US012390475B2

(12) **United States Patent**
Roscigno et al.

(10) **Patent No.: US 12,390,475 B2**
(45) **Date of Patent: *Aug. 19, 2025**

(54) **DRY POWDER TREPROSTINIL FOR THE TREATMENT OF PULMONARY HYPERTENSION**

(71) Applicant: **LIQUIDIA TECHNOLOGIES, INC.**, Morrisville, NC (US)

(72) Inventors: **Robert Frank Roscigno**, Melbourne Beach, FL (US); **Brian T. Farrer**, Apex, NC (US); **Jacob J. Sprague**, Cary, NC (US); **Benjamin Maynor**, Durham, NC (US)

(73) Assignee: **LIQUIDIA TECHNOLOGIES, INC.**, Morrisville, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **18/350,926**

(22) Filed: **Jul. 12, 2023**

(65) **Prior Publication Data**

US 2023/0346799 A1 Nov. 2, 2023

Related U.S. Application Data

(63) Continuation of application No. 17/390,514, filed on Jul. 30, 2021, now Pat. No. 11,744,836, which is a continuation of application No. 17/104,348, filed on Nov. 25, 2020, now Pat. No. 11,712,442, which is a continuation of application No. 16/099,135, filed as application No. PCT/US2017/031301 on May 5, 2017, now Pat. No. 10,898,494.

(60) Provisional application No. 62/472,204, filed on Mar. 16, 2017, provisional application No. 62/440,078, filed on Dec. 29, 2016, provisional application No. 62/404,960, filed on Oct. 6, 2016, provisional application No. 62/332,013, filed on May 5, 2016.

(51) **Int. Cl.**

A61K 9/00	(2006.01)
A61K 9/48	(2006.01)
A61K 31/557	(2006.01)
A61K 31/5575	(2006.01)
A61P 9/12	(2006.01)
A61K 47/02	(2006.01)
A61K 47/18	(2017.01)
A61K 47/26	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/557*** (2013.01); ***A61K 9/0075*** (2013.01); ***A61K 9/4858*** (2013.01); ***A61K 31/5575*** (2013.01); ***A61P 9/12*** (2018.01); *A61K 47/02* (2013.01); *A61K 47/183* (2013.01); *A61K 47/26* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,306,075	A	12/1981	Aristoff
5,153,222	A	10/1992	Tadepalli et al.
5,441,060	A	8/1995	Rose et al.
5,874,064	A	2/1999	Edwards et al.
6,242,482	B1	6/2001	Shorr et al.
6,503,480	B1	1/2003	Edwards et al.
6,521,212	B1	2/2003	Cloutier et al.
6,756,033	B2	6/2004	Cloutier et al.
6,765,117	B2	7/2004	Moriarty et al.
6,858,199	B1	2/2005	Edwards et al.
6,921,528	B2	7/2005	Edwards et al.
7,199,157	B2	4/2007	Wade et al.
7,879,909	B2	2/2011	Wade et al.
7,976,759	B2	7/2011	Henn et al.
8,128,393	B2	3/2012	Rolland et al.
8,158,728	B2	4/2012	DeSimone et al.
8,242,305	B2	8/2012	Batra et al.
8,263,129	B2	9/2012	DeSimone et al.
8,268,446	B2	9/2012	DeSimone et al.
8,350,079	B2	1/2013	Walsh
8,420,124	B2	4/2013	DeSimone et al.
8,444,907	B2	5/2013	Ermochkine et al.
8,454,939	B2	6/2013	Hrkach
8,497,393	B2	7/2013	Batra et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	3012679	A1	8/2017
EP	1161234	B1	7/2003

(Continued)

OTHER PUBLICATIONS

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2017/031301, mailed Jul. 19, 2017.

Channick, Richard N. et al. "Inhaled treprostinil: a therapeutic review," Drug Design, Development and Therapy, vol. 6 (2012): 19-28.

Voswinckel, Robert et al. "Favorable Effects of Inhaled Treprostinil in Severe Pulmonary Hypertension: Results From Randomized Controlled Pilot Studies," Journal of the American College of Cardiology, vol. 48, Issue 8 (2006): 1672-1681.

Michael Castagna, PharmD; "TECHNOSPHERE Drug Delivery Platform"; United Therapeutics Corporation; UTHR Science Day 2018 (16 pages).

(Continued)

Primary Examiner — Celeste A Roney

(74) *Attorney, Agent, or Firm* — Morgan, Lewis & Bockius LLP

(57) **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.

20 Claims, 22 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

8,518,316	B2	8/2013	Henn et al.	
8,765,813	B2	7/2014	Wade et al.	
8,887,715	B2	11/2014	Hrkach	
9,156,786	B2	10/2015	Batra et al.	
9,161,986	B2	10/2015	Mizushima et al.	
9,339,507	B2	5/2016	Olschewski et al.	
9,358,240	B2	6/2016	Olschewski et al.	
9,550,716	B2	1/2017	Giust et al.	
9,593,066	B2	3/2017	Batra et al.	
9,604,901	B2	3/2017	Batra et al.	
9,872,864	B2	1/2018	Sprogoe et al.	
9,957,220	B2	5/2018	Zhang et al.	
9,988,644	B2	6/2018	Heffner et al.	
10,016,338	B2	7/2018	Weikart et al.	
10,053,414	B2	8/2018	Zhang et al.	
10,071,123	B2	9/2018	Jeffs et al.	
10,071,209	B2	9/2018	Shlomo et al.	
10,080,804	B2	9/2018	Rudolph et al.	
10,086,001	B2	10/2018	Freissmuth et al.	
10,130,685	B2	11/2018	Leone-Bay et al.	
10,166,183	B2	1/2019	Sommer et al.	
10,167,247	B2	1/2019	Phares et al.	
10,245,244	B2	4/2019	Yacoub et al.	
10,246,403	B2	4/2019	Zhang et al.	
10,322,099	B2	6/2019	Batra et al.	
10,376,525	B2	8/2019	Olschewski et al.	
10,421,729	B2	9/2019	Wilson et al.	
10,478,410	B2	11/2019	Batra et al.	
10,494,327	B2	12/2019	Laing	
10,548,863	B2	2/2020	Batra et al.	
10,716,793	B2	7/2020	Olschewski et al.	
10,772,883	B2	9/2020	Grant et al.	
10,898,494	B2	1/2021	Roscigno et al.	
10,912,778	B2	2/2021	Weers et al.	
10,995,055	B2	5/2021	Malinin et al.	
11,723,887	B2	8/2023	Batra et al.	
12,102,610	B2	10/2024	Remick	
2003/0118567	A1	6/2003	Stewart	
2004/0265238	A1	12/2004	Chaudry	
2006/0073105	A1	4/2006	Yamashita et al.	
2006/0147520	A1	7/2006	Ruegg	
2008/0131692	A1	6/2008	Rolland et al.	
2008/0200449	A1*	8/2008	Olschewski	A61K 9/008 514/250
2009/0036465	A1	2/2009	Roscigno et al.	
2009/0165320	A1	7/2009	DeSimone et al.	
2009/0264389	A1	10/2009	Zeng	
2010/0003291	A1	1/2010	Rothrock	
2011/0311630	A1	12/2011	Krueger et al.	
2012/0114554	A1	5/2012	DeSimone et al.	
2012/0177693	A1	7/2012	Cipolla et al.	
2012/0189728	A1	7/2012	Rolland et al.	
2012/0256354	A1	10/2012	DeSimone et al.	
2013/0011618	A1	1/2013	DeSimone et al.	
2013/0202729	A1	8/2013	Rolland et al.	
2013/0209564	A1	8/2013	Rele et al.	
2013/0228950	A1	9/2013	DeSimone et al.	
2013/0241107	A1	9/2013	Ermochkine et al.	
2013/0249138	A1	9/2013	DeSimone et al.	
2014/0027948	A1	1/2014	Henn et al.	
2014/0065219	A1*	3/2014	Bosch	A61K 31/46 514/304
2014/0072632	A1	3/2014	DeSimone et al.	
2014/0127227	A1	5/2014	Chang	
2014/0127311	A1	5/2014	Beck-Broichsitter et al.	
2015/0021690	A1	1/2015	Jeffs et al.	
2015/0136130	A1	5/2015	DeHaan et al.	
2015/0166503	A1	6/2015	Becker et al.	
2015/0196516	A1	7/2015	Yacoub et al.	
2015/0246188	A1	9/2015	Steiner et al.	
2015/0273079	A1	10/2015	Hubby et al.	
2015/0328232	A1	11/2015	Malinin et al.	
2016/0045434	A1	2/2016	Caponetti et al.	
2017/0216538	A1	8/2017	Kinsey et al.	
2017/0304276	A1	10/2017	Armer et al.	
2019/0151332	A1	5/2019	Roscigno et al.	
2019/0321290	A1	10/2019	Guarneri et al.	
2020/0046917	A1	2/2020	Kinsey et al.	
2021/0022994	A1	1/2021	Franssen et al.	
2021/0077504	A1	3/2021	Roscigno et al.	
2021/0353640	A1	11/2021	Roscigno et al.	
2021/0353641	A1	11/2021	Roscigno et al.	
2022/0242811	A1	8/2022	Li et al.	

FOREIGN PATENT DOCUMENTS

JP	56-122328		9/1981	
JP	2009-537246	A	10/2009	
JP	4819224	B2	11/2011	
JP	2015-508808	A	3/2015	
JP	2016-501233	A	1/2016	
WO	00/54758	A2	9/2000	
WO	2005/007081	A9	1/2005	
WO	2005/058303	A1	6/2005	
WO	2006/014930	A2	2/2006	
WO	2007/134292	A2	11/2007	
WO	WO-2009117112	A2 *	9/2009	A61M 11/001
WO	2009152160	A1	12/2009	
WO	2012/095511	A1	7/2012	
WO	2013/128283	A2	9/2013	
WO	2014/085813	A1	6/2014	
WO	2014/167023	A1	10/2014	
WO	2015061720	A2	4/2015	
WO	WO-2015138423	A1 *	9/2015	A61K 31/192
WO	2016/057712	A1	4/2016	
WO	2017/132601	A1	8/2017	
WO	2019/237028	A1	12/2019	

OTHER PUBLICATIONS

Vijaya Iyer, Phd "Treprostinil Delivered Using Technosphere Portable Inhaler Seen as Safe In Early Clinical Trial"; Pulmonary Hypertension News; Jun. 11, 2018 (10 pages).

"MannKind Successfully Completes Phase 1 Trial of Treprostinil Technosphere for Pulmonary Arterial Hypertension Advancing Development to Next Phase"; Press Release Jun. 7, 2018; MannKind Corporation (2 pages).

Extended European Search Report, dated Feb. 27, 2020, for European Patent Application No. 17793448.6.

Fromen, CA, et al., Synthesis and Characterization of Monodisperse Uniformly Shaped Respirable Aerosols, AlChE Journal. 2013; 59(9): 3184-3194.

Garcia, A, et al., Microfabricated Engineered Particle Systems for Respiratory Drug Delivery and Other Pharmaceutical Applications, Journal of Drug Delivery. 2012; 2012: 941243.

Mack, PD, et.al., Formulation and In Vivo Evaluation of Treprostinil Dry Powder for Inhalation, Fabricated Using the PRINT® Particle Technology, Respiratory Drug Delivery. 2014; 2: 473-476.

Official Action, dated Dec. 9, 2020, for Israel Patent Application No. 262720.

Examination report dated Sep. 14, 2021, for European Patent Application No. 17793448.6.

Notice of Reasons for Rejection, dated Mar. 2, 2022, for Japanese Patent Application No. 2018-558275 (w/ translation).

Examination Report No. 1 dated Mar. 3, 2022 for Australian Patent Application No. 2017261317.

Official Action dated May 3, 2022 for Israel Patent Application No. 262720 (w/ translation).

Notice of Reasons for Rejection, dated Mar. 29, 2021, for Japanese Patent Application No. 2018-558275 (w/ translation).

Kumar et al. "A Comprehensive Review of Treprostinil Pharmacokinetics via Four Routes of Administration." Clin Pharmacokinet (2016) 55:1495-1505.

Nadler et al. "Inhaled treprostinil and pulmonary arterial hypertension." Vascular Health and Risk Management (2010) 6:1115-1124.

Gessler et al. "The potential for inhaled treprostinil in the treatment of pulmonary arterial hypertension." Therapeutic Advances in Respiratory Disease (2011) 5(3):195-206.

