of the animal exposure. This ensured that there was no dilution of the generated aerosol. An equal delivery of aerosol to each exposure position was achieved by employing a distribution network that was identical for each individual exposure position attached to the system.
(362) Inhalation System Monitoring
(363) Determinations of aerosol concentration, particle size distribution, oxygen concentration, relative humidity and temperature were measured on

samples collected from a representative port of the exposure chamber, with a collection sample flow-rate of 1 L/min. The sample flow rates were precisely controlled using variable area flow meters that were calibrated before use using a primary airflow calibrator. The absolute volume of each aerosol concentration sample was measured with a wet type gas meter.
(364) Oxygen Concentration
(365) The oxygen concentration of the generated atmosphere was measured once during each aerosol exposure. Oxygen concentrations of the exposure atmospheres were maintained between 19-23%.
(366) Relative Humidity/Temperature
(367) The temperature and relative humidity of the generated atmosphere were measured once during each aerosol exposure. Temperatures of the exposure atmospheres were maintained between 19-24° C.
(368) Determination of Aerosol Concentration
(369) At least one aerosol concentration filter sample was collected for all groups on each aerosol generation. The filter samples from Groups 3 to 5 were weighed in order to measure the gravimetric concentration of the test item in the generated aerosol. The filter samples were transferred to the analytical chemistry laboratory for chemical determination of Treprostinil and Trehalose concentrations. The filter samples from Group 2 were weighed in order to measure the gravimetric concentration of the control item in the generated aerosol. The filter samples were transferred to the analytical chemistry laboratory for chemical determination of Trehalose concentration and to confirm the absence of Treprostinil. The filter samples for Group 1 were not weighed gravimetrically and were only transferred to the analytical laboratory to confirm the absence of Treprostinil and Trehalose. The analysis in the analytical laboratory was performed using an analytical method (Study No. 41609 and 41635).
(370) Determination of Aerosol Homogeneity
(371) At least once during the study, atmosphere homogeneity in the exposure system was tested by collecting multiple aerosol samples from the top, middle and bottom tiers of the exposure system of Groups 2 to 5.
(372) Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)
(373) The distribution of particle size in the generated aerosols was measured at least once for Groups 2 to 5 by collecting samples into a 7-Stage Mercer Cascade Impactor. All sample substrates obtained from Groups 3 to 5 were weighed gravimetrically and then transferred to the analytical chemistry laboratory for chemical determination of particle size of aerosolized Treprostinil and Trehalose. All sample substrates obtained from Group 2 were weighed gravimetrically and then transferred to the analytical laboratory for determination of particle size of aerosolized Trehalose. The analysis in the analytical laboratory was performed using an analytical method (Study No. 41609 and 41635).
(374) The MMAD and the Geometric Standard Deviation (GSD) were calculated based on the results obtained from the impactor using a log-probit transformation.
(375) Reporting of Analytical Results
(376) The analytical report containing the results from the filter and particle size distribution sample analyses were prepared. Any samples not employed in the primary analysis or any remaining sample from the primary analysis were retained until it was determined by the analyst and Study Director that it was not required for confirmatory analysis. These samples were then discarded and their disposition was recorded in the raw data.
(377) Standard Operating Procedures
(378) All procedures, were performed in accordance with the Standard Operating Procedures and these were kept on file. Deviations to the Standard Operating Procedures were documented in the raw data.
RESULTS
(379) Inhalation System Monitoring
(380) Oxygen Concentration, Temperature, and Relative Humidity
(381) Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.
(382) TABLE-US-00054 Oxygen Humidity Temperature Concentration Group (% RH) (° C.) (%) Number Average Average Average 1 28.6 20.4 20.9 2 39.6 21.2 20.9 3 35.6 21.6 20.9 4 26.2 21.1 20.9 5 31.1 20.8 20.9
(383) Exposure atmosphere oxygen concentration, temperature and relative humidity were considered acceptable throughout the study.
(384) Aerosol Concentrations
(385) Achieved gravimetric test atmosphere concentrations were as follows:
(386) TABLE-US-00055 Achieved Targeted Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (mg\L) (mg\L) (%) Target 2 2.000* 1.966 34.7 98.3 3 0.200* 0.231 21.4 115.5 4 1.000* 0.928 20.5 92.8 5 2.000* 1.848 26.0 92.4 *Target aerosol concentrations were 0.140 mg/L for Group 3, 0.700 mg/L for Group 4 and 1.400 mg/L for Groups 2 and 5 for the first 2 days of exposure.
(387) Achieved test atmosphere concentrations for treprostinil were as follows:
(388) TABLE-US-00056 Targeted Achieved Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (µg\L) (µg\L) (%) Target 3 1.0 1.10 22.1 110.0 4 5.0 4.44 21.0 88.8 5 10.0 8.94 28.1 89.4
(389) Achieved test atmosphere concentrations for trehalose were as follows:
(390) TABLE-US-00057 Targeted Achieved Aerosol Mean Aerosol Coefficient Group Concentration Concentration of Variation % of No. (µg\L) (µg\L) (%) Target 2 1684.6 1832.13 36.4 108.8 3 175.2 216.30 22.1 123.5 4 876.2 869.99 21.2 99.3 5 1752.5 1735.84 29.4 99.0
(391) The overall achieved aerosol concentrations for all groups were within 20% of the targeted concentrations gravimetrically and for both treprostinil and trehalose, except for Group 3 for trehalose which was 23.5% greater than the targeted concentration. The generated atmospheres were considered stable over the treatment period even if all % CV were all above 20% as this was due the wrong targeted gravimetric concentrations being applied for the first 2 days of dosing. The overall aerosol concentrations were still considered acceptable for the study as there was a significant difference in aerosol concentration between groups.
(392) Aerosol Homogeneity
(393) Achieved gravimetric test atmosphere homogeneity concentrations were as follows:
(394) TABLE-US-00058 Aerosol Aerosol Aerosol Concentration Concentration Concentration Group of Top Tier of Middle Tier of Bottom Tier CV No. (mg/L) (mg/L) (mg/L) (%) 2 1.061 1.029 1.078 2.4 3 0.134 0.136 0.126 4.0 4 0.810 0.877 0.845 4.0 5 1.225 1.263 1.280 2.2
(395) Achieved test atmosphere homogeneity concentrations for treprostinil were as follows:
(396) TABLE-US-00059 Aerosol Aerosol Aerosol Concentration Concentration Concentration Group of Top Tier of Middle Tier of Bottom Tier CV No. (µg/L) (µg/L) (µg/L) (%) 3 0.63 0.64 0.59 4.3 4 3.90 4.16 4.04 3.2 5 5.73 6.03 6.12 3.4
(397) Achieved test atmosphere homogeneity concentrations for trehalose were as follows:
(398) TABLE-US-00060 Aerosol Aerosol Aerosol Concentration Concentration Concentration Group of Top Tier of Middle Tier of Bottom Tier CV No. (µg/L) (µg/L) (µg/L) (%) 2 916.21 888.61 919.90 1.9 3 113.82 111.38 102.88 5.3 4 774.67 887.67 780.97 7.8 5 1091.32 1153.45 1160.14 3.3
(399) Chamber homogeneity of the aerosol concentrations were considered acceptable since the coefficient of variance of aerosol concentration between samples was not greater than 20%.
(400) Particle Size Distribution
(401) The average gravimetric particle size distribution measurement data were as follows:
(402) TABLE-US-00061 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (µm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (µm) GSD µm 2 89.1 81.8 51.3 19.3 11.6 9.4 7.9 0.0 2.0 2.46 78 3 97.9 95.0 79.6 35.5 21.1 15.1 5.7 0.0 1.3 1.96 94 4 95.5 89.4 60.3 24.1 13.4 9.0 4.8 0.0 1.7 2.07 88 5 96.9 92.3 63.1 28.2 15.7 8.5 4.8 0.0 1.6 1.99 91 MMAD = Mass median aerodynamic diameter GSD =