(56) **References Cited**

OTHER PUBLICATIONS

Plaunt et al. "Development and Characterization of Treprostinil Palmitil Inhalation Aerosol for the Investigational Treatement of Pulmonary Arterial Hypertension." International Journal of Molecular Sciences (2021) 22, 548.
Obata et al. "Single Injection of a Sustained-release Prostacyclin Analog Improves Pulmonary Hypertension in Rats." American Journal of Respiratory and Critical Care Medicine. vol. 177. pp 195-201, 2008.
Leifer et al. "Inhaled Treprostinil-Prodrug Lipid Nanoparticle Formulations Provide Long-Acting Pulmonary Vasodilation." Drug Res 2018; 68: 605-614.
Notice of Reasons for Rejection, dated Mar. 2, 2022, for Japanese Patent Application No. 2018-558275 (w/ English translation).
Official Action dated May 3, 2022 for Israel Patent Application No. 262720 (English translation / summary).
Walpole et al. "The weight of nations: an estimation of adult human biomass," BMC Public Health, 2012, 12:439.
Declaration under 37 C.F.R. 1.132 filed Dec. 12, 2019 in U.S. Appl. No. 16/099,135.
Official Action dated Nov. 2, 2022 for Japanese Patent Application No. 2018-558275 (w/ English translation).
Office Action, dated May 24, 2023, for Canadian Patent Application No. 3,023,257.
Office Action, dated Jun. 11, 2024, for Australian Patent Application No. 2023201307.
Office Action, dated Jun. 3, 2024, for Canadian Patent Application No. 3,023,257.
Office Action, dated Jan. 30, 2024, for European Patent Application No. 17793448.6.
Office Action, dated Apr. 22, 2024, for Japanese Patent Application No. 2023-031081 (w/ translation).
Denyer, J., et al., "Adaptive Aerosol Delivery (AAD®) technology," Expert Opinion on Drug Delivery, 1:1, 165-176 ("Denyer 2004") [IPR2021-00406, Ex. 1101].
Byrne, N.M., et al., "Comparison of lung deposition of colomycin using the HaloLite and the Pari LC Plus nebulisers in patients with cystic fibrosis," Arch Dis Child 2003;88:715-718 ("Byrne 2003") [IPR2021-00406, Ex. 1102].
Leung, K., et al., "Comparison of Breath-Enhanced to Breath-Actuated Nebulizers for Rate, Consistency, and Efficiency," Chest 2004; 126:1619-1627 ("Leung 2004") [IPR2021-00406, Ex. 1103].
Sulica, R. and Poon, M., "Medical therapeutics for pulmonary arterial hypertension: from basic science and clinical trial design to evidence-based medicine," Expert Rev. Cardiovasc. Ther. 3(2), (2005) ("Sulica 2005") [IPR2021-00406, Ex. 1104].
European Society of Cardiology Annual Report 2005 [IPR2021-00406, Ex. 1105].
Reply Declaration of Nicholas Hill, M.D. [IPR2021-00406, Ex. 1106].
Reply Declaration of Igor Gonda, Ph.D. [IPR2021-00406, Ex. 1107].
Transcript from the Jan. 8, 2022 Deposition of Aaron Waxman, M.D., Ph.D., *Liquidia Technologies, Inc.* v. *United Therapeutics Corp.* [IPR2021-00406, Ex. 1108].
Transcript from the Jan. 11, 2022 Deposition of Jason McConville, *Liquidia Technologies, Inc.* v. *United Therapeutics Corp.* [IPR2021-00406, Ex. 1109].
Transcript from the Dec. 29, 2021 Deposition of Lyndsey Pilar Wyman, *Liquidia Technologies, Inc.* v. *United Therapeutics Corp.* [IPR2021-00406, Ex. 1110].
Reply Declaration of Sylvia Hall-Ellis, Ph.D. [IPR2021-00406, Ex. 1112].
Voswinckel JAHA Supplemental Author Index [IPR2021-00406, Ex. 1113].
American Heart Association 2004 Online Archive, pages available at https://web.archive.org/web/20041130084648/http://circ.ahajournals.org:80/;https://web.archive.org/web/20041206215947/http://aha.agora.com/abstractviewer/; https://web.archive.org/web/20041128050933/http://circ.ahajournals.org/contents-by-date.0.shtml; and https://web.archive.org/web/20041204145419/http://circ.ahajournals.org/content/vol110/issue17/ [IPR2021-00406, Ex. 1114].
Affidavit of Duncan Hall, Dec. 7, 2021 [IPR2021-00406, Ex. 1115].
Voswinckel JAHA British Library Declaration [IPR2021-00406, Ex. 1116].
Voswinckel JAHA Supplement PubMed Search Results [IPR2021-00406, Ex. 1117].
PubMed. gov search results for Sulica, R. and Poon, M., "Medical therapeutics for pulmonary arterial hypertension: from basic science and clinical trial design to evidence-based medicine," Expert Rev. Cardiovasc. Ther. 3(2), (2005) [IPR2021-00406, Ex. 1118].
Voswinckel JESC British Library Declaration [IPR2021-00406, Ex. 1119].
Voswinckel JESC Web of Science Search Results [IPR2021-00406, Ex. 1120].
Ghofrani 2005, British Library [IPR2021-00406, Ex. 1121].
Ghofrani 2005 Search Results [IPR2021-00406, Ex. 1122].
Circulation MARC record, British Library [IPR2021-00406, Ex. 1123].
Circulation MARC record, Library of Congress [IPR2021-00406, Ex. 1124].
Circulation MARC record, Stanford [IPR2021-00406, Ex. 1125].
Circulation MARC record, UC Davis [IPR2021-00406, Ex. 1126].
European Heart Journal bibliographic record, UWash [IPR2021-00406, Ex. 1127].
European Heart Journal MARC record, UWisc [IPR2021-00406, Ex. 1128].
European Heart Journal MARC record, British Library [IPR2021-00406, Ex. 1129].
Jan. 27, 2020 Press Release "Liquidia Submits New Drug Application for LIQ861 (treprostinil) inhalation powder to U.S. Food and Drug Administration for the Treatment of Pulmonary Arterial Hypertension (PAH)," available at https://www.liquidia.com/news-releases/news-release-details/liquidia-submits-new-drug-application-liq861-treprostinil [IPR2021-00406, Ex. 1130].
Michael Mellon et al., Comparable Efficacy of Administration with Face Mask or Mouthpiece of Nebulized Budesonide Inhalation Suspension for Infants and Young Children with Persistent Asthma, 162 Am. J. Respir. Care Med. 593 (2000) ("Mellon 2000") [IPR2021-00406, Ex. 1131].
Patent Owner Response, dated Nov. 10, 2021 [IPR2021-00406].
McLaughlin et al., Efficacy and safety of treprostinil: an epoprostenol analog for primary pulmonary hypertension, J. Cardiovascular Pharmacology, 41:293-299 (2003) [IPR2021-00406, Ex. 2036].
Springer website (from fn 13 of Hall-Ellis Decl) [IPR2021-00406, Ex. 2037].
Springer website (from fn 14 of Hall-Ellis Decl) [IPR2021-00406, Ex. 2039].
University of Wisconsin-Madison Library Catalog Search for holdings of Circulation: the journal of the American Heart Association [IPR2021-00406, Ex. 2040].
Declaration of Ms. Pilar Wyman [IPR2021-00406, Ex. 2041].
Ms. Pilar Wyman's curriculum vitae [IPR2021-00406, Ex. 2042].
Deposition Transcript of Sylvia Hall-Ellis, Ph. D. [IPR2021-00406, Ex. 2043].
American Heart Association Listing of Circulation Supplements [IPR2021-00406, Ex. 2044].
Chemical Abstracts Plus Search Results Transcript [IPR2021-00406, Ex. 2045].
Ovid Search Results for "Voswinckel" [IPR2021-00406, Ex. 2046].
PubMed Search Results for "Voswinckel" [IPR2021-00406, Ex. 2047].
Compilation Showing Search Results for Descriptor Terms [IPR2021-00406, Ex. 2048].
Oxford Academic Listing of European Heart Journal Supplements [IPR2021-00406, Ex. 2049].
Simonneau et al., Updated Clinical Classification of Pulmonary Hypertension., J Am. College of Cardiol, 62(25)D34-D42 at D34-D35 (2013) [IPR2021-00406, Ex. 2050].
Sitbon and Noordegraaf, Epoprostenol and pulmonary arterial hypertension: 20 years of clinical experience, Eur. Respir Rev. 26:160055 (2017) [IPR2021-00406, Ex. 2051].

(56) **References Cited**

OTHER PUBLICATIONS

Second Declaration of Dr. Aaron Waxman [IPR2021-00406, Ex. 2052].
Declaration of Dr. Jason McConville [IPR2021-00406, Ex. 2053].
Dr. McConville's curriculum vitae [IPR2021-00406, Ex. 2054].
Deposition of Dr. Nicholas Hill (Oct. 17, 2021), IPR2021-00406 [IPR2021-00406, Ex. 2055].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC 2022-2133, Decision on Appeal from the United States Patent and Trademark Office, Patent Trial and Appeal Board in No. IPR2020-00770, decided Jun. 27, 2024.
Petitioner's Notice of Cross-Appeal, dated Aug. 29, 2022 [IPR2020-00770].
Patent Owner's Notice of Appeal, dated Aug. 15, 2022 [IPR2020-00770].
Decision Denying Patent Owner's Request for Rehearing of Final Written Decision, dated Jun. 14, 2022 [IPR2020-00770].
Patent Owner's Request for Panel Rehearing Pursuant to 37 CFR 42.71(d), dated Nov. 8, 2021 [IPR2020-00770].
Final Written Decision: Determining Some Challenged Claims Unpatentable, Denying Petition's Request to Strike, Denying Patent Owner's Motion to Exclude, Granting Petitioner's Motion to Submit Supplemental Information, dated Oct. 8, 2021 [IPR2020-00770].
Record of Jun. 23, 2021 Oral Hearing, entered Oct. 1, 2021 [IPR2020-00770].
Petitioner's Updated Demonstrative Exhibit, dated Jun. 23, 2021 [IPR2020-00770, Exhibit 1056].
Patent Owner's Updated Demonstrative Exhibit, dated Jun. 23, 2021 [IPR2020-00770, Exhibit 2037].
Petitioner's Demonstrative Exhibit [IPR2020-00770, Exhibit 1055].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, No. 20-755-RGA-JLH (D.Del), Claim Construction Order, Jun. 16, 2021 [IPR2020-00770, Exhibit 2035].
Patent Owner's Demonstrative Exhibit [IPR2020-00770, Exhibit 2036].
Patent Owner's Opposition to Petitioner's Motion to Submit Supplemental Information, dated Jun. 21, 2021 [IPR2020-00770].
Petitioner's Motion to Submit Supplemental Evidence, dated Jun. 15, 2021 [IPR2020-00770].
Transcript from Jun. 4, 2021 Markman Hearing in *United Therapeutics Corp.* v. *Liquidia Technologies, Inc.*, No. 20-755-RGA-JLH (D. Del) [IPR2020-00770, Exhibit 1053].
Patent Owner's Reply to Opposition to Motion to Exclude, dated Jun. 8, 2021 [IPR2020-00770].
Patent Owner's Objections to Petitioner's Supplemental Evidence Submitted with Petitioner's Opposition to Patent Owner's Motion to Exclude, dated Jun. 8, 2021 [IPR2020-00770].
Petitioner's Opposition to Patent Owner's Motion to Exclude, dated Jun. 1, 2021 [IPR2020-00770].
Certified English translation of Japanese Patent App. No. 56-122328A to Kawakami, et al., Ex. 1007 in *Steadymed Ltd.* v. *United Therapeutics Corp.*, IPR2016-00006 [IPR2020-00770, Exhibit 1048].
Affidavit of Boris Levine certifying Translation of Japanese Patent App. No. 56-122328A to Kawakami, et al., previously submitted as Ex. 1011 in *Steadymed Ltd.* v. *United Therapeutics Corp.*, IPR2016-00006 [IPR2020-00770, Exhibit 1049].
Declaration of James Dowdle Under 37 C.F.R. § 42.63(B), previously submitted as Ex. 1019 in *Steadymed Ltd.* v. *United Therapeutics Corp.*, IPR2016-00006 [IPR2020-00770, Exhibit 1050].
Declaration of Boris Levine 37 C.F.R. § 42.63(B), previously submitted as Ex. 1020 in *Steadymed Ltd.* v. *United Therapeutics Corp.*, IPR2016-00006 [IPR2020-00770, Exhibit 1051].
Supplemental Declaration of Sylvia Hall-Ellis, Ph.D. [IPR2020-00770, Exhibit 1052].
Patent Owner's Motion to Exclude, dated May 24, 2021 [IPR2020-00770].
Joint Paper Concerning Petitioner's Request to Strike Portions of Patent Owner's Paper Nos. 12 and 25 and Exhibits 2002 and 2025, dated May 17, 2021 [IPR2020-00770].
Patent Owner's Sur-Reply, dated Apr. 19, 2021 [IPR2020-00770].
Linked-In Profile of Ken Phares, https://www.linkedin.com/in/ken-phares-4b62198 (last accessed Apr. 5, 2021) [IPR2020-00770, Exhibit 2029].
Linked-In Profile of David Mottola, https://www.linkedin.com/in/david-mottola-phd (last accessed Apr. 5, 2021) [IPR2020-00770, Exhibit 2030].
Linked-In Profile of Bob Moriarty, https://www.linkedin.com/in/bob-moriarty-7581539 (last accessed Apr. 5, 2021) [IPR2020-00770, Exhibit 2031].
Second Winkler Deposition Transcript, Apr. 5, 2021 [IPR2020-00770, Exhibit 2032].
Supplemental Declaration of Robert R. Ruffolo, Ph.D., Apr. 2, 2021 (*United Therapeutics Co.* v. *Liquidia Techs., Inc.*, Case No. 1:20-cv-00755-RGA (D. Del.)) [IPR2020-00770, Exhibit 2033].
Ruffolo Deposition Transcript, Apr. 15, 2021 (*United Therapeutics Co.* v. *Liquidia Techs., Inc.*, Case No. 1:20-cv-00755-RGA (D. Del.)) [IPR2020-00770, Exhibit 2034].
Decision Denying Patent Owner's Request on Rehearing of Decision on Institution, dated Mar. 1, 2021 [IPR2020-00770].
Petitioner's Reply, dated Mar. 1, 2021 [IPR2020-00770].
Reply Declaration of Jeffrey D. Winkler, Ph.D. [IPR2020-00770, Exhibit 1017].
Deposition transcript of Rodolfo Pinal, dated Feb. 10, 2021 [IPR2020-00770, Exhibit 1018].
Gao, K., "Synthesis of A-Galceramides, (-)-Treprostinil, and Design and Synthesis of Anti-Viral Agents," Thesis submitted as partial fulfillment of the requirements for the degree of Doctor Of Philosophy in Chemistry in the Graduate College of the University of Illinois at Chicago, 2006 [IPR2020-00770, Exhibit 1019].
Parks, B.W., et al., "Convenient Synthesis of 6,6-Bicyclic Malonamides: A New Class of Conformationally Preorganized Ligands for f-Block Ion Binding," J. Org. Chem., 71:9622-27 (2006) [IPR2020-00770, Exhibit 1020].
Hanessian, S., et al., "Structure-Based Organic Synthesis of a Tricyclic N-Malayamycin Analogue," J. Org. Chem., 71:9807-17 (2006) [IPR2020-00770, Exhibit 1021].
Frost, J.M., et al., "Synthesis and Structure—Activity Relationships of 3,8-Diazabicyclo[4.2.0]octane Ligands, Potent Nicotinic Acetylcholine Receptor Agonists," J. Med. Chem., 49:7843-53 (2006) [IPR2020-00770, Exhibit 1022].
Regan, J., et al., "Quinol-4-ones as Steroid A-Ring Mimetics in Nonsteroidal Dissociated Glucocorticoid Agonists," J. Med. Chem., 49:7887-96 (2006) [IPR2020-00770, Exhibit 1023].
Paulekuhn, G.S., et al., "Trends in Active Pharmaceutical Ingredient Salt Selection based on Analysis of the Orange Book Database," J. Med. Chem., 50:6665-72 (2007) [IPR2020-00770, Exhibit 1024].
Mak, K.K.W., et al., "Mannich Reactions in Room Temperature Ionic Liquids (RTILs): An Advanced Undergraduate Project of Green Chemistry and Structural Elucidation," J. Chem. Ed., 83(6):943-46 (2006) [IPR2020-00770, Exhibit 1025].
Supplemental Materials to Mak, K.K.W., et al., "Mannich Reactions in Room Temperature Ionic Liquids (RTILs): An Advanced Undergraduate Project of Green Chemistry and Structural Elucidation," J. Chem. Ed., 83(6):943-46 (2006) [IPR2020-00770, Exhibit 1026].
Baar, M.R., et al., "Enantiomeric Resolution of (±)-Mandelic Acid by (1R,2S)-(−)-Ephedrine," J. Chem. Ed., 82 (7):1040-42 (2005) [IPR2020-00770, Exhibit 1027].
Supplemental Materials for Baar, M.R., et al., "Enantiomeric Resolution of (±)-Mandelic Acid by (1R,2S)-(−)-Ephedrine," J. Chem. Ed., 82(7):1040-42 (2005) [IPR2020-00770, Exhibit 1028].
Brigandi, L.M., et al., "Synthesis and Analysis of Copper Hydroxy Double Salts," J. Chem. Educ., 82(11):1662 (2005) [IPR2020-00770, Exhibit 1029].
Supplemental Material for Online Publication for Brigandi, L.M., et al., "Synthesis and Analysis of Copper Hydroxy Double Salts," J. Chem. Educ., 82(11):1662 (2005) [IPR2020-00770, Exhibit 1030].
Hamilton, "Experiment #5: Resolution of (R,S)-1-Phenylethylamine via Diastereoisomer formation with (2R),(3R)-Tartaric Acid," Laboratory Manual for Chemistry 202, Organic Chemistry Laboratory I at Mount Holyoke College (2006) [IPR2020-00770, Exhibit 1031].

(56) **References Cited**

OTHER PUBLICATIONS

Yadav, J.S., et al., "A concise and stereoselective synthesis of both enantiomers of altholactone and isoaltholactone," Tetrahedron Letters, 44:5831-33 (2003) [IPR2020-00770, Exhibit 1032].
Declaration of Sylvia Hall-Ellis, Ph.D. [IPR2020-00770, Exhibit 1015].
Prosecution History of the U.S. Pat. No. 8,497,393 [IPR2020-00770, Exhibit 1016].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-22-2133, Opening Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 32, Nov. 28, 2022.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-22-2133, Response Brief Filed by Appellee Liquidia Technologies, Inc., Doc. No. 36, Jan. 6, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-22-2133, Intervenor Brief Filed by Intervenor Katherine K. Vidal., Doc. No. 38, Feb. 16, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-22-2133, Reply Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 43, Mar. 30, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-22-2133, Corrected Reply Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 47, Apr. 7, 2023.
Decision Denying Institution of Inter Partes Review 35 U.S.C. § 314, dated Oct. 13, 2020 [IPR2020-00769].
Patent Owner Preliminary Response Under 35 U.S.C. § 313 and 37 C.F.R. § 42.107, dated Jul. 14, 2020 [IPR2020-00769].
Declaration of Rodolfo Pinal, Ph.D. [IPR2020-00769, Exhibit 2002].
Curriculum Vitae of Rodolfo Pinal, Ph.D. [IPR2020-00769, Exhibit 2003].
21 C.F.R. §210.3 (Apr. 1, 2007 edition) [IPR2020-00769, Exhibit 2004].
Office Action, U.S. Appl. No. 15/423,021 dated Jan. 11, 2018 [IPR2020-00769, Exhibit 2005].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 1 of 6 [IPR2020-00769, Exhibit 2006].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 2 of 6 [IPR2020-00769, Exhibit 2006].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 3 of 6 [IPR2020-00769, Exhibit 2006].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 4 of 6 [IPR2020-00769, Exhibit 2006].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 5 of 6 [IPR2020-00769, Exhibit 2006].
Complete Prosecution History of U.S. Pat. No. 9,593,066, part 6 of 6 [IPR2020-00769, Exhibit 2006].
Stahl, P. H., & Wermuth, C. G. (Eds.). (2001). Handbook of Pharmaceutical Salts (1st ed.). Weinheim, Germany: Wiley-VCH, pp. 1-7, 41-81, 135-220, 249-63 [IPR2020-00769, Exhibit 2008].
Batra, H., et al., Crystallization Process Development for a Stable Polymorph of Treprostinil Diethanolamine (UT-15C) by Seeding, Org. Proc. Res. Dev., 13, 242-49 (2009) [IPR2020-00769, Exhibit 2009].
Wiberg, K., Laboratory Technique in Organic Chemistry (1960), pp. 75-119. [IPR2020-00769, Exhibit 2010].
Schoffstall, A. M., et al., Microscale and Miniscale Organic Chemistry Laboratory Experiments, 2nd ed. (2004), pp. 22-27, 537-77 [IPR2020-00769, Exhibit 2011].
Comparing IPR2020-00769 EX1002 to the Petition in IPR2020-00769 [IPR2020-00769, Exhibit 2012].
U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: ANDAs: Pharmaceutical Solid Polymorphism, Chemistry, Manufacturing, and Controls Information (Jul. 2007) ("Polymorph Guidance") [IPR2020-00769, Exhibit 2016].
Petition for Inter Partes Review of U.S. Pat. No. 9,593,066, dated Mar. 30, 2020 [IPR2020-00769].
Declaration of Jeffrey D. Winkler, Ph.D. ("Winkler Decl.") [IPR2020-00769, Exhibit 1002].
Curriculum Vitae of Dr. Jeffrey D. Winkler [IPR2020-00769, Exhibit 1003].
SteadyMed Ltd. v. *United Therapeutics Corp.*, IPR2016-00006, Paper 82 (PTAB Mar. 31, 2017) ("IPR2016-00006") [IPR2020-00769, Exhibit 1005].
Prosecution History of U.S. Pat. No. 9,593,066 [IPR2020-00769, Exhibit 1006].
Moriarty, R.M., et al., "The Intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzindene Prostacyclins: Synthesis of UT-15 (Treprostinil)," J. Org. Chem. vol. 69, No. 6,1890-1902 (2004) ("Moriarty") [IPR2020-00769, Exhibit 1009].
Wiberg, K., Laboratory Technique in Organic Chemistry (1960), p. 112 ("Wiberg") [IPR2020-00769, Exhibit 1010].
Schoffstall, A.M et al., Microscale and Miniscale Organic Chemistry Laboratory Experiments, 2d ed. (2004) pp. 200-202 [IPR2020-00769, Exhibit 1011].
Ege, S., Organic Chemistry Second Edition, Ch. 14 Carboxylic Acids and Their Derivates I. Nucleophilic Substitution Reactions at the Carbonyl Group (1989) pp. 543-547 ("Ege") [IPR2020-00769, Exhibit 1013].
Declaration of Sylvia Hall-Ellis, Ph.D. [IPR2020-00769, Exhibit 1015].
Prosecution History of U.S. Pat. No. 8,497,393 [IPR2020-00769, Exhibit 1016].
Takadoi, M., et al., "Synthetic studies of himbacine, a potent antagonist of the muscarinic M2 subtype receptor 1. Stereoselective total synthesis and antagonistic activity of enantiomeric pairs of himbacine and (2'S,6'R)-diepihimbacine, 4-epihimbacine, and novel himbacine congeners," Tetrahedron 58 (2002) 9903-23 [IPR2020-00770, Exhibit 1033].
Berge, S.M., et al., "Pharmaceutical Salts," J. Pharm. Scis., 66(1):1-19 (1977) [IPR2020-00770, Exhibit 1034].
Excerpts from Loewenthal, H.J.E., et al., A Guide for the Perplexed Organic Experimentalist, Chapter 4: Running Small-scale Reactions in the Research Laboratory, pp. 87-119, 2d ed. (1990) [IPR2020-00770, Exhibit 1035].
Heidelberger, M., An Advanced Laboratory Manual of Organic Chemistry (1923) [IPR2020-00770, Exhibit 1036].
Product Information for Remodulin (2006) ("Remodulin Label") [IPR2020-00770, Exhibit 1037].
Excerpt from Hawley's Condensed Chemical Dictionary, 15th ed. (2007) [IPR2020-00770, Exhibit 1038].
Refiled Declaration of Jeffrey D. Winkler, Ph.D. (Ex. 1002) [IPR2020-00770, Exhibit 1039].
Patent Owner's Response to Petition, dated Dec. 21, 2020 [IPR2020-00770].
Anderson, Practical Process Research and Development, Second Ed., Academic Press, Oxford, UK, 2012 [IPR2020-00770, Exhibit 2017].
Gad, Pharmaceutical Manufacturing Handbook, Production and Processes, John Wiley & Sons, Hoboken, NJ (2008) [IPR2020-00770, Exhibit 2018].
Levin, Pharmaceutical Process Scale-Up, Taylor & Francis, Boca Raton, FL (2006) [IPR2020-00770, Exhibit 2019].
Bennett, Pharmaceutical Production. An Engineering Guide, Institution of Chemical Engineers (ICHEME), Rugby, Warwickshire, UK (2003) [IPR2020-00770, Exhibit 2020].
Qiu, et al., Identification of pharmaceutical impurities, J. Liquid Chromatograph. Rel. Techs., 30, 877-935 [IPR2020-00770, Exhibit 2021].
Ahuja, et al., Assuring quality of drugs by monitoring impurities, Adv. Drug Delivery Rev., 59, 3-11 (2007) [IPR2020-00770, Exhibit 2022].
Paul, et al., Design of Reaction Systems for Specialty Organic Chemicals, Chem. Engineer. Sci., 43, 1773-82 (1988) ("Paul") [IPR2020-00770, Exhibit 2023].
Wadekar, et al., Evaluating Impurities in Drugs (Part I of III), Pharm. Tech., 36(2), 46-51 (2012) [IPR2020-00770, Exhibit 2024].
Declaration of Rodolfo Pinal, Ph.D. Supporting Patent Owner Response [IPR2020-00770, Exhibit 2025].
Winkler Deposition Transcript, Dec. 14, 2020 [IPR2020-00770, Exhibit 2026].

(56) **References Cited**

OTHER PUBLICATIONS

Prosecution History of U.S. Appl. No. 13/933,623 [IPR2020-00770, Exhibit 2028].
Patent Owner's Objections To Petitioner's Evidence Submitted With Petition, dated Oct. 27, 2020 [IPR2020-00770].
Patent Owner's Request for Rehearing Under 37 C.F.R. § 42.71, dated Oct. 27, 2020 [IPR2020-00770].
Decision Granting Institution of Inter Partes Review 35 U.S.C. § 314, dated Oct. 13, 2020 [IPR2020-00770].
Patent Owner Preliminary Response Under 35 U.S.C. § 313 and 37 C.F.R. § 42.107, dated Jul. 14, 2020 [IPR2020-00770].
Declaration of Rodolfo Pinal, Ph.D. [IPR2020-00770, Exhibit 2002].
Curriculum Vitae of Rodolfo Pinal, Ph.D. [IPR2020-00770, Exhibit 2003].
21 C.F.R. § 210.3 (Apr. 1, 2007 edition) [IPR2020-00770, Exhibit 2004].
Office Action, U.S. Appl. No. 15/423,021 dated Jan. 11, 2018 [IPR2020-00770, Exhibit 2005].
Prosecution History of U.S. Pat. No. 9,604,901, Part 1 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 2 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 3 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 4 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 5 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 6 of 7 [IPR2020-00770, Exhibit 2007].
Prosecution History of U.S. Pat. No. 9,604,901, Part 7 of 7 [IPR2020-00770, Exhibit 2007].
Stahl, P. H., & Wermuth, C. G. (Eds.). (2002). Handbook of Pharmaceutical Salts (1st ed.). Weinheim, Germany: Wiley-VCH, pp. 1-7, 41-81, 135-220, 259-63 [IPR2020-00770, Exhibit 2008].
Batra, H., et al., Crystallization Process Development for a Stable Polymorph of Treprostinil Diethanolamine (UT-15C) by Seeding, Org. Proc. Res. Dev., 13, 242-49 (2009) [IPR2020-00770, Exhibit 2009].
Wiberg, K., Laboratory Technique in Organic Chemistry (1960), pp. 75-119 [IPR2020-00770, Exhibit 2010].
Schoffstall, A. M., et al., Microscale and Miniscale Organic Chemistry Laboratory Experiments, 2nd ed. (2004), pp. 22-27, 537-59 [IPR2020-00770, Exhibit 2011].
Comparing IPR2020-00770 Ex. 1002 to the Petition in IPR2020-00770 [IPR2020-00770, Exhibit 2013].
U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry: ANDAs: Pharmaceutical Solid Polymorphism, Chemistry, Manufacturing, and Controls Information (Jul. 2007) [IPR2020-00770, Exhibit 2016].
Petition for Inter Partes Review of U.S. Pat. No. 9,604,901, dated Mar. 30, 2020 [IPR2020-00770].
Declaration of Jeffrey D. Winkler, Ph.D. ("Winkler Decl.") [IPR2020-00770, Exhibit 1002].
Curriculum Vitae of Dr. Jeffrey D. Winkler [IPR2020-00770, Exhibit 1003].
SteadyMed Ltd. v. *United Therapeutics Corp.*, IPR2016-00006, Paper 82 (PTAB Mar. 31, 2017) ("IPR2016-00006") [IPR2020-00770, Exhibit 1005].
Prosecution History of the U.S. Pat. No. 9,604,901 [IPR2020-00770, Exhibit 1006].
Moriarty, R.M., et al., "The Intramolecular Asymmetric Pauson-Khand Cyclization as a Novel and General Stereoselective Route to Benzindene Prostacyclins: Synthesis of UT-15 (Treprostinil)," J. Org. Chem. vol. 69, No. 6, 1890-1902 (2004) ("Moriarty") [IPR2020-00770, Exhibit 1009].
Wiberg, K., Laboratory Technique in Organic Chemistry (1960), p. 112 ("Wiberg") [IPR2020-00770, Exhibit 1010].
Schoffstall, A.M. et al., Microscale and Miniscale Organic Chemistry Laboratory Experiments, 2d ed. (2004) pp. 200-202 ("Schoffstall") [IPR2020-00770, Exhibit 1011].
Certified English translation of Japanese Patent App. No. 56-122328A to Kawakami, et al. ("Kawakami") [IPR2020-00770, Exhibit 1012].
Ege, S., Organic Chemistry Second Edition, Ch. 14 Carboxylic Acids and Their Derivates I. Nucleophilic Substitution Reactions at the Carbonyl Group (1989) pp. 543-547 ("Ege") [IPR2020-00770, Exhibit 1013].
Decision of Rejection, mailed Jan. 6, 2025, for Japanese Patent Application No. 2023-031081 w/ translation.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC 2023-1805, Order denying petition for writ of certiorari, entered Oct. 7, 2024.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC 2023-1805, Decision on Appeal from the United States Patent and Trademark Office, Patent Trial and Appeal Board in No. IPR2021-00406, decided Dec. 20, 2023.
Patent Owner's Notice of Appeal to the Federal Circuit, dated Apr. 5, 2023 [IPR2021-00406].
Final Written Decision: Denying Patent Owners Request on Rehearing of Final Written Decision 37 C.F.R. sec. 42.71d, dated Feb. 2, 2023 [IPR2021-00406].
Patent Owner's Request for Rehearing, dated Aug. 18, 2022 [IPR2021-00406].
Final Written Decision Determining All Challenged Claims Unpatentable, entered Jul. 19, 2022 [IPR2021-00406].
Record of May 13, 2022 Oral Hearing, entered Jun. 22, 2022 [IPR2021-00406].
Patent Owner's Demonstrative Exhibit [IPR2021-00406, Ex. 2109].
Petitioner's Demonstrative Exhibit [IPR2021-00406, Ex. 1134].
DiNatale Declaration regarding EX2094 [IPR2021-00406, Ex. 2105].
Internet Archive Declaration regarding EX2100 and EX2101 [IPR2021-00406, Ex. 2106].
Revilla Declaration regarding EX2102 [IPR2021-00406, Ex. 2107].
Patent Owner's Opposition to Petitioner's Motion to Exclude, dated Apr. 27, 2022 [IPR2021-00406].
Petitioner's Opposition to Patent Owner's Motion to Exclude, dated Apr. 27, 2022 [IPR2021-00406].
Patent Owner's Motion to Exclude Evidence, dated Apr. 20, 2022 [IPR2021-00406].
Petitioner's Motion to Exclude Exhibits, dated Apr. 20, 2022 [IPR2021-00406].
Deposition of Dr. Nicholas Hill (Apr. 13, 2022), IPR2021-00406 [IPR2021-00406, Ex. 2108].
Patent Owner's Observations on Apr. 13, 2022 Deposition of Dr. Hill, dated Apr. 15, 2022 [IPR2021-00406].
Petitioner's Observations on the Cross-Examination of Dr. Hill, dated Apr. 15, 2022 [IPR2021-00406].
Patent Owner's Identification of Portions of Petitioner's Reply to Which Identified Sur-Reply Evidence and Arguments Are Responsive, dated Apr. 14, 2022 [IPR2021-00406].
Petitioner's Identification of Exhibits and Portions of PO's Sur-Reply that Exceed the Scope of Allowable Sur-Reply Evidence, dated Apr. 7, 2022 [IPR2021-00406].
Petition's Objections to Evidence, dated Mar. 23, 2022 [IPR2021-00406].
Patent Owner Sur-Reply, dated Mar. 16, 2022 [IPR2021-00406].
Deposition of Dr. Sylvia Hall-Ellis, Ph. D. (Mar. 11, 2022), IPR2021-00406, with exhibits: Deposition Exhibit 2092: British Library Communication from Rupert Lee; Deposition Exhibit 2093: McLaughlin et al., Addition of Inhaled Treprostinil to Oral Therapy for Pulmonary Arterial Hypertension, JACC 55(18):1915-1922 (2010) [IPR2021-00406, Ex. 2094].
Deposition of Christopher Butler (Mar. 10, 2022), IPR2021-00406 [IPR2021-00406, Ex. 2095].
Deposition of Igor Gonda, Ph.D. (Mar. 14, 2022), IPR2021-00406 [IPR2021-00406, Ex. 2099].
Schill: Multisonic, Inhaling with ultrasonic infraControl, Instructions for Use (2004) (Exhibit from Mar. 14, 2022 Deposition of Dr. Gonda, referenced as "Tab 6") [IPR2021-00406, Ex. 2100].