Geometric standard deviation.
(403) The chemical determinations of particle size distribution for treprostinil were as follows:
(404) TABLE-US-00062 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (μm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (μm) GSD μm 3 98.8 95.7 80.3 35.2 20.2 14.2 5.0 0.0 1.3 1.88 96 4 96.3 90.1 59.9 22.2 11.1 6.9 2.9 0.0 1.7 1.95 89 5 97.2 92.8 63.3 26.7 13.6 6.4 2.8 0.0 1.6 1.89 92 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(405) The chemical determinations of particle size distribution for trehalose were as follows:
(406) TABLE-US-00063 Cumulative % Less Than Stated Effective Mean % Group Cut-Off Diameter (μm) MMAD below 4 No. 4.60 3.00 2.10 1.60 1.10 0.70 0.33 0.00 (μm) GSD μm 2 89.3 85.2 52.1 17.7 8.1 4.0 0.0 0.0 2.1 1.74 87 3 96.6 93.3 77.4 31.0 15.4 12.0 3.4 0.0 1.5 1.94 93 4 97.3 90.9 59.3 19.9 8.0 5.3 2.7 0.0 1.8 1.88 90 5 97.2 94.4 62.6 25.0 12.1 5.6 2.8 0.0 1.6 1.87 92 MMAD = Mass median aerodynamic diameter GSD = Geometric standard deviation.
(407) The particle size distribution was considered respirable for this study as all MMADs were below 4 μm and the GSDs were within 1.5 and 3.
(408) Estimation of Achieved Dose Levels
(409) Overall achieved doses for treprostinil are presented below:
(410) TABLE-US-00064 Targeted Dose Duration of Body Estimated % of Group Level Exposure Weight Achieved Doses Targeted No. (mg/kg/day) (min) Sex (kg) (mg/kg/day) Dose Level 3 0.15 240 Male 0.324 0.16 106.7 Female 0.227 0.17 113.3 Combined 0.276 0.17 113.3 4 0.75 240 Male 0.316 0.66 88.0 Female 0.229 0.70 93.3 Combined 0.273 0.68 90.7 5 1.5 240 Male 0.315 1.33 88.7 Female 0.228 1.42 94.7 Combined 0.272 1.37 91.3
(411) Overall achieved doses for trehalose are presented below:
(412) TABLE-US-00065 Targeted Dose Duration of Body Estimated % of Group Level Exposure Weight Achieved Doses Targeted No. (mg/kg/day) (min) Sex (kg) (mg/kg/day) Dose Level 2 262.9 240 Male 0.316 273.4 104.0 Female 0.229 290.8 110.6 Combined 0.273 281.2 107.0 3 26.3 240 Male 0.324 32.1 122.1 Female 0.227 34.4 130.8 Combined 0.276 33.1 125.9 4 131.4 240 Male 0.316 129.8 98.8 Female 0.229 138.1 105.1 Combined 0.273 133.5 101.6 5 262.9 240 Male 0.315 259.2 98.6 Female 0.228 275.7 104.9 Combined 0.272 266.6 101.4
(413) Overall achieved doses for leucine are presented below:
(414) TABLE-US-00066 Targeted Dose Duration of Body Estimated % of Group Level Exposure Weight Achieved Doses.sup.a Targeted No. (mg/kg/day) (min) Sex (kg) (mg/kg/day) Dose Level 2 11.2 240 Male 0.316 11.7 104.5 Female 0.229 12.4 110.7 Combined 0.273 12.0 107.1 3 1.1 240 Male 0.324 1.2 109.1 Female 0.227 1.3 118.2 Combined 0.276 1.3 118.2 4 5.7 240 Male 0.316 5.0 87.7 Female 0.229 5.3 93.0 Combined 0.273 5.1 89.5 5 11.3 240 Male 0.315 10.0 88.5 Female 0.228 10.7 94.7 Combined 0.272 10.3 91.2 .sup.a= Calculated with a content of 4% of Leucine in PRINT-Tre and PRINT Placebo (using Trehalose percentage of 93.5% in PRINT Placebo for Group 2 and Treprostinil percentage of 0.53% in PRINT-Tre for Groups 3 to 5)
(415) Average achieved dose levels for all groups were within 20% of the targeted dose levels, except for Group 3 for trehalose which was 26% above the targeted dose level; however, the dose levels were considered acceptable for the study as a clear dose differentiation between groups for each sex was achieved.
(416) Mortality
(417) There were no mortalities during the study.
(418) Clinical Signs
(419) There were no clinical signs observed during the study.
(420) Body Weight
(421) The only differences in body weight or weight gain potentially related to administration of the test or control item were slightly less growth (weight gain) in males given PRINT-Tre at 0.68 mg/kg/day and in both sexes given to PRINT-Tre at 1.37 mg/kg/day, relative to the air control group. The pattern of differences implicates the active ingredient treprostinil, not one of the excipients in PRINT-Tre.
(422) These data are summarized in the table below, with differences potentially related to treprostinil in bold.
(423) TABLE-US-00067 Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Males Starting weight (Day 1) (g) 301 309 315 312 312 After 4 doses (Day 5) (g) 324 326 337 324 322 Change Absolute (g) +23 +17 +22 +12 +10 Relative to Air Control — −6 −1 **−11 −13** After 7 doses (fasted Day 8) (g) 300 302 310 301 298 Change Absolute (g)* −1 −7 −5 −10 −14 Relative to Air Control — −6 −4 **−9 −13** Females Starting weight (Day 1) (g) 220 223 220 227 226 After 4 doses (Day 5) (g) 231 235 232 237 231 Change Absolute (g) +11 +12 +12 +10 +5 Relative to Air Control — +1 +1 −1 **−6** After 7 doses (fasted Day 8) (g) 205 209 204 213 211 Change Absolute (g)* −15 −14 −16 −14 −15 Relative to Air Control — +1 −1 −1 0 *All animals were fasted overnight prior to necropsy.
(424) Remaining differences were considered incidental and of no biological significance.
(425) Hematology
(426) The only differences in mean hematology parameters potentially related to administration of the test or control item were greater mean reticulocyte counts in all groups given PRINT-Tre, relative to the air control group. The magnitude of difference was dose-related but statistically significant only in males. The pattern of differences implicates the active ingredient treprostinil, not one of the excipients in PRINT-Tre.
(427) These data are summarized in the table below, with differences potentially related to treprostinil in bold.
(428) TABLE-US-00068 Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Reticulocyte count Males Mean (×10.sup.12/L) 0.226 0.228 0.309 0.333 0.337 Relative to Air Control — +1% **+37**% **+47**% **+49**% Females Mean (×10.sup.12/L) 0.179 0.176 0.214 0.290 0.283 Relative to Air Control — +1% **+20**% **+62**% **+58**%
(429) An increase in reticulocyte count is an appropriate response to an increased demand for RBCs. In this study, greater reticulocyte counts were not associated with differences in circulating erythron mass (i.e., no differences in RBC count, haemoglobin concentration, or haematocrit). This suggests that the increased release of reticulocytes was accompanied by, and probably a response to, an increased rate of RBC loss, and that the erythropoietic response was adequate to maintain normal circulating RBC numbers.
(430) Remaining differences among mean hematology parameters were considered incidental and of no biological significance.
(431) Coagulation
(432) There were no differences in mean coagulation parameters that were considered to be related to administration of the test or control item. All differences were considered incidental and of no biological significance.
(433) Clinical Chemistry
(434) There were no differences in mean clinical chemistry parameters that were considered to be related to administration of the test or control item. All differences were considered incidental and of no biological significance.
(435) Urinalysis
(436) There were no differences in the urinalysis parameters that were considered to be related to administration of the test or control item. All differences were considered incidental and of no biological significance.
(437) Organ Weights
(438) Differences in mean organ weight potentially related to administration of the test or control item were noted for lungs, adrenal glands, thymus, and testes.
(439) Remaining differences in mean organ weight were considered incidental and of no biological significance.