(56) **References Cited**

OTHER PUBLICATIONS

Schill: Multisonic, Inhaling with ultrasonic infraControl, Instructions for Use (Exhibit from Mar. 14, 2022 Deposition of Dr. Gonda, referenced as "Tab 5") [IPR2021-00406, Ex. 2101].
DeVilbiss, UltraNeb, Ultrasonic Nebulizer manual (Exhibit from Mar. 14, 2022 Deposition of Dr. Gonda, referenced as "Tab 9") [IPR2021-00406, Ex. 2102].
Lieberman, et al., In Vitro Performance of the MyNeb® Nebulizer: A New Portable Aerosol Delivery System (Exhibit from Mar. 14, 2022 Deposition of Dr. Gonda, referenced as "Tab 4") [IPR2021-00406, Ex. 2103].
Transcript of Telephonic Hearing (Mar. 1, 2022) [IPR2021-00406, Ex. 2104].
Petitioner's Identification of Portions of Patent Owner's Response to Which Identified Reply Evidence and Arguments are Responsive, dated Feb. 28, 2022 [IPR2021-00406].
Patent Owner's Objections to Petitioner's Evidence Submitted with Petitioner Reply, dated Feb. 17, 2022 [IPR2021-00406].
Patent Owner's Identification of Non-Responsive Evidence and Arguments, dated Feb. 22, 2022 [IPR2021-00406].
Reply in Support of Petition for Inter Partes Review of U.S. Pat. No. 10,716,793 B2, filed Feb. 10, 2022 [IPR2021-00406].
Butler Affidavit [IPR2021-00406, Ex. 1087].
Tyvaso® Label 2021 [IPR2021-00406, Ex. 1088].
Voswinckel JESC, UWash [IPR2021-00406, Ex. 1089].
Voswinckel JESC, UWisc [IPR2021-00406, Ex. 1090].
Voswinckel JESC, British Library [IPR2021-00406, Ex. 1091].
Voswinckel JESC, Additional Pages [IPR2021-00406, Ex. 1092].
Voswinckel JAHA, British Library [IPR2021-00406, Ex. 1093].
Voswinckel JAHA, Library of Congress [IPR2021-00406, Ex. 1094].
Voswinckel JAHA, Stanford [IPR2021-00406, Ex. 1095].
Voswinckel JAHA, UC Davis [IPR2021-00406, Ex. 1096].
Rhind, G.B. et al., "Effect of Spirometry of Distilled Water and Cromoglycate Solutions Nebulised by a Small Portable Ultrasonic Nebuliser," Respiration, 51:86-90 (1987) ("Rhind 1987") [IPR2021-00406, Ex. 1097].
Hager, J., et al., "Measurement of Particle and Mass Distribution of Pentamidine Aerosol by Ultrasonic and Air Jet Nebulizers," Journal of Aerosol Medicine, vol. 5, No. 2 (1992) ("Hager 1992") [IPR2021-00406, Ex. 1098].
Leigh, T.R., et al., "Performance characteristics of the DeVilbiss Ultraneb 99 ultrasonic nebuliser with reference to use in sputum induction," International Journal of Pharmaceutics, 67 (1991) 275-282 ("Leigh 1991") [IPR2021-00406, Ex. 1099].
Ventavis EU Summary of Product Characteristics [IPR2021-00406, Ex. 1100].
Chattaraj, S.C., "Treprostinil sodium Pharmacia," Current Opinion in Investigational Drugs, 3(4):582-86 (Apr. 2002), available at https://pubmed.ncbi.nlm.nih.gov/12090728/ ("Chattaraj") [IPR2021-00406, Ex. 1035].
Declaration of Sylvia Hall-Ellis, Ph.D. dated Jan. 7, 2021 ("Hall-Ellis Decl.") [IPR2021-00406, Ex. 1036].
English translation of OptiNeb® User Manual 2005 [IPR2021-00406, Ex. 1037].
Atkins, P.J., "Dry Powder Inhalers: An Overview," Respiratory Care, 50(10):1304-12 (2005) ("Atkins") [IPR2021-00406, Ex. 1038].
Frijlink, H.W. and De Boer, A.H., "Dry powder inhalers for pulmonary drug delivery," Expert Opinion on Drug Delivery, 1 (1):67-86 (2004) ("Frijlink and De Boer") [IPR2021-00406, Ex. 1039].
Chew N. and Chan H.-K., "Pharmaceutical Dry Powder Aerosol Delivery," KONA, No. 19, pp. 46-56 (2001) ("Chew and Chan") [IPR2021-00406, Ex. 1040].
Jan. 27, 2020 Press Release, "Liquidia Submits New Drug Application for LIQ861 (Treprostinil) Inhalation Powder to U.S. Food And Drug Administration for the Treatment of Pulmonary Arterial Hypertension (PAH)," available at https://investors.liquidia.com/news-releases/news-release-details/liquidia-submits-new-drug-application-liq861-treprostinil [IPR2021-00406, Ex. 1042].
2009 Tyvaso® Label, available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/022387s015lbl.pdf [IPR2021-00406, Ex. 1043].
U.S. Pat. No. 9,358,240 Patent Prosecution History (excerpted) [IPR2021-00406, Ex. 1044].
Hoeper, M.M., et al., "Long-Term Treatment of Primary Pulmonary Hypertension with Aerosolized Iloprost, a Prostacyclin Analogue," N Engl J Med, 342:1866-70 (2000) ("Hoeper") [IPR2021-00406, Ex. 1047].
Walmrath, D., et al., "Aerosolised prostacyclin in adult respiratory distress syndrome," Lancet, 342:961-62 (1993) ("Walmrath 1993") [IPR2021-00406, Ex. 1048].
Apr. 8, 2020 Press Release, "Liquidia Announces FDA Acceptance of New Drug Application for LIQ861 (Treprostinil) Inhalation Powder for the Treatment of Pulmonary Arterial Hypertension," available at https://investors.liquidia.com/news-releases/news- release-details/liquidia-announces-fda-acceptance-new-drug-application-liq861 [IPR2021-00406, Ex. 1049].
Pulmozyme® Label [IPR2021-00406, Ex. 1050].
Farber, H.W. and Loscalzo, J., "Pulmonary Arterial Hypertension," N Engl J Med, 351:1655-65 (2004) ("Farber and Loscalzo") [IPR2021-00406, Ex. 1051].
Rubin, L.J. and Badesch, D.B., "Evaluation and Management of the Patient with Pulmonary Arterial Hypertension," Ann Intern Med., 143:282-92 (2005) ("Rubin and Badesch") [IPR2021-00406, Ex. 1052].
Flolan® Label [IPR2021-00406, Ex. 1053].
Gonda, I., "A semi-empirical model of aerosol deposition in the human respiratory tract for mouth inhalation," J. Pharm. Pharmacol., 33:692-96 (1981) ("Gonda 1981") [IPR2021-00406, Ex. 1054].
Gonda, I., "Study of the effects of polydispersity of aerosols on regional deposition in the respiratory tract," J. Pharm. Pharmacol., 33 (Suppl.) 52P (1981) ("Gonda 1981b") [IPR2021-00406, Ex. 1055].
Telko, M.J. and Hickey, A.J., "Dry Powder Inhaler Formulation," Respiratory Care, 50(9):1209-27 (2005) ("Telko and Hickey") [IPR2021-00406, Ex. 1056].
Oct. 24, 2005 Press Release, "Aradigm Corporation And United Therapeutics Corporation Sign Development and Commercialization Agreement Targeting Pulmonary Hypertension," available at https://www.biospace.com/article/releases/aradigm-corporation-and-united-therapeutics-corporation-sign-development-and-commercialization-agreement-targeting-pulmonary-hypertension-/ [IPR2021-00406, Ex. 1057].
Ziegler, J. and Wachtel, H., "Comparison of Cascade Impaction and Laser Diffraction for Particle Size Distribution Measurements," Journal of Aerosol Medicine, 18(3):311-24 (2005) ("Ziegler and Wachtel") [IPR2021-00406, Ex. 1058].
Nauser, T.D., "Pulmonary Hypertension: New Perspectives," CHF, 9:155-62 (2003) ("Nauser 2003") [IPR2021-00406, Ex. 1059].
Pitcairn, G., et al., "Deposition of Corticosteroid Aerosol in the Human Lung by Respimat® Soft Mist™ Inhaler Compared to Deposition by Metered Dose Inhaler or by Turbuhaler® Dry Powder Inhaler," Journal of Aerosol Medicine, 18(3):264-72 (2005) ("Pitcairn") [IPR2021-00406, Ex. 1060].
Dalby, R., et al., "A review of the development of Respimat® Soft MistTM Inhaler," International Journal of Pharmaceutics, 283:1-9 (2004) ("Dalby") [IPR2021-00406, Ex. 1061].
Gessler, T., et al., "Ultrasonic versus jet nebulization of iloprost in severe pulmonary hypertension," Eur Respir J, 17:14-19 (2001) ("Gessler") [IPR2021-00406, Ex. 1062].
Dolovich, M.B., et al., "Device Selection and Outcomes of Aerosol Therapy: Evidence-Based Guidelines," Chest, 127:335-71 (2005) ("Dolovich") [IPR2021-00406, Ex. 1064].
Olschewski, H., et al., "Inhaled Iloprost for Severe Pulmonary Hypertension," N Engl J Med, 347(5):322-29 (2002) ("Olschewski 2002") [IPR2021-00406, Ex. 1065].
AccuNeb® Label [IPR2021-00406, Ex. 1066].
Anderson, P.J., "History of Aerosol Therapy: Liquid Nebulization to MDIs to DPIs," Respiratory Care, 50(9):1139-49 (2005) ("Anderson 2005") [IPR2021-00406, Ex. 1067].

(56) **References Cited**

OTHER PUBLICATIONS

Vachiéry, J.-L., et al., "Transitioning From IV Epoprostenol to Subcutaneous Treprostinil in Pulmonary Arterial Hypertension," Chest, 121:1561-65 (2002) ("Vachiéry 2002") [IPR2021-00406, Ex. 1068].
Zierenberg, B. and Eicher, J., Chapter 78 "The Respimat, a New Soft Mist Inahler for Delivering Drugs to The Lungs," Modified-Release Drug Delivery Technology (2002) pp. 925-933 ("Zierenberg") [IPR2021-00406, Ex. 1069].
Beasley, R., et al., "Preservatives in Nebulizer Solutions: Risks without Benefit," Pharmacotherapy, 18(1):130-39 (1998) ("Beasley") [IPR2021-00406, Ex. 1070].
Prober, C.G., et al., "Technical Report: Precautions Regarding the Use of Aerosolized Antibiotics," Pediatrics, 106 (6):1-6 (2000) ("Prober") [IPR2021-00406, Ex. 1071].
Aradigm Corporation Form 10-Q for the quarterly period ended Jun. 30, 2009, available at https://www.sec.gov/Archives/edgar/data/1013238/00009501230903 1361/f53244e10vq.htm [IPR2021-00406, Ex. 1073].
Orenitram® Label, available at https://www.orenitram.com/pdf/Orenitram-Prescribing-Information.pdf [IPR2021-00406, Ex. 1074].
Nov. 17, 2008 Press Release, "Eli Lilly and Company Licenses U.S. Rights for Tadalafil PAH Indication to United Therapeutics Corporation," available at https://www.fiercebiotech.com/biotech/eli-lilly-and-company- licenses-u-s-rights-for-tadalafil-pah-indication-to-united [IPR2021-00406, Ex. 1075].
Oct. 23, 2017 Press Release, "United Therapeutics Announces FDA Approval Of Third Generation Nebulizer For The Tyvaso® Inhalation System," available at https://www.prnewswire.com/news-releases/united-therapeutics-announces-fda-approval-of-third-generation-nebulizer-for-the-tyvaso-inhalation-system- 300540953.html [IPR2021-00406, Ex. 1076].
Boyle, M.P., "So Many Drugs, So Little Time. The Future Challenge of Cystic Fibrosis Care," Chest, 123(1):3-5 (2003) ("Boyle 2003") [IPR2021-00406, Ex. 1077].
Azmacort® Label 2003 [IPR2021-00406, Ex. 1078].
Hill, N.S., et al., "Inhaled Therapies for Pulmonary Hypertension," Respiratory Care, 60(6):794-805 (2015) ("Hill 2015") [IPR2021-00406, Ex. 1079].
Patent Owner's Objections to Petitioner's Evidence Submitted with Petition, dated Aug. 25, 2021 [IPR2021-00406].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Opening Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 17, Aug. 4, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Response Brief Filed by Appellee Liquidia Technologies, Inc., Doc. No. 21, Sep. 6, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Reply Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 29, Oct. 11, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Corrected Opening Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 39, Oct. 20, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Corrected Response Brief Filed by Appellee Liquidia Technologies, Inc., Doc. No. 45, Nov. 1, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Corrected Reply Brief Filed by Appellant United Therapeutics Corporation, Doc. No. 48, Nov. 2, 2023.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Petition for en banc rehearing, for panel rehearing filed by Appellant United Therapeutics Corporation, Doc. No. 54, Jan. 19, 2024.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Response of Appellee Liquidia Technologies, Inc. to Appellant's Petition for Panel Rehearing and Rehearing en banc, Doc. No. 57, Feb. 26, 2024.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, CAFC-23-1805, Order filed denying [54] petition for panel rehearing; rehearing en banc filed by United Therapeutics Corporation, Doc. No. 63, Mar. 12, 2024.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-21 (public docket) [IPR2021-00406, Ex. 2017].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-41 (public docket) [IPR2021-00406, Ex. 2018].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-49 (public docket) [IPR2021-00406, Ex. 2019].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-68 (public docket) [IPR2021-00406, Ex. 2020].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-71 (public docket) [IPR2021-00406, Ex. 2021].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-40 (public docket) [IPR2021-00406, Ex. 2022].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-47 (public docket) [IPR2021-00406, Ex. 2023].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-75 (public docket) [IPR2021-00406, Ex. 2024].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-80 (public docket) [IPR2021-00406, Ex. 2025].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-81 (public docket) [IPR2021-00406, Ex. 2026].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-92 (public docket) [IPR2021-00406, Ex. 2027].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-74 (public docket) [IPR2021-00406, Ex. 2028].
Hess et al., 2007, A guide to aerosol delivery devices for respiratory therapists. American Association for Respiratory Care [IPR2021-00406, Ex. 2029].
Dennis JH, 2002, Standardization issues: in vitro assessment of nebulizer performance. Respir. Care. 47 (12):1455-1458 [IPR2021-00406, Ex. 2030].
Hess et al., 1996, Medication nebulizer performance. Effects of diluent volume nebulizer flow, and nebulizer brand. Chest, 110(2):498-505 [IPR2021-00406, Ex. 2031].
Rubin BK et al., 2008 Treatment Delivery Systems (in Clinical Asthma), available at https://www.sciencedirect.com/topics/medicine-anddentistry/nebulizer [IPR2021-00406, Ex. 2032].
Gardenhire, D.S. et al., 2017, A Guide to Aerosol Delivery Devices for Respiratory Therapists (4th Ed.) American Association for Respiratory Care [IPR2021-00406, Ex. 2033].
Tyvaso® Label 2021 [IPR2021-00406, Ex. 2034].
Bourge et al., Cardiovascular Therapeutics, 31:38-44 (2013) [IPR2021-00406, Ex. 2035].
Petition for Inter Partes Review of U.S. Pat. No. 10,716,793 B2, filed Jan. 7, 2021 [IPR2021-00406].
Declaration of Dr. Nicholas Hill, dated Jan. 7, 2021 ("Hill Decl.") [IPR2021-00406, Ex. 1002].
Curriculum Vitae of Dr. Nicholas Hill (May 2020) [IPR2021-00406, Ex. 1003].
Declaration of Dr. Igor Gonda, dated Jan. 7, 2021 ("Gonda Decl.") [IPR2021-00406, Ex. 1004].
Curriculum Vitae of Dr. Igor Gonda (2020) [IPR2021-00406, Ex. 1005].
Voswinckel, R., et al., Abstract 218: "Inhaled treprostinil is a potent pulmonary vasodilator in severe pulmonary hypertension," European Heart Journal 25:22 (2004) ("Voswinckel JESC") [IPR2021-00406, Ex. 1007].

(56) **References Cited**

OTHER PUBLICATIONS

Robert Voswinckel, et al., Abstract 1414: "Inhaled Treprostinil Sodium (TRE) For the Treatment of Pulmonary Hypertension," Abstracts from the 2004 Scientific Sessions of the American Heart Association, Circulation, 110(17 Suppl.):III-295 (Oct. 26, 2004) [IPR2021-00406, Ex. 1008].
Robert Voswinckel, Hossein A. Ghofrani, Friedrich Grimminger, and Werner Seeger, "Clinical Observations" on "Inhaled Treprostinil for Treatment of Chronic Pulmonary Arterial Hypertension," "Letters" Section of the Annals of Internal Medicine, 144(2):149-50 (Jan. 2006) [IPR2021-00406, Ex. 1009].
Hossein Ardeschir Ghofrani, Robert Voswinckel, et al., Neue Therapieoptionen in der Behandlung der pulmonalarteriellen Hypertonie, 30(4) HERZ, 30(4):296-302 (Jun. 2005) ("Ghofrani") (Foreign article and English translation attached) [IPR2021-00406, Ex. 1010].
First Amended Complaint filed Jul. 22, 2020 in *United Therapeutics Corporation* v. *Liquidia Technologies, Inc*., Case No. 1:20-cv-00755-RGA (D. Del.) [IPR2021-00406, Ex. 1011].
United Therapeutics Corporation's Answer to Defendant Liquidia Technologies, Inc.'s Counterclaims filed in *United Therapeutics Corporation* v. *Liquidia Technologies, Inc*., Case No. 1:20-cv-00755-RGA (D. Del.) [IPR2021-00406, Ex. 1012].
United Therapeutics Corporation's Opening Brief in Support of its Motion to Dismiss Defendant's Counterclaim filed Aug. 26, 2020 in *United Therapeutics Corporation* v. *Liquidia Technologies, Inc*., Case No. 1:20-cv-00755-RGA (D. Del.) [IPR2021-00406, Ex. 1013].
Memorandum Order denying United Therapeutics Corporation's Motion to Dismiss Defendants Counterclaim filed Nov. 3, 2020 in *United Therapeutics Corporation* v. *Liquidia Technologies, Inc*., Case No. 1:20-cv-00755-RGA (D. Del.) [IPR2021-00406, Ex. 1014].
U.S. Pat. No. 10,716,793 Patent Prosecution History [IPR2021-00406, Ex. 1015].
U.S. Pat. No. 10,376,525 Patent Prosecution History (excerpted) [IPR2021-00406, Ex. 1016].
U.S. Pat. No. 9,339,507 Patent Prosecution History (excerpted) [IPR2021-00406, Ex. 1017].
Remodulin® 2004 Label [IPR2021-00406, Ex. 1018].
Stein, S.W., et al., "The History of Therapeutic Aerosols: A Chronological Review," Journal of Aerosol Medicine and Pulmonary Drug Delivery, 30(1):20-41 (2017) ("Stein") [IPR2021-00406, Ex. 1019].
Clark, A.R., "Medical Aerosol Inhalers: Past, Present, and Future," Aerosol Science and Technology, 22:374-91 (1995) ("Clark") [IPR2021-00406, Ex. 1020].
Ruan, C.-H., et al., "Prostacyclin Therapy for Pulmonary Arterial Hypertension," Texas Heart Institute Journal, 37 (4):391-99 (2010) ("Ruan") [IPR2021-00406, Ex. 1021].
Walmrath, D., et al., "Direct Comparison of Inhaled Nitric Oxide and Aerosolized Prostacyclin in Acute Respiratory Distress Syndrome," American Journal of Respiratory Critical Care Medicine, 153:991-96 (1996) ("Walmrath 1996") [IPR2021-00406, Ex. 1022].
Olschewski, H., et al., "Inhaled Prostacyclin and Iloprost in Severe Pulmonary Hypertension Secondary to Lung Fibrosis," American Journal of Respiratory Critical Care Medicine, 160:600-07 (1999) ("Olschewski 1999") [IPR2021-00406, Ex. 1023].
Haché, M., et al., "Inhaled epoprostenol (prostacyclin) and pulmonary hypertension before cardiac surgery," Journal of Thoracic and Cardiovascular Surgery, 125:642-49 (2003) ("Hache") [IPR2021-00406, Ex. 1024].
De Wet, C.J., et al., "Inhaled prostacyclin is safe, effective, and affordable in patients with pulmonary hypertension, right heart dysfunction, and refractory hypoxemia after cardiothoracic surgery," Journal of Thoracic and Cardiovascular Surgery, 127:1058-67 (2004) ("De Wet") [IPR2021-00406, Ex. 1025].
Denver, J. and Dyche, T., "The Adaptive Aerosol Delivery (AAD) Technology: Past, Present, and Future," Journal of Aerosol Medicine and Pulmonary Drug Delivery, 23(1 suppl):S-1-S10 (2010) ("Denver and Dyche") [IPR2021-00406, Ex. 1026].
Ventavis® Label 2004 [IPR2021-00406, Ex. 1029].
Newman, S.P., "Aerosols", Chapter from Encyclopedia of Respiratory Medicine pp. 58-64 (2006) ("Newman") [IPR2021-00406, Ex. 1030].
Geller, D.E., "Comparing Clinical Features of the Nebulizer, Metered-Dose Inhaler, and Dry Powder Inhaler," Respiratory Care, 50(10):1313-21 (2005) ("Geller 2005") [IPR2021-00406, Ex. 1031].
Bender, B., et al., "Nonadherence in asthmatic patients: is there a solution to the problem?" Annals of Allergy, Asthma & Immunology, 79:177-86 (1997) ("Bender 1997") [IPR2021-00406, Ex. 1032].
Rau, J.L., "Determinants of Patient Adherence to an Aerosol Regimen," Respiratory Care 50(10):1346-56 (2005) ("Rau 2005") [IPR2021-00406, Ex. 1033].
Geller, D., et al., "Bolus Inhalation of rhDNase with the AERx System in Subjects with Cystic Fibrosis," Journal of Aerosol Medicine, 16(2):175-82 (2003) ("Geller 2003") [IPR2021-00406, Ex. 1034].
Deposition of Igor Gonda, Ph. D. (Oct. 26, 2021), IPR2021-00406 [IPR2021-00406, Ex. 2056].
Vital Signs (Body Temperature, Pulse Rate, Respiration Rate, Blood Pressure), Johns Hopkins Medicine, available at https://www.hopkinsmedicine.org/health/conditions-and-diseases/vital-signs-body-temperature-pulse-rate-respiration-rate-blood-pressure [IPR2021-00406, Ex. 2057].
Pharmacokinetics of Inhaled Drugs, available at https://media.lanecc.edu/users/driscolln/RT127/Softchalk/Pharmcology_SFTCHLK_Lesson/Pharmacology_lesson10.html [IPR2021-00406, Ex. 2058].
Waxman et al., Inhaled Treprostinil in Pulmonary Hypertension Due to Interstitial Lung Disease, N. Eng. J. Med. 384:325-334 (2021) [IPR2021-00406, Ex. 2060].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01622, Declaration of Dr. Robert Roscigno (EX2048) [IPR2021-00406, Ex. 2061].
Declaration of Dr. Werner Seeger regarding U.S. Appl. No. 11/748,205 [IPR2021-00406, Ex. 2065].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621 Declaration of Dr. Werner Seeger (EX2020) [IPR2021-00406, Ex. 2066].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621/-01622 Declaration of Dr. Hossein A. Ghofrani (EX2026) [IPR2021-00406, Ex. 2067].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621/-01622 Declaration of Dr. Frank Reichenberger (EX2027) [IPR2021-00406, Ex. 2068].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621/-01622 Declaration of Dr. Friedich Grimminger (EX2028) [IPR2021-00406, Ex. 2069].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621 Declaration of Dr. Werner Seeger (EX2097) [IPR2021-00406, Ex. 2070].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621 Second Declaration of Dr. Werner Seeger (EX2098) [IPR2021-00406, Ex. 2071].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621 (EX2101) [IPR2021-00406, Ex. 2072].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621 (EX2102) [IPR2021-00406, Ex. 2073].
Watson Labs., Inc. v. *United Therapeutics Inc*., IPR2017-01621/-01622 Second Declaration of Dr. Hossein A. Ghofrani (EX2099) [IPR2021-00406, Ex. 2074].
Le Brun et al., A review of the technical aspects of drug nebulization, Pharmacy World & Science, 22(3):75-81 (2000) [IPR2021-00406, Ex. 2075].
Kendrick, et al., Selecting and Using Nebuliser Equipment, Thorax, 52(Suppl 2):S92-S101 (1997) [IPR2021-00406, Ex. 2076].
Rau et al., Performance Comparison of Nebulizer Designs: Constant-Output, Breath-Enhanced, and Dosimetric, Respiratory Care, 49(2):174-179 (2004) [IPR2021-00406, Ex. 2077].
Rau, The Inhalation of Drugs: Advantages and Problems, Respiratory Care, 50(3):367-382 (2005) [IPR2021-00406, Ex. 2078].
Hess et al., Medication Nebulizer Performance, Laboratory and Animal Investigations, 110(2):498-505 (1996) [IPR2021-00406, Ex. 2079].
FDA Guidance 2002 [IPR2021-00406, Ex. 2080].