(440) Lungs
(441) Mean lungs/trachea weights (absolute and relative to body weight) were greater in all groups given the test or control item, compared to the air control group. The differences were greater with PRINT-Tre than with PRINT Placebo, and the differences were dose-related for PRINT-Tre. This pattern suggests that administration of the excipients (likely trehalose) resulted in a slight (15% to 17%) increase in lung weight, which was exacerbated by co-administration of treprostinil as the lung weights of PRINT-Tre groups were increased compared to the lung weights of the PRINT Placebo group.
(442) There was a histopathologic finding in the lungs that might have accounted for the greater lung weight; specifically, increased alveolar macrophages with basophilic vacuolated cytoplasm in the lungs of all rats given PRINT Placebo or PRINT-Tre at ≥0.68 mg/kg/day. However, neither the distribution of this histopathologic finding across groups nor the grade of the finding correlated well with the differences in mean lung weight, suggesting that some other factor was responsible. Because lungs were weighed before fixation, it is possible that some material responsible for the greater weight was removed during tissue fixation and processing.
(443) Lung weight data are summarized in the table below, with differences potentially related to PRINT Placebo and PRINT-Tre in bold.
(444) TABLE-US-00069 Mean Lung Weight Data Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Males Absolute weight (g) 1.39 1.60 1.70 1.78* 1.90* Relative to Air Control — **+15**% **+22**% **+28**% **+37**% Relative weight (% body weight) 0.46 0.53 0.55 0.59* 0.64* Relative to Air Control — **+15**% **+20**% **+28**% **+39**% Females Absolute weight (g) 1.09 1.27 1.31* 1.48* 1.44* Relative to Air Control — **+17**% **+20**% **+36**% **+32**% Relative weight (% body weight) 0.53 0.61 0.64 0.70* 0.68* Relative to Air Control — **+15**% **+21**% **+32**% **+28**% *Statistically significant compared to air control; Dunnett's 2-sided, $p < 0.05$
Thymus
(445) Mean thymus weights (absolute and relative to body weight) were slightly lower in both sexes given PRINT-Tre at 0.68 mg/kg/day and in both sexes given to PRINT-Tre at 1.37 mg/kg/day, relative to the air control group (though not statistically significantly different). The pattern of differences implicates the active ingredient treprostinil, not one of the excipients in PRINT-Tre as differences were also seen between PRINT-Tre groups and PRINT Placebo group. Lower thymus weight was not associated with lower lymphocyte count or with any histopathologic findings.
(446) Lower thymus weight is one common manifestation of nonspecific physiological or psychological stress (Everds et al., 2013). Because this finding was associated with reduced weight gain (growth) and sometimes also with greater adrenal glands weight, it was most likely secondary to stress and not a direct effect of treprostinil.
(447) Thymus weight data are summarized in the table below, with differences potentially related to treprostinil in bold.
(448) TABLE-US-00070 Mean Thymus Weight Data Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Males Absolute weight (mg) 504 593 537 388 429 Relative to Air Control — +18% +7% **−23**% **−15**% Relative weight (% body 0.168 0.198 0.173 0.128 0.143 weight) Relative to Air Control — +18% +3% **−24**% **−15**% Females Absolute weight (mg) 469 437 465 428 352 Relative to Air Control — −7% −1% −9% **−25**% Relative weight (% body 0.229 0.210 0.228 0.202 0.166 weight) Relative to Air Control — −8% ±0% −12% **−28**%
Adrenal Glands
(449) Mean adrenal glands weight (absolute and relative to body weight) was greater in males given PRINT-Tre at 0.17 mg/kg/day and in both sexes given to PRINT-Tre at 1.37 mg/kg/day, relative to the air control group (though not statistically significantly different). While the differences may have been due to chance and a consequence of the small group sizes (3/sex), the pattern of differences raises the possibility that they are related to administration of treprostinil, at least at the high-dose level as differences were also seen between the high dose PRINT-Tre group and the PRINT Placebo group. Greater adrenal glands weight was not associated with any histopathologic findings.
(450) Greater adrenal glands weight is one common manifestation of nonspecific physiological or psychological stress (Everds et al., 2013). Because this finding was associated with reduced weight gain (growth) and lower thymus weight at the high-dose level, it was most likely secondary to stress and not a direct effect of treprostinil.
(451) Adrenal glands weight data are summarized in the table below, with differences potentially related to treprostinil in bold.
(452) TABLE-US-00071 Mean Adrenal Glands Weight Data Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Males Absolute weight (mg) 62 71 76 68 78 Relative to Air Control — +15% **+23**% +10% **+26**% Relative weight (% body 0.021 0.023 0.025 0.023 0.027 weight) Relative to Air Control — +10% **+19**% +10% **+29**% Females Absolute weight (mg) 74 73 70 76 89 Relative to Air Control — −1% −5% +3% **+20**% Relative weight (% body 0.036 0.035 0.035 0.036 0.042 weight) Relative to Air Control — −3% −3% ±0% **+17**%
Testes
(453) There was a trend toward slightly lower mean testes weight (absolute and relative to body weight) in groups given PRINT-Tre at ≥0.68 mg/kg/day, relative to the air control group. While the differences may have been due to chance and a consequence of the small group sizes (3/sex), the pattern of differences raises the possibility that they are related to administration of treprostinil as differences were also seen between the mid and high dose PRINT-Tre groups and the PRINT Placebo group. Slightly lower testes weight was not associated with any histopathologic findings.
(454) Testes weight data are summarized in the table below, with differences potentially related to treprostinil in bold.
(455) TABLE-US-00072 Mean Testes Weight Data Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Absolute weight (g) 3.51 3.40 3.39 3.25 3.15 Relative to Air Control — −3% −3% **−7**% **−10**% Relative weight (% body 1.17 1.13 1.10 1.08 1.06 weight) Relative to Air Control — −3% −6% **−8**% **−9**%
Macroscopic Findings
(456) There was no evidence of test item-related macroscopic findings at necropsy.
(457) All findings were considered to be incidental as they were not dose-related, of low incidence, or occurred in the air control, placebo control and treated animals.
(458) Microscopic Findings
(459) Treatment-related findings were observed in the lungs, anterior nasal cavity, and nasopharynx. All other microscopic findings were considered to be incidental or procedure-related.
(460) Lungs
(461) In the lungs, minimal to mild increased alveolar macrophages with basophilic vacuolated cytoplasm were observed in all rats given PRINT Placebo or PRINT-Tre at ≥0.68 mg/kg/day. The pattern of this finding across groups indicates that it is a response to the excipients (likely trehalose). There were no associated inflammatory changes in the lungs. Increased alveolar macrophages are a common finding in inhalation toxicity studies with powders. It reflects normal pulmonary clearance of inhaled particles and is not considered to be adverse.
(462) These data are summarized in the table below, with differences potentially related to test or control item in bold.
(463) TABLE-US-00073 Incidence and Grade of Increased Alveolar Macrophages Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Males Incidence 0/3 **3/3** 0/3 **3/3** **3/3** Mean grade — **1.7** — **1.0** **1.7** Females Incidence 0/3 **3/3** 0/3 **3/3** **3/3** Mean grade — **2.0** — **1.0** **1.7**
Nasal Cavity and Nasopharynx
(464) Goblet-cell hypertrophy/hyperplasia was seen in the cranial portion of the nasal cavity and in the nasopharynx of at least one rat in all groups given PRINT Placebo or PRINT-Tre, but the incidence was greater in groups given PRINT-Tre at ≥0.68 mg/kg/day, and the mean grade was greater in the group given PRINT-Tre at 1.37 mg/kg/day. This pattern suggests that administration of the excipients (likely trehalose) resulted in occasional

goblet-cell changes, which were exacerbated by co-administration of treprostinil at higher dose levels.
(465) Goblet cell hypertrophy/hyperplasia in the anterior nasal cavity and nasopharynx is one of the most frequently observed lesions in rodents exposed to irritant compounds. This finding generally is considered a nonspecific protective or adaptive response and not adverse.
(466) These data are summarized in the table below, with differences potentially related to test or control item in bold.
(467) TABLE-US-00074 Incidence and Grade of Goblet-cell Hypertrophy/Hyperplasia Test Material = PRINT Air Placebo PRINT-Tre Treprostinial Dose Level (mg/kg/day) = 0 0 0.17 0.68 1.37 Trehalose Dose Level (mg/kg/day) = 0 281 33 134 267 Nasal Cavity Males Incidence 0/3 **1/3 1/3 3/3 3/3** Mean grade — 1.0 1.0 1.0 **1.3** Females Incidence 0/3 0/3 0/3 **1/3 2/3** Mean grade — — — 1.0 **1.5** Nasopharynx Males Incidence (all graded minimal) 0/3 0/3 0/3 **3/3 3/3** Females Incidence(all graded minimal) 0/3 **1/3** 0/3 **3/3 3/3**
Discussion and Conclusions
(468) Rats tolerated daily administration of PRINT Placebo or PRINT-Tre at up to 1.37 mg/kg/day by 4-hour inhalation for 7 days.
(469) The only findings potentially related to administration of excipients (likely trehalose) were: Increased alveolar macrophages with basophilic vacuolated cytoplasm in all rats given PRINT Placebo or PRINT-Tre at ≥0.68 mg/kg/day; i.e., in rats given trehalose at ≥134 mg/kg/day. The mean grade of this finding increased with trehalose dose level. This finding was not associated with inflammatory changes in the lungs and was considered to reflect normal pulmonary clearance of inhaled particles. It was not considered to be adverse. Greater mean lung weight in groups given PRINT Placebo or PRINT-Tre. The weight differences were unrelated to trehalose dose level. Instead, they were greater with PRINT-Tre than with PRINT Placebo and were dose-related for PRINT-Tre. This pattern suggests that administration of the excipients (likely trehalose) resulted in a slight (15% to 17%) increase in lung weight, which was exacerbated by co-administration of treprostinil. Of note, the pattern of differences in lung weight across groups is distinct from the pattern of increased alveolar macrophages across groups, indicating that the weight differences were not a consequence of increased macrophages. There were no histopathologic findings in the lungs that might have accounted for the greater lung weight. Because lungs were weighed before fixation, it is possible that some material responsible for the greater weight was removed during tissue fixation and processing. Minimal goblet-cell hypertrophy/hyperplasia in the cranial portion of the nasal cavity of at least one rat in all groups given PRINT Placebo or PRINT-Tre. The incidence of this finding was unrelated to trehalose dose level. Instead, the incidence was greater with PRINT-Tre at ≥0.68 mg/kg/day, and the mean grade was greater with PRINT-Tre at 1.37 mg/kg/day. This pattern suggests that administration of the excipients (likely trehalose) resulted in occasional goblet-cell changes, which were exacerbated by co-administration of treprostinil at higher dose levels. Goblet-cell hypertrophy/hyperplasia was considered a nonspecific protective or adaptive response and not adverse.
(470) Besides exacerbating lung weight differences and goblet-cell hypertrophy/hyperplasia in the nasal cavity and nasopharynx, the following other findings were potentially related to administration of treprostinil as PRINT-Tre: Slightly less growth (weight gain) in males at 0.68 mg/kg/day and in both sexes at 1.37 mg/kg/day. Greater mean reticulocyte counts at all dose levels, with the magnitude of difference increasing with dose level. This was not considered adverse in and of itself; however, it likely reflected an appropriate adaptive response to an increased rate of RBC loss or turnover. Greater mean adrenal glands weight in males at 0.17 mg/kg/day, lower mean thymus weight in both sexes at 0.68 mg/kg/day, and greater mean adrenal glands weight and lower mean thymus weight in both sexes at 1.37 mg/kg/day. There were no associated differences in lymphocyte count or histopathologic findings in either organ. These organ weight differences most likely reflected stress and were not a direct effect of treprostinil.
(471) Based on these results, it is recommended that an upcoming 14-day GLP inhalation toxicology study in rats target similar dose levels as used in the current study.
(472) Clinical Study: LIQ861
(473) Randomized, Placebo-controlled, Single-ascending Dose Study Evaluating Pharmacokinetics (PK) and Safety in Healthy Male and Female Volunteers
(474) A clinical study was conducted to (1) determine the single-dose safety and tolerability and (2) evaluate the single-dose pharmacokinetics of particles of the invention upon administration to healthy male and female subjects.
(475) Six cohorts were evaluated: dose levels of 25, 50, 75, 100, 125 and 150 μg of treprostinil respectively. In each cohort, eight subjects were randomly assigned in a 3:1 blinded ratio and received a single dose of either particles of the invention (N=6) or placebo particles (N=2).
(476) Blood was collected for PK evaluation at T=0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes and 3, 4, 6, and 8 hours post-dose.
(477) Cohort 1
(478) Eight subjects were enrolled and dosed in Cohort 1. Six subjects received active treatment and 2 received placebo. Active treatment was administered by dry powder inhalation (DPI) as a single capsule of 25 μg treprostinil strength, and placebo treatments were administered by DPI as a single capsule of the placebo formulation. All inhalations were administered using the RS00 inhaler.
(479) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.
(480) The table shown in FIG. **3**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 1. Preliminary non-compartmental PK parameters for treprostinil are summarized in the table shown in FIG. **3**B. The highest concentrations for three of the six subjects occurred at 0.33 hours post-inhalation; one subject each had a T.sub.max of 0.167, 0.25, and 0.417 hours post-dose. Concentrations subsequently decayed with a single-phase disposition profile, as shown in the log-linear plots. At two hours after inhalation, two of six active subjects had measurable concentrations of treprostinil and only one subject had measurable concentrations at 2.5 and 3 hours after inhalation. No subjects had quantifiable concentrations after the 3 hour timepoint.
(481) The C.sub.max averaged 0.364 ng/mL and the most frequent T.sub.max was 0.33 hours after inhalation. AUCinf values averaged 0.301 h*ng/mL with a CV % of 30.2%. The apparent volume of distribution (Vz/F) averaged 68.1 L. Oral clearance (CL/F) averaged 91.0 L/h and ranged from 59.1 to 150. Variability in the CL/F value had a CV % of 35.8%.
(482) Cohort 2
(483) Nine subjects were enrolled and dosed in Cohort 2. At least six subjects received active treatment and at least 2 received placebo; 1 subject withdrew before the 2 hour PK sample and was replaced. Subjects with truncated sampling schedules have been excluded in this interim analysis. Active treatment was administered by dry powder inhalation (DPI) as a single capsule of 50 μg treprostinil strength, and placebo treatments were administered by DPI as a single capsule of the placebo formulation. All inhalations were administered using the RS00 inhaler.
(484) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.
(485) The table shown in FIG. **4**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 2.
(486) The highest concentrations for four of the six subjects occurred at 0.17 hours post-inhalation; of the remaining subjects, one had Tmax at 0.083 hours post-dose and one at 0.417 hours post-dose. At 2.5 hours after inhalation, 2 of 6 active subjects had measurable concentrations of treprostinil and only one subject had measurable concentrations at 3 hours after inhalation. No subjects had quantifiable concentrations after the 3 hour timepoint.
(487) Preliminary non-compartmental PK parameters for treprostinil for Cohort 2 are summarized in the table shown in FIG. **4**B. The Cmax averaged