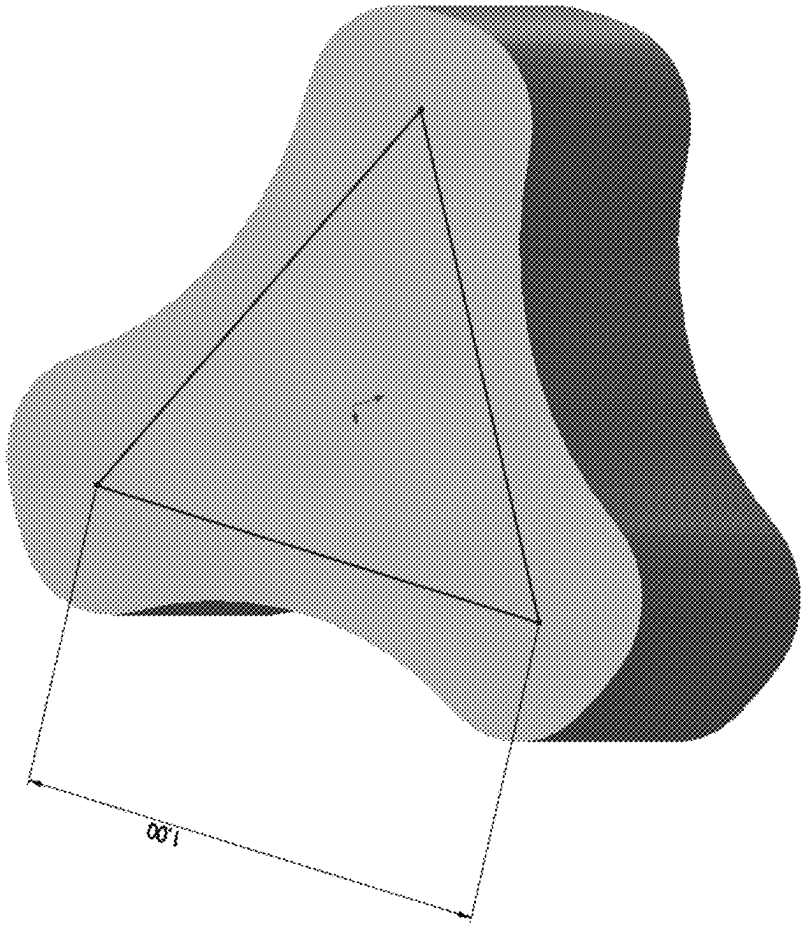
(56) **References Cited**

OTHER PUBLICATIONS

Newman et al., Efficient Delivery to the Lungs of Flunisolide Aerosol from a New Portable Hand-Held Multidose Nebulizer, 1996 85(9) J. Pharm Sciences 960 (1996) [IPR2021-00406, Ex. 2081].
Dubus et al., Aerosol Deposition in Neonatal Ventilation, Pediatric Research, 58(1):10-15 (2005) [IPR2021-00406, Ex. 2082].
Treprostinil, PubChem, available at https://pubchem.ncbi.nlm.nih.gov/compound/Treprostinil [IPR2021-00406, Ex. 2083].
Roscigno et al., 2020 Pharmacokinetics and tolerability of LIQ861, a novel dry-powder formulation of treprostinil. Pulmonary Circulation, 10(4):1-9 (2020) [IPR2021-00406, Ex. 2084].
Roscigno et al., Comparative bioavailability of inhaled treprostinil administered as LIQ861 and Tyvaso® in healthy subjects, Vascular Pharmacology 138:106840 (2021) [IPR2021-00406, Ex. 2085].
Declaration of Dr. Roham T. Zamanian regarding U.S. Appl. No. 12/591,200, Part 1 [IPR2021-00406, Ex. 2086].
Declaration of Dr. Roham T. Zamanian regarding U.S. Appl. No. 12/591,200, Part 2 [IPR2021-00406, Ex. 2086].
Sandifer et al., Potent Effects of aerosol compared with intravenous Treprostinil on the pulmonary circulation, J. Appl. Physiol. 99:2363-2368 (2005) [IPR2021-00406, Ex. 2087].
Liquidia SEC Form 10-K (2020) [IPR2021-00406, Ex. 2089].
Preston et al., Safety and efficacy of transition from inhaled treprostinil to parenteral treprostinil in selected patients with pulmonary arterial hypertension, Pulm Cir. 4(3):456-461 (2014) [IPR2021-00406, Ex. 2090].
Expert Report of Dr. Igor Gonda (D. Del) (excerpts) [IPR2021-00406, Ex. 2091].
Decision Granting Institution of Inter Partes Review of U.S. Pat. No. 10,716,793 B2, dated Aug. 11, 2021 [IPR2021-00406].
Patent Owner United Therapeutic Corporation's Preliminary Response, filed May 17, 2021 [IPR2021-00406].
Declaration of Dr. Aaron Waxman [IPR2021-00406, Ex. 2001].
Dr. Waxman's curriculum vitae [IPR2021-00406, Ex. 2002].
Declaration of Dr. Werner Seeger [IPR2021-00406, Ex. 2003].
Declaration of Dr. Hossein A. Ghofrani [IPR2021-00406, Ex. 2004].
Declaration of Dr. Frank Reichenberger [IPR2021-00406, Ex. 2005].
Declaration of Dr. Friedrich Grimminger [IPR2021-00406, Ex. 2006].
Tyvaso Orange Book listing [IPR2021-00406, Ex. 2007].
Hill, N., 2005, Therapeutic Options for the Treatment of Pulmonary Hypertension, Medscape Pulmonary Medicine 9(2). [IPR2021-00406, Ex. 2008].
Substantive Submission filed in U.S. Appl. No. 12/591,200 (Mar. 9, 2015) [IPR2021-00406, Ex. 2009].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-1 (public docket) [IPR2021-00406, Ex. 2010].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-11 (public docket) [IPR2021-00406, Ex. 2011].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-16 (public docket) [IPR2021-00406, Ex. 2012].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), unnumbered docket entry dated Jul. 30, 2020 [IPR2021-00406, Ex. 2013].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-20 (public docket) (excerpted) [IPR2021-00406, Ex. 2014].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-29 (public docket) [IPR2021-00406, Ex. 2015].
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del.), ECF-45 (public docket) [IPR2021-00406, Ex. 2016].
Shapiro, S. et al. "Safety And Feasibility Of Doses Greater Than 54 Mcg QID Of Inhaled Treprostinil In Pulmonary Arterial Hypertension," Abstract, American Journal of Respiratory and Critical Care Medicine 2012;185:A4804.
Parikh KS, Rajagopal S, Fortin T, Tapson VF, Poms AD. "Safety and Tolerability of High-dose Inhaled Treprostinil in Pulmonary Hypertension." J Cardiovasc Pharmacol. Apr. 2016; 67(4):322-5.
Mandras, S. et al. "Survival and Drug Persistence in Patients With Pulmonary Hypertension Receiving Inhaled Treprostinil At Doses Greater Than 54 MCG (9 Breaths) 4 Times Daily." Chest, vol. 156, Issue 4, Supplement A283-A284 Oct. 2019.
Shapiro, S. et al. "Survival and drug persistence in patients receiving inhaled treprostinil at doses greater than 54 mg (nine breaths) four times daily." Pulmonary Circulation 2021; 11(4) 1-7.
United Therapeutics Corporation v. *Liquidia Technologies, Inc.*, Case No. 1:20-cv-00755-RGA-JLH (D. Del. 2022), Document No. 433, Trial Opinion, dated Aug. 31, 2022.
Sarala, N et al. "Technosphere: new drug delivery system for inhaled insulin," Future Prescriber, vol. 13(1) 14-16, 2012.

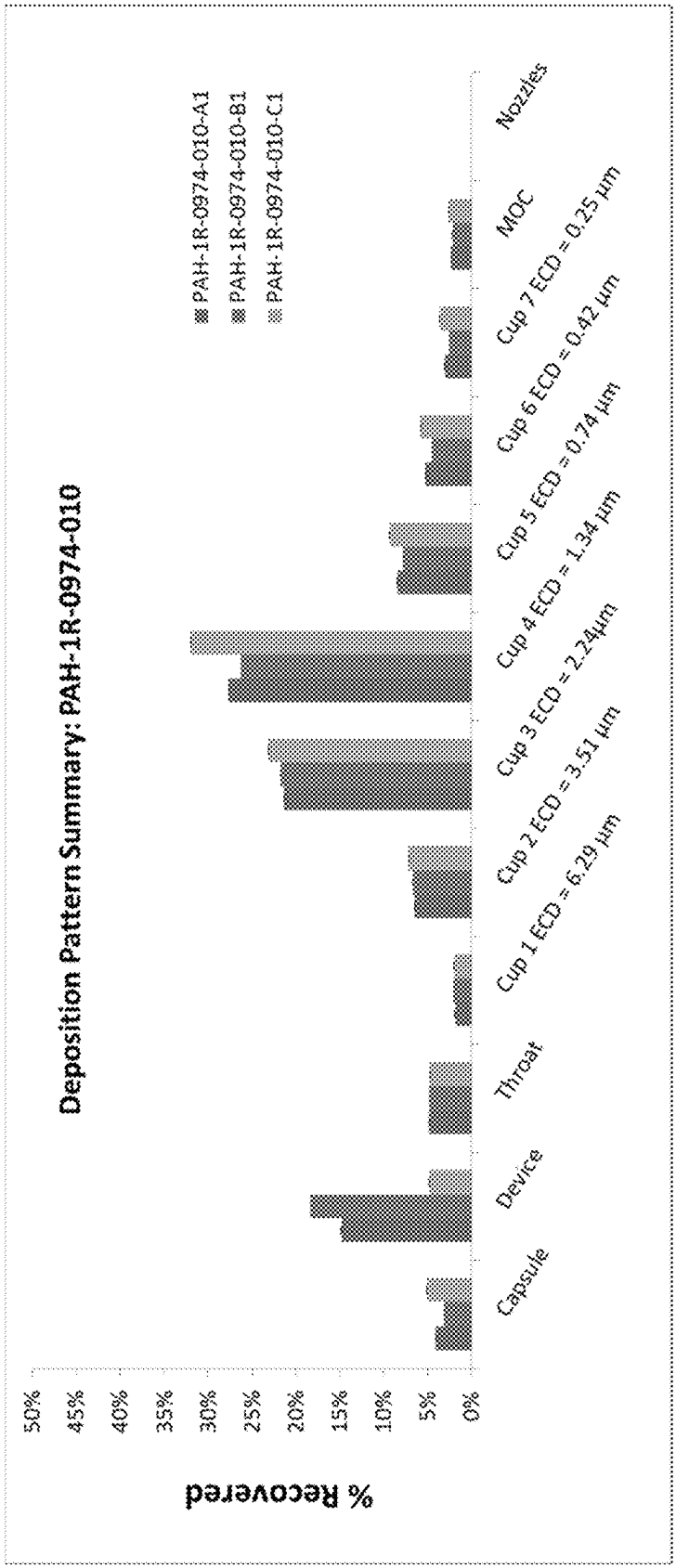
* cited by examiner

FIGURE 1



1 micrometer 'pollen' particle

FIGURE 2



Example NGI distribution for active particles (PAH-1R-0943-010) made. The NGI shows beginning of run (A1-red, left bar), middle of run (B1-blue, center bar), end of run (C1-green, right bar). Data obtained using Monodose Model 8 device (95L/min, 2sec).

FIGURE 3A

		Time (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject Alias**	**Treprostinil (ng/mL)**																
Cohort 1	1-A	0.00	0.164	NR	0.166	0.197	0.150	0.146	0.0992	0.0553	0.0314	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1-B	0.00	0.243	0.251	0.266	0.326	0.268	0.278	0.136	0.124	0.0688	0.0573	0.0292	0.0328	0.00	0.00	0.00	0.00
	1-C	0.00	0.200	0.272	0.412	0.414	0.251	0.176	0.139	0.108	0.0460	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1-D	0.00	0.474	0.493	0.458	0.443	0.511	0.383	0.258	0.138	0.0629	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1-E	0.00	0.200	0.28	0.224	0.255	0.172	0.148	0.114	0.105	0.0519	0.0308	0.00	0.00	0.00	0.00	0.00	0.00
	1-F	0.00	0.402	NR	0.456	0.427	0.313	0.265	0.188	0.106	0.0483	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N	**6**	**6**	**4**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.281**	**0.324**	**0.330**	**0.344**	**0.278**	**0.233**	**0.156**	**0.106**	**0.0516**	**0.0147**	**0.00487**	**0.00547**	**0.00**	**0.00**	**0.00**	**0.00**
	SD	**0.00**	**0.127**	**0.113**	**0.127**	**0.101**	**0.130**	**0.0934**	**0.0585**	**0.028**	**0.0132**	**0.0242**	**0.0119**	**0.0134**	**0.00**	**0.00**	**0.00**	**0.00**
	Min	**0.00**	**0.164**	**0.251**	**0.166**	**0.197**	**0.150**	**0.146**	**0.0992**	**0.0553**	**0.0314**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.222**	**0.276**	**0.339**	**0.370**	**0.260**	**0.221**	**0.138**	**0.107**	**0.0501**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Max	**0.00**	**0.474**	**0.493**	**0.458**	**0.443**	**0.511**	**0.383**	**0.258**	**0.138**	**0.0688**	**0.0573**	**0.0292**	**0.0328**	**0.00**	**0.00**	**0.00**	**0.00**
	CV%	**NC**	**45.1**	**35.0**	**38.6**	**29.5**	**46.7**	**40.1**	**37.6**	**26.4**	**25.6**	**165.1**	**244.9**	**244.9**	**NC**	**NC**	**NC**	**NC**

NC= Not calculated; NR = Not Reported

FIGURE 3B

Cohort	Subject Alias	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 1	1-A	0.197	0.33	0.454	0.146	0.167	150	98.2
	1-B	0.326	0.33	0.795	0.321	0.359	69.7	80.0
	1-C	0.414	0.33	0.460	0.242	0.272	91.8	60.9
	1-D	0.511	0.42	0.377	0.389	0.423	59.1	32.1
	1-E	0.280	0.17	0.648	0.218	0.247	101	94.7
	1-F	0.456	0.25	0.399	0.308	0.336	74.4	42.8
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.364**	**0.306**	**0.522**	**0.271**	**0.301**	**91.0**	**68.1**
	SD	**0.117**	**0.086**	**0.164**	**0.086**	**0.091**	**32.6**	**27.4**
	Min	**0.20**	**0.17**	**0.38**	**0.15**	**0.17**	**59.1**	**32.1**
	Median	**0.37**	**0.33**	**0.46**	**0.28**	**0.30**	**83.1**	**70.4**
	Max	**0.51**	**0.42**	**0.80**	**0.39**	**0.42**	**150**	**98.2**
	CV%	**32.3**	**28.2**	**31.5**	**31.8**	**30.2**	**35.8**	**40.2**
	Geometric Mean	**0.347**	**0.294**	**0.503**	**0.258**	**0.288**	**86.8**	**63.0**
	CV% Geometric Mean	**36.52**	**33.02**	**29.96**	**35.77**	**33.91**	**33.9**	**47.67**