0.572 ng/mL and the most frequent Tmax was 0.167 hours after inhalation. AUCinf values averaged 0.422 h*ng/mL with a CV % of 62.8%. The apparent volume of distribution (Vz/F) averaged 110 L. Oral clearance (CL/F) averaged 208 L/h and ranged from 67 to 624. Variability in the CL/F value had a CV % of 101.5%.

(488) By comparison, the Cmax for Cohort 1 averaged 0.364 ng/mL and the AUCinf values averaged 0.301 h*ng/mL. Thus, a doubling of the treprostinil dose resulted in an approximate 50% increase in exposure. The Vz/F and the CL/F values were considerably higher for Cohort 2 and with greater variability.

(489) Cohort 3

(490) Eight subjects were enrolled and dosed in Cohort 3. Six subjects received active treatment and two received placebo. Active treatment was administered by dry powder inhalation (DPI) as a single capsule of 75 μg treprostinil strength and placebo treatments were administered by DPI as a single capsule of the placebo formulation. All inhalations were administered using the RS00 inhaler.

(491) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.

(492) The table shown in FIG. **5**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the 6 active subjects in Cohort 3.

(493) The highest concentrations for three of the six subjects occurred at 0.25 hours post-inhalation; of the remaining subjects, 1 had Tmax at 0.083 hours post-dose, 1 at 0.17 hours post-dose, and 1 at 0.417 hours post-dose. At 3 hours after inhalation, two of six active subjects had measurable concentrations of treprostinil. No subjects had quantifiable concentrations after the 3 hour timepoint.

(494) Preliminary non-compartmental PK parameters for treprostinil for Cohort 3 are summarized in the table shown in FIG. **5**B. The Cmax averaged 0.728 ng/mL and the most frequent Tmax was 0.25 hours after inhalation. AUCinf values averaged 0.757 h*ng/mL with a CV % of 39.4%. The apparent volume of distribution (Vz/F) averaged 97 L. Oral clearance (CL/F) averaged 112 L/h and ranged from 58 to 161. Variability in the CL/F value had a CV % of 39.4%.

(495) By comparison, the Cmax for Cohort 1 and Cohort 2 averaged 0.364 ng/mL and 572 ng/mL, respectively, while the AUCinf values averaged 0.301 h*ng/mL and 0.422 h*ng/mL. Thus, a tripling of the dose from Cohort 1 resulted in an approximate 100-150% increase in exposure. The CL/F values were for Cohort 3 were more consistent with Cohort 1, and with similar variability, than what was observed in Cohort 2. The results indicate that both C.sub.max and AUCinf may be increasing proportionately to the increase in the dose and that the CL is independent of dose over the range of 25 to 75 μg treprostinil.

(496) Cohort 4

(497) Eight subjects were enrolled and dosed in Cohort 4. Six subjects received active treatment and two received placebo. Active treatment of 100 μg treprostinil was administered by dry powder inhalation (DPI) as 2 capsules of 50 μg treprostinil strength and placebo treatments were administered by DPI as 2 capsules of the placebo formulation. All inhalations were administered using the RS00 inhaler.

(498) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.

(499) The table shown in FIG. **6**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 4. The highest concentrations for 2 of the 6 subjects occurred at 0.25 hours post-inhalation; of the remaining subjects, 2 had Tmax at 0.5 hours post-dose, 1 at 0.17 hours post-dose, and 1 at 0.33 hours post-dose. At 4 hours after inhalation, 3 of 6 active subjects had measurable concentrations of treprostinil. No subjects had quantifiable concentrations at the 6 or 8 hour timepoints.

(500) Preliminary non-compartmental PK parameters for treprostinil for Cohort 4 are summarized in FIG. **6**B. The Cmax averaged 1.08 ng/mL and the most frequent Tmax values were observed at 0.25 hours and 0.5 hours after inhalation. AUCinf values averaged 1.22 h*ng/mL with a CV % of 18.4%. The apparent volume of distribution (Vz/F) averaged 96 L. Oral clearance (CL/F) averaged 84.8 L/h and ranged from 68.3 to 122. Variability (CV %) in the CL/F value was 22.8%.

(501) By comparison, the Cmax for Cohorts 1, 2, and 3 averaged 0.364 ng/mL, 0.572 ng/mL, and 0.728 ng/mL respectively, while the AUCinf values averaged 0.301 h*ng/mL, 0.422 h*ng/mL, and 0.757 h*ng/mL. Thus, a quadrupling of the dose from Cohort 1 resulted in an approximate 200-300% increase in exposure, while a doubling of the dose from Cohort 2 resulted in an approximate 2-fold increase in exposure. The results indicate that both Cmax and AUCinf may be increasing proportionately to the increase in the dose and that the CL/F is independent of dose over the range of 25 to 100 μg treprostinil.

(502) Cohort 5

(503) Eight subjects were enrolled and dosed in Cohort 5. Six subjects received active treatment and two received placebo. Active treatment of 125 μg treprostinil was administered by dry powder inhalation (DPI) as 1 capsule of 75 μg and 1 capsule of 50 μg treprostinil strength and placebo treatments were administered by DPI as 2 capsules of the placebo formulation. All inhalations were administered using the RS00 inhaler.

(504) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.

(505) The table shown in FIG. **7**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the 6 active subjects in Cohort 5. The highest concentrations for 3 of the 6 subjects occurred at 0.17 hours post-inhalation; of the remaining subjects, 2 had Tmax at 0.33 hours post-dose, and 1 at 0.42 hours post-dose. At 3.5 and 4 hours after inhalation, only 1 of 6 active subjects had measurable concentrations of treprostinil. No subjects had quantifiable concentrations at the 6 or 8 hour timepoints.

(506) Preliminary non-compartmental PK parameters for treprostinil for Cohort 5 are summarized in FIG. **7**B. The Cmax averaged 1.19 ng/mL and the most frequent Tmax values were observed at 0.17 hours after inhalation. AUCinf values averaged 1.15 h*ng/mL with a CV % of 44.9%. The apparent volume of distribution (Vz/F) averaged 101 L. Oral clearance (CL/F) averaged 141 L/h and ranged from 65.7 to 336. Variability (CV %) in the CL/F value was 69.9%.

(507) By comparison, the Cmax for Cohorts 1, 2, 3, and 4 averaged 0.364 ng/mL, 0.572 ng/mL, 0.728 ng/mL, and 1.08 ng/mL respectively, while the AUCinf values averaged 0.301 h*ng/mL, 0.422 h*ng/mL, 0.757 h*ng/mL, and 1.22 h*ng/mL. Thus, a quintupling of the dose from Cohort 1 resulted in an approximate 220-280% increase in exposure. The results indicate that both Cmax and AUCinf may be increasing proportionately to the increase in the dose and that the CL/F is independent of dose over the range of 25 to 125 μg treprostinil.

(508) Cohort 6

(509) Cohort 6 was conducted as an original and a repeat. In each Cohort 6 (original and repeat), eight subjects were enrolled and dosed. Six subjects received active treatment and two received placebo. Active treatment of 150 μg treprostinil was administered by dry powder inhalation (DPI) as 2 capsules of 75 μg treprostinil strength and placebo treatments were administered by DPI as 2 capsules of the placebo formulation. All inhalations were administered using the RS00 inhaler. Cohort 6 original included some mechanical device failures and subject non-compliance with instructions, giving rise to Cohort 6 repeat.

(510) Blood samples were obtained prior to dosing and at nominal times of 0.083, 0.167, 0.25, 0.33, 0.417, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 hours after inhalation. Analysis of plasma concentration versus time data for calculation of standard pharmacokinetic (PK) parameters following inhalation was conducted using Phoenix WinNonlin version 6.3 using scheduled blood sampling times. Plasma concentrations were provided only for subjects on active dose and random subject numbers were assigned by the bioanalytical laboratory to retain the study blind.
(511) The table shown in FIG. **8**A contains a summary of the treprostinil concentration-time data for individual subjects with descriptive statistics for the six active subjects in Cohort 6-R. The highest concentrations for 2 of the 6 subjects occurred at 0.25 hours post-inhalation and at 0.33 hours post-inhalation. In the remaining 2 subjects, Tmax occurred at the 0.167 and 0.417 hours post-dose timepoints. At 4 hours after inhalation, 4 of 6 active subjects had measurable concentrations of treprostinil. No subjects had quantifiable concentrations at the 6 or 8 hour timepoints.
(512) Preliminary non-compartmental PK parameters for treprostinil for Cohort 6-R are summarized in FIG. **8**B. Preliminary non-compartmental PK parameters for treprostinil for Cohort 6-Original are summarized in FIG. **8**C. Mean concentration-time data for each of the six cohorts is displayed on a linear scale in FIG. **8**D. The Cmax averaged 1.45 ng/mL and the most frequent Tmax values were observed at 0.25 and 0.33 hours after inhalation. AUCinf values averaged 1.62 h*ng/mL with a CV % of 68.3%. The apparent volume of distribution (Vz/F) averaged 107 L. Oral clearance (CL/F) averaged 126 L/h and ranged from 51.8 to 245. Variability (CV %) in the CL/F value was 68.3%.
(513) By comparison, the Cmax for Cohorts 1, 2, 3, 4, 5, and the combined Cohort 6 averaged 0.364 ng/mL, 0.572 ng/mL, 0.728 ng/mL, 1.08 ng/mL, 1.19 ng/mL, and 1.21 ng/mL, respectively (FIG. **8**E), while the AUCinf values averaged 0.301 h*ng/mL, 0.422 h*ng/mL, 0.757 h*ng/mL, 1.22 h*ng/mL, 1.15 h*ng/mL, and 1.37 h*ng/mL (FIG. **8**F). Thus, a 6-fold increase of the dose from Cohort 1 to the combined Cohort 6 observations resulted in an approximate 260-400% increase in exposure, while tripling from Cohort 2 and doubling from Cohort 3 resulted in approximate increases of exposure by 130-255% and 81-98%, respectively. Moreover, plots of the relationship between dose and Cmax and AUCinf are displayed in FIG. **8**E and FIG. **8**F, respectively. The results indicate that both Cmax and AUCinf may be increasing proportionately to the increase in the dose. It was observed at the CRU during the original 150 μg dosing, however, that there were several apparent device failures that may have resulted in incomplete and/or inefficient exposures. It should be noted that no device failures were noted during the repeat dosing and, while mean values may be higher than in the initial cohort, the variability in the repeated cohort is greater. A plot of the relationship between dose and CL/F (FIG. **8**G) shows that the CL/F is independent of dose over the range of 25 to 150 μg treprostinil, which suggests that PK of treprostinil has a proportional relationship to dose over the range of 25 to 150 μg treprostinil.
CLINICAL CONCLUSIONS
(514) LIQ861 was dosed at levels of 25, 50, 75, 100, 125 and 150 μg treprostinil from either a single capsule (25, 50 and 75 mcg doses) or a combination of two lower capsular strengths (for 100, 125 and 150 mcg doses), each capsule either undergoing either a single breath or two breaths. According to embodiments of the present invention, novel capsule to particle powder to active ingredient ratios, and breath per capsule and powder per breath ratios for human dosing are included in the following table.
(515) TABLE-US-00075 Patient presentation of particle powder and active agent per capsule per breath for particle formulation having 0.5 percent active agent load Capsules 1 1 1 2 2 2 Particle 5 10 15 Combination Combination Combination Powder of two 50 mcg of, ex., 1 at of, ex., two in mg capsules or 50 mcg capsules one 25 mcg and 1 at 75 at 75 mcg and one 75 mcg mcg Active Agent 25 50 75 Varies, see Varies, see Varies, see Load in mcg above above above Breaths to 1 to 2 1 to 2 1 to 2 1 to 2 per 1 to 2 per 1 to 2 per Deliver capsule capsule capsule Particle 2.5-5 5-10 7.5-15 Varies, up to Varies, up to Varies, up to Powder 15 15 15 per Breath in mg Active Agent 12.5-25 25-50 37.5-75 Varies, up to Varies, up to Varies, up to per Breath 75 75 75 in mcg
(516) According to such embodiments, as shown in the above table, each breath can receive from 2.5-15 mg of particle power and from 12.5-75 mcg of active agent.
(517) For the given treprostinil delivered in the given mass of particle powder loaded into a capsule and delivered through a dry powder inhaler results in the human clinical outcomes are included in the following table for LIQ861.
(518) TABLE-US-00076 LIQ861 Clinical C.sub.max T.sub.max.sup.a t.sub.1/2 AUC.sub.last AUC.sub.inf CL/F Vz/F Outcomes (ng/mL) (h) (h) (h * ng/mL) (h * ng/mL) (L/h) (L) 25 mcg Treprostinil 0.36 (0.12) 0.33 (0.17, 0.42) 0.52 (0.16) 0.27 (0.09) 0.3 (0.09) 91 (32.6) 68.1 (27.4) 50 mcg Treprostinil 0.57 (0.37) 0.17 (0.08, 0.42) 0.45 (0.12) 0.4 (0.26) 0.42 (0.27) 208 (211) 110 (66.6) 75 mcg Treprostinil 0.73 (0.3) 0.25 (0.08, 0.42) 0.62 (0.18) 0.72 (0.31) 0.76 (0.31) 112 (38.5) 97 (29.1) 100 mcg Treprostinil 1.08 (0.31) 0.29 (0.17, 0.5) 0.78 (0.13) 1.18 (0.22) 1.22 (0.23) 84.8 (19.3) 95.5 (28.2) 125 mcg Treprostinil 1.19 (0.53) 0.25 (0.17, 0.42) 0.53 (0.07) 1.12 (0.51) 1.15 (0.52) 141 (98.8) 101 (58.7) 150 mcg Treprostinil 1.21 (0.3) 0.29 (0.08, 0.42) 0.66 (0.15) 1.33 (0.44) 1.37 (0.42) 119 (35.8) 115 (51.4) 150 mcg Treprostinil 1.45 (0.63) 0.29 (0.17, 0.42) 0.64 (0.11) 1.58 (0.85) 1.62 (0.87) 126 (80.3) 107 (54) Abbreviations: SD = standard deviation; C.sub.max = maximum observed plasma concentration; T.sub.max = time to C.sub.max; t.sub.1/2 = half-life; AUC = area under the curve; CL/F = apparent clearance; Vz/F = apparent volume of distribution All values except for T.sub.max are reported as arithmetic means with SD in parentheses. .sup.aT.sub.max reports median values with minimum and maximum values in parentheses
(519) For comparison, TYVASO (United Therapeutics, Inc.) provides the current standard of treatment for inhaled treprostinil. Such treprostinil is delivered through a nebulizer for the treatment of PAH and is limited to deliver 6 mcg of treprostinil per breath, utilizing 9 breaths to reach a 54 mcg dose. The current standard of inhaled treatment has shown to be dose limited to a maximum tolerated dose of 84 mcg of treprostinil, which required 14 breaths to reach such dose. See, Channick, R. et al., Inhaled Treprostinil: a therapeutic review, Drug Design, Development and Therapy 2012:6 19-28; and Nelsen AC, et al., Pharmacokinetics Of Inhaled Treprostinil Sodium In Healthy Volunteers. Am J Respir Crit Care Med. 2010; 181:A3348; both of which are incorporated herein by reference in their entirety.
(520) In alternative embodiments, particles of the present invention may include 1% treprostinil load, as compared to 0.5% treprostinil load of the LIQ861 particles. According to an embodiment of the present invention, a plurality of 1% treprostinil particles were fabricated from a solution comprising, weight percent solids in water, of: 1.06% treprostinil sodium, 92.44% trehalose dihydrate, 2% polysorbate 80, 4% L leucine, 0.27% sodium citrate dihydrate, and 0.23% sodium chloride.
(521) According to a 1 percent treprostinil particle formulation of the present invention, particle powder mass and active agent presented to a patient comprise the following novel capsule to particle powder to active ingredient ratios, and breath per capsule and powder per breath ratios for human dosing.
(522) TABLE-US-00077 Patient presentation of particle powder and active agent per capsule per breath for particle formulation having 1 percent active agent load Capsules 1 1 1 1 1 1 Particle 2.5 5 7.5 10 12.5 15 Powder in mg Active Agent 25 50 75 100 125 150 Load in mcg Breaths to 1 to 2 1 to 2 1 to 2 1 to 2 1 to 2 1 to 2 Deliver Particle 1.25-2.5 2.5-5 3.75-7.5 5-10 6.25-12.5 7.5-15 Powder per Breath in mg Active Agent 12.5-25 25-50 37.5-75 50-100 62.5-125 75-150 per Breath in mcg
(523) According to such embodiments, as shown in the above table, each breath can receive from 1.25-15 mg of particle power and from 12.5-150 mcg of active agent.
(524) For the powder mass found acceptable in LIQ861 initial clinical trial associated with delivery of the 150 mcg dose, at a 1% active drug particle a dose of 300 mcg of active drug can be administered in a safe and acceptable powder mass and excipient quantity.
(525) Kits
(526) According to embodiments of the present invention the dry powder inhaler device can be combined into a kit with capsules for use therein. The capsules can be packaged in blister packs with or without desiccant to ensure controlled environment for the LIQ861 particle powder while the traveling with a user. The blister packs can include capsules for a single dosing or multiple capsules for a day, week or month of doses. Typically a patient will treat 4 times per day for the PAH indication. The kit can include capsules comprising dosage strengths of 25, 50, 75, 100, 125, 150, 200,