FIGURE 4A

		Time (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject Alias**	**Treprostinil (ng/mL)**																
Cohort 2	2-A	0.00	NR	0.99	0.93	0.77	0.70	0.58	0.37	0.30	0.12	0.08	0.03	0.00	0.00	0.00	0.00	0.00
	2-B	0.00	0.94	1.03	0.95	0.83	0.64	0.41	0.30	0.18	0.09	0.05	0.03	0.03	0.00	0.00	0.00	0.00
	2-C	0.00	0.48	0.41	0.32	0.31	0.26	0.22	0.13	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2-D	0.00	0.45	0.53	0.50	0.46	0.45	0.44	0.30	0.22	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00
	2-E	0.00	0.28	0.29	0.26	0.23	0.21	0.20	0.13	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2-F	0.00	0.00	0.09	0.11	0.11	0.12	0.11	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N	**6**	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.429**	**0.556**	**0.511**	**0.452**	**0.395**	**0.326**	**0.214**	**0.149**	**0.0624**	**0.0277**	**0.0108**	**0.00507**	**0.00**	**0.00**	**0.00**	**0.00**
	SD	**0.00**	**0.342**	**0.382**	**0.355**	**0.295**	**0.240**	**0.180**	**0.123**	**0.101**	**0.0433**	**0.0333**	**0.0168**	**0.0124**	**0.00**	**0.00**	**0.00**	**0.00**
	Min	**0.00**	**0.00**	**0.0853**	**0.111**	**0.108**	**0.116**	**0.110**	**0.0600**	**0.0270**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.453**	**0.470**	**0.410**	**0.385**	**0.351**	**0.313**	**0.216**	**0.134**	**0.0603**	**0.0186**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Max	**0.00**	**0.937**	**1.03**	**0.948**	**0.833**	**0.703**	**0.583**	**0.367**	**0.298**	**0.124**	**0.0794**	**0.0332**	**0.0304**	**0.00**	**0.00**	**0.00**	**0.00**
	CV%	**NC**	**79.7**	**68.8**	**69.4**	**65.1**	**60.8**	**55.2**	**57.6**	**68.1**	**69.3**	**120.3**	**155.0**	**244.9**	**NC**	**NC**	**NC**	**NC**

NC= Not calculated; NR = Not Reported

FIGURE 4B

Cohort	Subject	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 2	2-A	0.991	0.17	0.482	0.723	0.746	67.0	46.6
	2-B	1.03	0.17	0.624	0.663	0.690	72.5	65.2
	2-C	0.477	0.08	0.474	0.254	0.282	177	121
	2-D	0.530	0.17	0.410	0.466	0.488	102	60.5
	2-E	0.288	0.17	0.478	0.212	0.242	206	142
	2-F	0.116	0.42	0.247	0.0706	0.0802	624	222
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.572**	**0.195**	**0.452**	**0.398**	**0.422**	**208**	**110**
	SD	**0.370**	**0.114**	**0.123**	**0.262**	**0.265**	**211**	**66.6**
	Min	**0.116**	**0.0830**	**0.247**	**0.0706**	**0.0802**	**67.0**	**46.6**
	Median	**0.504**	**0.167**	**0.476**	**0.360**	**0.385**	**140**	**93.3**
	Max	**1.03**	**0.417**	**0.624**	**0.723**	**0.746**	**624**	**222**
	CV%	**64.7**	**58.6**	**27.2**	**65.8**	**62.8**	**101.5**	**60.7**
	Geometric Mean	**0.453**	**0.173**	**0.436**	**0.308**	**0.334**	**150**	**94.3**
	CV% Geometric Mean	**98.2**	**54.9**	**31.8**	**107.4**	**100.4**	**100.4**	**65.7**

FIGURE 5A

		NTime (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject Alias**	**Treprostinil (ng/mL)**																
Cohort 3	3-A	0.00	0.63	0.62	0.55	0.47	0.43	0.42	0.32	0.21	0.11	0.06	0.03	0.00	0.00	0.00	0.00	0.00
	3-B	0.00	0.44	0.52	0.51	0.51	0.53	0.47	0.39	0.26	0.13	0.12	0.03	0.00	0.00	0.00	0.00	0.00
	3-C	0.00	0.75	1.07	1.11	1.01	NR	0.80	0.47	0.35	0.14	0.07	0.05	0.03	0.00	0.00	0.00	0.00
	3-D	0.00	NR	0.40	0.43	0.40	0.37	0.34	0.29	0.20	0.10	0.05	0.00	0.00	0.00	0.00	0.00	0.00
	3-E	0.00	0.45	0.52	0.54	0.57	0.54	0.48	0.36	0.28	0.15	0.11	0.03	0.00	0.00	0.00	0.00	0.00
	3-F	0.00	0.94	1.05	1.10	1.10	1.07	1.03	0.80	0.53	0.30	0.15	0.07	0.04	0.00	0.00	0.00	0.00
	N	**6**	**5**	**6**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.641**	**0.697**	**0.706**	**0.677**	**0.587**	**0.591**	**0.437**	**0.304**	**0.155**	**0.0951**	**0.0349**	**0.0124**	**0.00**	**0.00**	**0.00**	**0.00**
	SD	**0.00**	**0.209**	**0.290**	**0.312**	**0.299**	**0.279**	**0.267**	**0.187**	**0.123**	**0.0755**	**0.0389**	**0.0225**	**0.0192**	**0.00**	**0.00**	**0.00**	**0.00**
	Min	**0.00**	**0.437**	**0.399**	**0.428**	**0.400**	**0.371**	**0.344**	**0.285**	**0.199**	**0.0976**	**0.0547**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.630**	**0.571**	**0.545**	**0.540**	**0.532**	**0.474**	**0.379**	**0.269**	**0.136**	**0.0916**	**0.0342**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Max	**0.00**	**0.936**	**1.07**	**1.11**	**1.10**	**1.07**	**1.03**	**0.796**	**0.529**	**0.304**	**0.149**	**0.0682**	**0.0395**	**0.00**	**0.00**	**0.00**	**0.00**
	CV%	**NC**	**32.6**	**41.7**	**44.2**	**44.2**	**47.5**	**45.2**	**42.8**	**40.5**	**48.8**	**40.9**	**64.6**	**155.4**	**NC**	**NC**	**NC**	**NC**

NC= Not calculated; NR = Not Reported

FIGURE 5B

Cohort	Subject Alias	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 3	3-A	0.630	0.08	0.609	0.538	0.568	132	116
	3-B	0.532	0.42	0.568	0.611	0.638	118	96.3
	3-C	1.11	0.25	0.980	0.906	0.955	78.5	111
	3-D	0.428	0.25	0.522	0.411	0.452	166	125
	3-E	0.567	0.33	0.531	0.619	0.639	117	89.9
	3-F	1.10	0.25	0.520	1.26	1.29	58.1	43.6
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.728**	**0.264**	**0.622**	**0.724**	**0.757**	**112**	**97.0**
	SD	**0.299**	**0.111**	**0.179**	**0.309**	**0.310**	**38.5**	**29.1**
	Min	**0.428**	**0.0833**	**0.520**	**0.411**	**0.452**	**58.1**	**43.6**
	Median	**0.599**	**0.250**	**0.550**	**0.615**	**0.639**	**117**	**104**
	Max	**1.11**	**0.417**	**0.980**	**1.26**	**1.29**	**166**	**125**
	CV%	**41.1**	**42.0**	**28.7**	**42.7**	**41.0**	**34.5**	**30.0**
	Geometric Mean	**0.681**	**0.238**	**0.605**	**0.676**	**0.711**	**106**	**92.1**
	CV% Geometric Mean	**41.2**	**60.0**	**24.8**	**41.5**	**39.4**	**39.4**	**40.0**

FIGURE 6A

		NTime (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject**	**Treprostinil (ng/mL)**																
Cohort 4	4-A	0.00	0.294	0.534	0.648	0.684	0.722	0.763	0.575	0.358	0.150	0.0789	0.0523	0.0332	0.00	0.00	0.00	0.00
	4-B	0.00	0.629	0.872	0.959	1.04	1.02	0.924	0.622	0.495	0.273	0.144	0.0710	0.0449	0.0300	0.00	0.00	0.00
	4-C	0.00	0.552	0.725	0.704	0.718	0.688	0.729	0.625	0.557	0.346	0.205	0.143	0.0908	0.0445	0.0286	0.00	0.00
	4-D	0.00	1.30	1.54	1.44	1.35	1.29	1.08	0.790	0.559	0.290	0.134	0.0709	0.0728	0.0333	0.00	0.00	0.00
	4-E	0.00	0.877	1.14	1.16	1.14	0.969	0.970	0.657	0.513	0.270	0.166	0.0968	0.0524	0.0367	0.0256	0.00	0.00
	4-F	0.00	1.13	1.21	1.23	1.09	0.915	0.826	0.662	0.521	0.253	0.125	0.140	0.0698	0.0324	0.0837	0.00	0.00
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.797**	**1.00**	**1.02**	**1.00**	**0.934**	**0.882**	**0.655**	**0.501**	**0.264**	**0.142**	**0.0957**	**0.0607**	**0.0295**	**0.0230**	**0.00**	**0.00**
	SD	**0.00**	**0.377**	**0.365**	**0.310**	**0.257**	**0.220**	**0.134**	**0.0730**	**0.0742**	**0.0642**	**0.0422**	**0.0382**	**0.0210**	**0.0153**	**0.0326**	**0.00**	**0.00**
	Min	**0.00**	**0.294**	**0.534**	**0.648**	**0.684**	**0.688**	**0.729**	**0.575**	**0.358**	**0.150**	**0.0789**	**0.0523**	**0.0332**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.753**	**1.01**	**1.06**	**1.07**	**0.942**	**0.875**	**0.641**	**0.517**	**0.272**	**0.139**	**0.0839**	**0.0611**	**0.0329**	**0.0128**	**0.00**	**0.00**
	Max	**0.00**	**1.30**	**1.54**	**1.44**	**1.35**	**1.29**	**1.08**	**0.790**	**0.559**	**0.346**	**0.205**	**0.143**	**0.0908**	**0.0445**	**0.0837**	**0.00**	**0.00**
	CV%	**NC**	**47.3**	**36.3**	**30.3**	**25.6**	**23.5**	**15.2**	**11.1**	**14.8**	**24.4**	**29.7**	**40.0**	**34.7**	**51.9**	**141.8**	**NC**	**NC**

NC= Not calculated

FIGURE 6B

Cohort	Subject	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 4	4-A	0.763	0.50	0.801	0.781	0.819	122	141
	4-B	1.04	0.33	0.805	1.13	1.17	85.8	99.6
	4-C	0.729	0.50	0.702	1.18	1.21	82.8	83.8
	4-D	1.54	0.17	0.583	1.44	1.46	68.3	57.4
	4-E	1.16	0.25	0.968	1.26	1.30	77.1	108
	4-F	1.23	0.25	0.794	1.28	1.37	72.8	83.4
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**1.08**	**0.333**	**0.775**	**1.18**	**1.22**	**84.8**	**95.5**
	SD	**0.305**	**0.139**	**0.127**	**0.221**	**0.225**	**19.3**	**28.2**
	Min	**0.729**	**0.167**	**0.583**	**0.781**	**0.819**	**68.3**	**57.4**
	Median	**1.10**	**0.292**	**0.798**	**1.22**	**1.25**	**79.9**	**91.7**
	Max	**1.54**	**0.500**	**0.968**	**1.44**	**1.46**	**122**	**141**
	CV%	**28.3**	**41.8**	**16.4**	**18.7**	**18.4**	**22.8**	**29.5**
	Geometric Mean	**1.04**	**0.309**	**0.766**	**1.16**	**1.20**	**83.2**	**92.0**
	CV% Geometric Mean	**29.5**	**45.5**	**17.0**	**21.2**	**20.7**	**20.7**	**30.8**

FIGURE 7A

		NTime (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject**	**Treprostinil (ng/mL)**																
Cohort 5	5-A	0.00	1.13	1.96	1.80	1.64	1.54	1.38	0.971	0.752	0.394	0.238	0.136	0.0833	0.0583	0.0345	0.00	0.00
	5-B	0.00	0.562	1.26	1.26	1.45	1.40	1.24	0.876	0.605	0.312	0.195	0.0732	0.0351	0.00	0.00	0.00	0.00
	5-C	0.00	0.478	0.763	0.979	1.02	0.976	0.909	NR	0.409	0.294	0.128	0.0658	0.0433	0.00	0.00	0.00	0.00
	5-D	0.00	1.04	1.42	1.26	1.08	1.02	0.824	0.635	0.467	0.252	0.146	0.0615	0.0304	0.00	0.00	0.00	0.00
	5-E	0.00	0.543	0.728	0.730	0.743	0.754	0.699	0.502	0.397	0.230	0.113	0.0500	0.0262	0.00	0.00	0.00	0.00
	5-F	0.00	0.502	0.510	0.427	0.399	0.333	0.265	0.201	0.120	0.0565	0.0306	0.00	0.00	0.00	0.00	0.00	0.00
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.709**	**1.11**	**1.08**	**1.06**	**1.00**	**0.886**	**0.637**	**0.458**	**0.256**	**0.142**	**0.0644**	**0.0364**	**0.00972**	**0.00575**	**0.00**	**0.00**
	SD	**0.00**	**0.294**	**0.542**	**0.478**	**0.453**	**0.438**	**0.399**	**0.307**	**0.214**	**0.113**	**0.0714**	**0.0437**	**0.0273**	**0.0238**	**0.0141**	**0.00**	**0.00**
	Min	**0.00**	**0.478**	**0.510**	**0.427**	**0.399**	**0.333**	**0.265**	**0.201**	**0.120**	**0.0565**	**0.0306**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.553**	**1.01**	**1.12**	**1.05**	**0.998**	**0.867**	**0.635**	**0.438**	**0.273**	**0.137**	**0.0637**	**0.0328**	**0.00**	**0.00**	**0.00**	**0.00**
	Max	**0.00**	**1.13**	**1.96**	**1.80**	**1.64**	**1.54**	**1.38**	**0.971**	**0.752**	**0.394**	**0.238**	**0.136**	**0.0833**	**0.0583**	**0.0345**	**0.00**	**0.00**
	CV%	**NC**	**41.5**	**48.9**	**44.4**	**43.0**	**43.6**	**45.0**	**48.2**	**46.6**	**44.2**	**50.4**	**67.9**	**74.9**	**244.9**	**244.9**	**NC**	**NC**