250, 300 mcg or beyond for the treatment of PAH. The particles of the powder in the capsules of the kits can be particles comprising 0.5% treprostinil or 1% treprostinil.
(527) Abbreviations and Nomenclature Cross-references
(528) TABLE-US-00078 6MWD 6 Minute Walk Distance AE Adverse Event AUC Area Under the Curve AUCinf Area Under the Concentration-Time Curve Extrapolated to Time Infinity AUClast Area Under the Concentration-Time Curve to the Last Measured Timepoint AUCext Percentage of Area Under the Curve Extrapolated Beyond Last Measureable Concentration AVT Acute Pulmonary Vasodilator Testing BA Bioavailability BDI Borg Dyspnea Index BLQ Below the Limit of Quantitation BMPR2 Bone Morphogenic Protein Receptor Type II Gene BP British Pharmacopoeia BTO Benzidine Triol CAS Chemical Abstracts Service CFR Code of Federal Regulations CFU Colony Forming Unit cGMP Current Good Manufacturing Practice CI Cardiac Index CL Clearance Cmax Maximum Concentration CMC Chemistry Manufacturing and Controls CO Cardiac Output COPD Chronic Obstructive Pulmonary Disease Css Concentration at Steady State CTEPH Chronic Thromboembolic Pulmonary Hypertension CYP Cytochrome P450 DMF Drug Master File DP Drug Product DPI Dry Powder Inhaler DPPA Diphenylphosphinic Acid DRF Dose Range Finding DS Drug Substance DUSA Dosage Unit Sampling Apparatus eCMH Extended Cochran-Mantel-Haenszel Test ECG Electrocardiogram ED Emitted Dose Emax Maximum Effect EP European Pharmacopoeia ERA Endothelin Receptor Agonists ET3 Polyethylene Terephthalate Cyclic Trimer EU Endotoxin Unit ESRD End-Stage Renal Disease F Bioavailability FCR Fluorocur ® FDA Food and Drug Administration FPF Fine Particle Fraction Frel Relative Bioavailability FT-IR Fourier Transform Infrared Spectroscopy GC Gas Chromatography GLP Good Laboratory Practice GMP Good Manufacturing Practice GSD Geometric Standard Deviation HIAC High Accuracy Particle Counter HIV Human Immunodeficiency Virus H-L Hodges-Lehmann HPLC High Performance Liquid Chromatography HPMC Hydroxypropyl Methylcellulose HR Heart Rate ICH International Conference on Harmonisation IM Intramuscular IND Investigational New Drug INR International Normalized Ratio IR Infrared ISO International Organization for Standardization IV Intravenous JP Japanese Pharmacopoeia KF Karl Fischer Titration LC Liquid Chromatography LC-MS Liquid Chromatography with Mass Spectrometry LV Left Ventricular LVdP/dt Left Ventricular Contractility mcg Micrograms, μg or ug MDI Metered Dose Inhaler MeOH Methanol MLWHF Minnesota Living With Heart Failure Questionnaire MMAD Mass Median Aerodynamic Diameter MTD Maximum Tolerated Dose PAPm Mean Pulmonary Arterial Pressure NDA New Drug Application NF National Formulary NGI Next Generation Impactor ™ NMR Nuclear Magnetic Resonance NMT Not More Than NO Nitric Oxide NOAEL No Observed Adverse Effect Level NRF Normal Renal Function NT Not Tested NT-proBNP N-Terminal of the Prohormone Brain Natriuretic Peptide NYHA New York Heart Association OHSAS Occupational Health and Safety Advisory Services OPP Oriented Polypropylene PAH Pulmonary Arterial Hypertension PAP Pulmonary Arterial Pressure PCW Pulmonary Capillary Wedge pressure PD Pharmacodynamics PDE5 Phosphodiesterase Type 5 Inhibitors PE Polyethylene PET Polyethylene Terephthalate PGI.sub.2 Prostaglandin I2 (Prostacyclin) PH Pulmonary Hypertension PK Pharmacokinetics PPM Parts Per Million PRINT Particle Replication In Nonwetting Templates PTFE Polytetrafluoroethylene PVR Pulmonary Vascular Resistance QID Quarter in Die (Four Times Daily) (Q)SAR Quantitative Structure-Activity Relationship QTc Corrected QT Interval RH Relative Humidity RLD Reference Listed Drug SAC Single Actuation Content SAE Serious Adverse Event SAP Systemic Arterial Pressure SC Subcutaneous SEM Standard Error of the Mean SOP Standard Operating Procedure SVR Systemic Vascular Resistance t½ Half-life TBD To Be Determined TK Toxicokinetics Tmax Time of Maximal Concentration TMB acid Trimethylbenzoic Acid TMB-Ald Trimethylbenzaldehyde TMP Trimethylbenzoyl Diphenylphosphine Oxide TRIUMPH Treprostinil Sodium Inhalation Used in the Management of Pulmonary Arterial Hypertension (clinical trial) TTC Threshold for Toxicological Concern IUPAC International Union of Pure and Applied Chemistry μg or ug Micrograms or mcg US United States USP United States Pharmacopeia WHO World Health Organization WRS Wilcoxon Rank Sum Test WT Weight XRD X-ray Diffractometer
(529) TABLE-US-00079 Product Nomenclature and Reference Table Term Designation Exemplary Embodiment Drug Substance DS or Treprostinil, supplied as treprostinil treprostinil sodium for manufacture of the LIQ861 drug product-intermediate Drug Product-Intermediate DP-intermediate or Dry powder particles of precise size and LIQ861 DP-intermediate shape containing an integrated matrix of treprostinil and excipients that is produced using Liquidia's PRINT ® Technology manufacturing process (bulk dry powder prior to capsule filling) Placebo Drug Product- Placebo DP-intermediate Identical formulation as the DP- Intermediate Intermediate, but treprostinil is replaced with an equal mass of trehalose Inhalation Powder Drug DP or LIQ861 DP-intermediate Product LIQ861 Drug Product filled into Size 3 HPMC capsules for oral or LIQ861 inhalation, but prior to integration with Inhalation Device Placebo Drug Product Placebo Placebo DP-intermediate filled into Size 3 hydroxypropylmethylcellulose (HPMC) capsules, but prior to integration with the Inhalation Device Drug Product Strength Treprostinil Amount of treprostinil in drug product or Dose in LIQ861 Packaged Drug Product None Drug Product in the Primary Packaging Inhalation Device Device Device that is used to deliver the Drug Product Premetered Dry Powder DPI Drug Product integrated with the Inhaler Inhalation Device; i.e., the final product for patient use

Claims

1. A system for administrating treprostinil or a pharmaceutically acceptable salt thereof to a patient, the system comprising: a dosage unit containing a predetermined amount of dry powder particles having a mass median aerodynamic diameter between 1 μm and 5 μm and a fine particle fraction between 82 percent and 89 percent of the predetermined amount of dry powder particles, the dry powder particles comprising: an excipient matrix; and treprostinil or a pharmaceutically acceptable salt thereof; and a dry powder inhaler comprising: a body configured to receive the dosage unit; and a mouthpiece that is moveable with respect to the body to an aligned position, wherein the dry powder inhaler allows delivery of the dry powder particles from the dosage unit through the mouthpiece to the patient via inhalation over one to two breaths when the mouthpiece is in the aligned position.
2. The system of claim 1, wherein the dry powder inhaler is actuatable by the patient to open the dosage unit while the dosage unit is received in the body to allow release of the dry powder particles from the dosage unit.
3. The system of claim 1, wherein the mouthpiece is rotatable with respect to the body.
4. The system of claim 1, wherein the system is configured to deliver from about 2.5 mg to about 5 mg of the dry powder particles to the patient per breath.
5. The system of claim 1, wherein the system is configured to deliver from about 5 mg to about 10 mg of the dry powder particles to the patient per breath.
6. The system of claim 1, wherein the system is configured to deliver from about 7.5 mg to about 15 mg of the dry powder particles to the patient per breath.
7. The system of claim 1, wherein the dosage unit contains from about 10 μg to about 220 ug treprostinil or the pharmaceutically acceptable salt thereof.
8. The system of claim 1, wherein the dry powder particles comprise about 0.53% by weight treprostinil or the pharmaceutically acceptable salt thereof.
9. The system of claim 1, wherein the dry powder particles comprise about 1.06% by weight treprostinil or the pharmaceutically acceptable salt thereof.
10. The system of claim 1, wherein the dry powder particles have a mass median aerodynamic diameter of 3 μm or less.
11. The system of claim 1, wherein the excipient matrix is formed from a dried solution comprising one or more excipients.
12. The system of claim 11, wherein the one or more excipients comprises one or more of a bulking agent, a wetting agent, a hydrophobicity modifier, a pH modifier, and a buffer.

13. The system of claim 12, wherein the one or more excipients comprises a bulking agent and a pH modifier.
14. The system of claim 1, wherein the dry powder particles are dried to less than 5 percent water content.
15. The system of claim 1, wherein the dosage unit comprises a capsule.
16. The system of claim 1, wherein the treprostinil or a pharmaceutically acceptable salt thereof becomes pharmaceutically available in less than 10 seconds upon delivery to the patient via inhalation.
17. The system of claim 16, wherein the treprostinil or a pharmaceutically acceptable salt thereof becomes pharmaceutically available in less than 5 seconds upon delivery to the patient via inhalation.
18. The system of claim 16, wherein the treprostinil or a pharmaceutically acceptable salt thereof becomes pharmaceutically available in less than 1 second upon delivery to the patient via inhalation.
19. The system of claim 1, further comprising a second dosage unit receivable in the body of the dry powder inhaler, the second dosage unit containing a second predetermined amount of the dry powder particles.
20. The system of claim 19, wherein each of the dosage unit and the second dosage unit contains from about 10 μg to about 220 μg treprostinil or the pharmaceutically acceptable salt thereof.