NC= Not calculated; NR = Not Reported

FIGURE 7B

Cohort	Subject	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 5	5-A	1.96	0.17	0.647	1.87	1.90	65.7	61.3
	5-B	1.45	0.33	0.495	1.42	1.45	86.2	61.6
	5-C	1.02	0.33	0.561	1.05	1.08	115	93.3
	5-D	1.42	0.17	0.526	1.18	1.20	104	78.9
	5-E	0.754	0.42	0.473	0.874	0.891	140	95.6
	5-F	0.510	0.17	0.448	0.352	0.372	336	217
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**1.19**	**0.264**	**0.525**	**1.12**	**1.15**	**141**	**101**
	SD	**0.528**	**0.111**	**0.0716**	**0.512**	**0.516**	**98.8**	**58.7**
	Min	**0.510**	**0.167**	**0.448**	**0.352**	**0.372**	**65.7**	**61.3**
	Median	**1.22**	**0.250**	**0.511**	**1.11**	**1.14**	**110**	**86.1**
	Max	**1.96**	**0.417**	**0.647**	**1.87**	**1.90**	**336**	**217**
	CV%	**44.5**	**42.0**	**13.6**	**45.6**	**44.9**	**69.9**	**57.9**
	Geometric Mean	**1.08**	**0.245**	**0.521**	**1.00**	**1.03**	**121**	**91.3**
	CV% Geometric Mean	**52.4**	**44.9**	**13.2**	**62.6**	**60.9**	**60.9**	**49.3**

FIGURE 8A

		NTime (h)																
		0.00	**0.08**	**0.17**	**0.25**	**0.33**	**0.42**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**6.00**	**8.00**
Cohort	**Subject**	**Treprostinil (ng/mL)**																
Cohort 6-R	6-A	0.00	0.244	0.474	0.596	0.622	0.704	0.61	0.459	0.345	0.177	0.085	0.0387	0.00	0.00	0.00	0.00	0.00
	6-B	0.00	0.877	0.907	0.75	0.657	0.538	0.459	0.287	0.166	0.0915	0.0488	0.00	0.00	0.00	0.00	0.00	0.00
	6-C	0.00	0.547	1.47	1.68	1.67	1.59	1.41	1.00	0.762	0.406	0.232	0.144	0.0885	0.0603	0.0376	0.00	0.00
	6-D	0.00	1.41	1.7	1.89	1.86	1.75	1.69	1.24	0.9	0.463	0.22	0.116	0.0673	0.0758	0.0404	0.00	0.00
	6-E	0.00	1.21	1.83	2.12	2.34	2.06	1.9	1.65	1.25	0.699	0.431	0.244	0.14	0.106	0.093	0.00	0.00
	6-F	0.00	0.615	0.938	1.09	1.19	1.12	1.11	0.912	0.682	0.412	0.207	0.122	0.0677	0.0502	0.0325	0.00	0.00
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**0.00**	**0.817**	**1.22**	**1.35**	**1.39**	**1.29**	**1.2**	**0.925**	**0.684**	**0.375**	**0.204**	**0.111**	**0.0606**	**0.0487**	**0.0339**	**0.00**	**0.00**
	SD	**0.00**	**0.436**	**0.529**	**0.631**	**0.688**	**0.605**	**0.58**	**0.501**	**0.389**	**0.217**	**0.135**	**0.0854**	**0.0539**	**0.0422**	**0.0342**	**0.00**	**0.00**
	Min	**0.00**	**0.244**	**0.474**	**0.596**	**0.622**	**0.538**	**0.459**	**0.287**	**0.166**	**0.0915**	**0.0488**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Median	**0.00**	**0.746**	**1.20**	**1.39**	**1.43**	**1.36**	**1.26**	**0.956**	**0.722**	**0.409**	**0.214**	**0.119**	**0.0675**	**0.0553**	**0.0351**	**0.00**	**0.00**
	Max	**0.00**	**1.41**	**1.83**	**2.12**	**2.34**	**2.06**	**1.90**	**1.65**	**1.25**	**0.699**	**0.431**	**0.244**	**0.14**	**0.106**	**0.093**	**0.00**	**0.00**
	CV%	**NC**	**53.4**	**43.4**	**46.6**	**49.5**	**46.8**	**48.4**	**54.2**	**56.8**	**57.8**	**66.1**	**77.1**	**89**	**86.6**	**100.8**	**NC**	**NC**

NC= Not calculated; NR = Not Reported

FIGURE 8B

Cohort	Subject	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 6-R	6-A	0.704	0.42	0.456	0.696	0.721	208	137
	6-B	0.907	0.17	0.566	0.573	0.613	245	200
	6-C	1.68	0.25	0.768	1.80	1.85	81.3	90.1
	6-E	1.89	0.25	0.628	2.10	2.13	70.3	63.6
	6-F	2.34	0.33	0.734	2.80	2.90	51.8	54.9
	6-G	1.19	0.33	0.665	1.50	1.53	97.9	93.8
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**1.45**	**0.292**	**0.636**	**1.58**	**1.62**	**126**	**107**
	SD	**0.626**	**0.087**	**0.114**	**0.849**	**0.869**	**80.3**	**54.0**
	Min	**0.704**	**0.167**	**0.456**	**0.573**	**0.613**	**51.8**	**54.9**
	Median	**1.44**	**0.292**	**0.646**	**1.65**	**1.69**	**89.6**	**92.0**
	Max	**2.34**	**0.417**	**0.768**	**2.8**	**2.90**	**245**	**200**
	CV%	**43.1**	**30.0**	**18.0**	**53.8**	**53.5**	**63.9**	**50.7**
	Geometric Mean	**1.33**	**0.28**	**0.627**	**1.36**	**1.41**	**107**	**96.5**
	CV% Geometric Mean	**48.6**	**32.9**	**19.2**	**70**	**68.3**	**68.3**	**50.9**

FIGURE 8C

Cohort	Subject	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)	CL/F (L/h)	Vz/F (L)
Cohort 6 - Original	6-A (ORIGINAL)	1.55	0.33	0.607	1.96	1.99	75.6	66.2
	6-B (ORIGINAL)	1.16	0.08	0.946	0.868	0.987	152	208
	6-C (ORIGINAL)	1.17	0.42	0.534	1.28	1.30	115	88.9
	6-D (ORIGINAL)	0.968	0.42	0.649	1.30	1.33	113	105
	6-E (ORIGINAL)	1.55	0.17	0.658	1.68	1.71	87.9	83.5
	6-F (ORIGINAL)	0.835	0.25	0.565	0.871	0.891	168	137
	N	**6**	**6**	**6**	**6**	**6**	**6**	**6**
	Mean	**1.21**	**0.278**	**0.660**	**1.33**	**1.37**	**119**	**115**
	SD	**0.295**	**0.136**	**0.148**	**0.435**	**0.418**	**35.8**	**51.4**
	Min	**0.835**	**0.0833**	**0.534**	**0.868**	**0.891**	**75.6**	**66.2**
	Median	**1.17**	**0.292**	**0.628**	**1.29**	**1.32**	**114**	**97.2**
	Max	**1.55**	**0.417**	**0.946**	**1.96**	**1.99**	**168**	**208**
	CV%	**24.4**	**49.0**	**22.5**	**32.8**	**30.6**	**30.2**	**44.8**
	Geometric Mean	**1.18**	**0.242**	**0.648**	**1.27**	**1.31**	**114**	**107**
	CV% Geometric Mean	**25.2**	**69.5**	**20.4**	**34.2**	**31.4**	**31.4**	**42.4**

FIGURE 8D

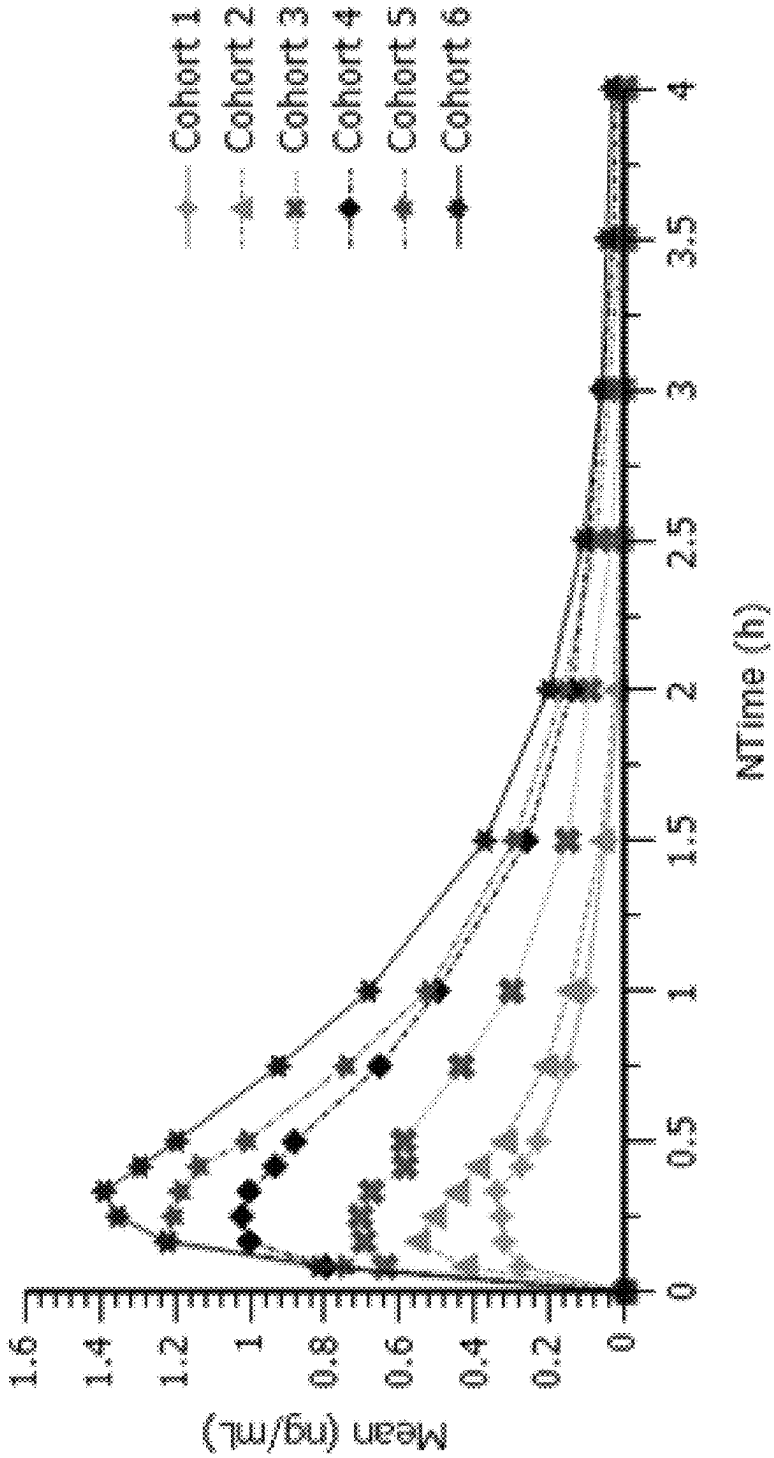

Cohort 1
Cohort 2
Cohort 3
Cohort 4
Cohort 5
Cohort 6
Mean (ng/mL)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
NTime (h)

FIGURE 8E

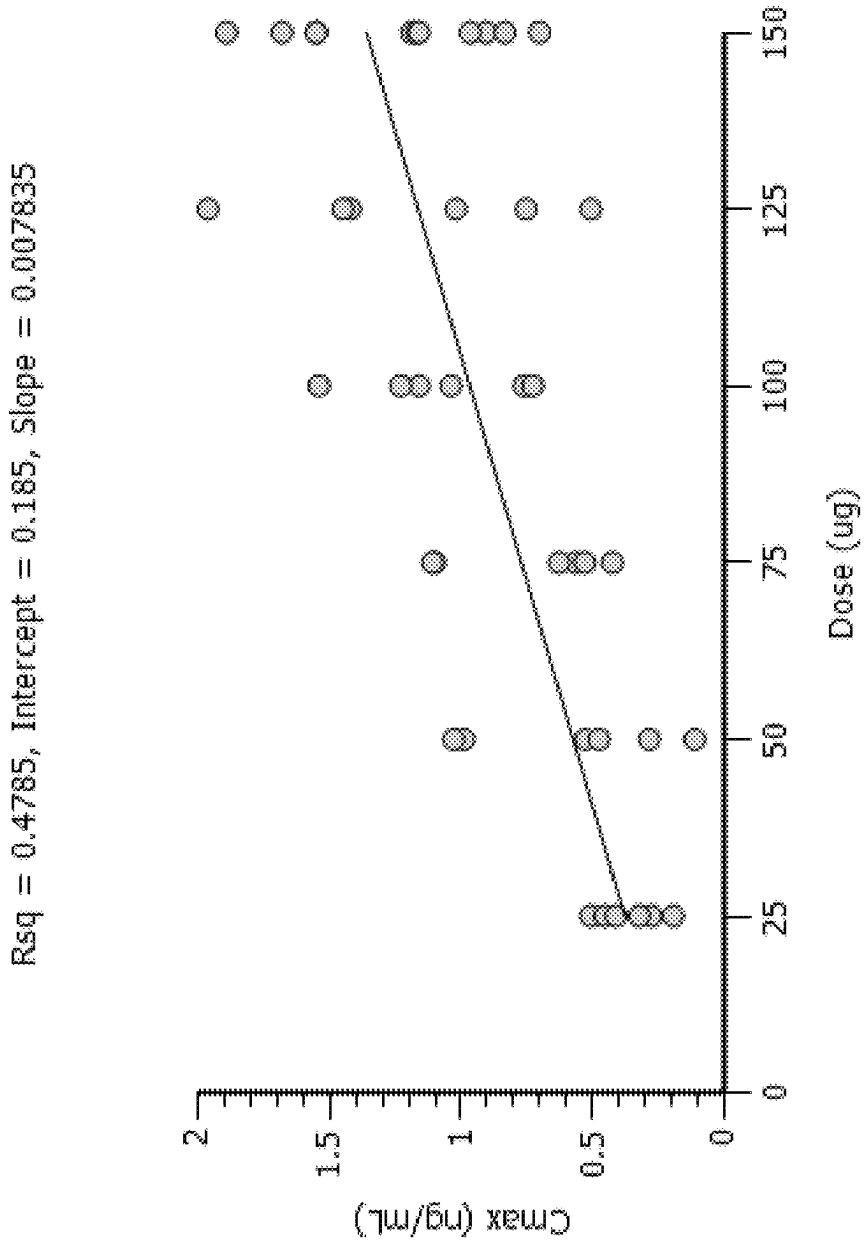

Rsq = 0.4785, Intercept = 0.185, Slope = 0.007835
Cmax (ng/mL)
2
1.5
1
0.5
0
0
25
50
75
100
125
150
Dose (ug)

FIGURE 8F

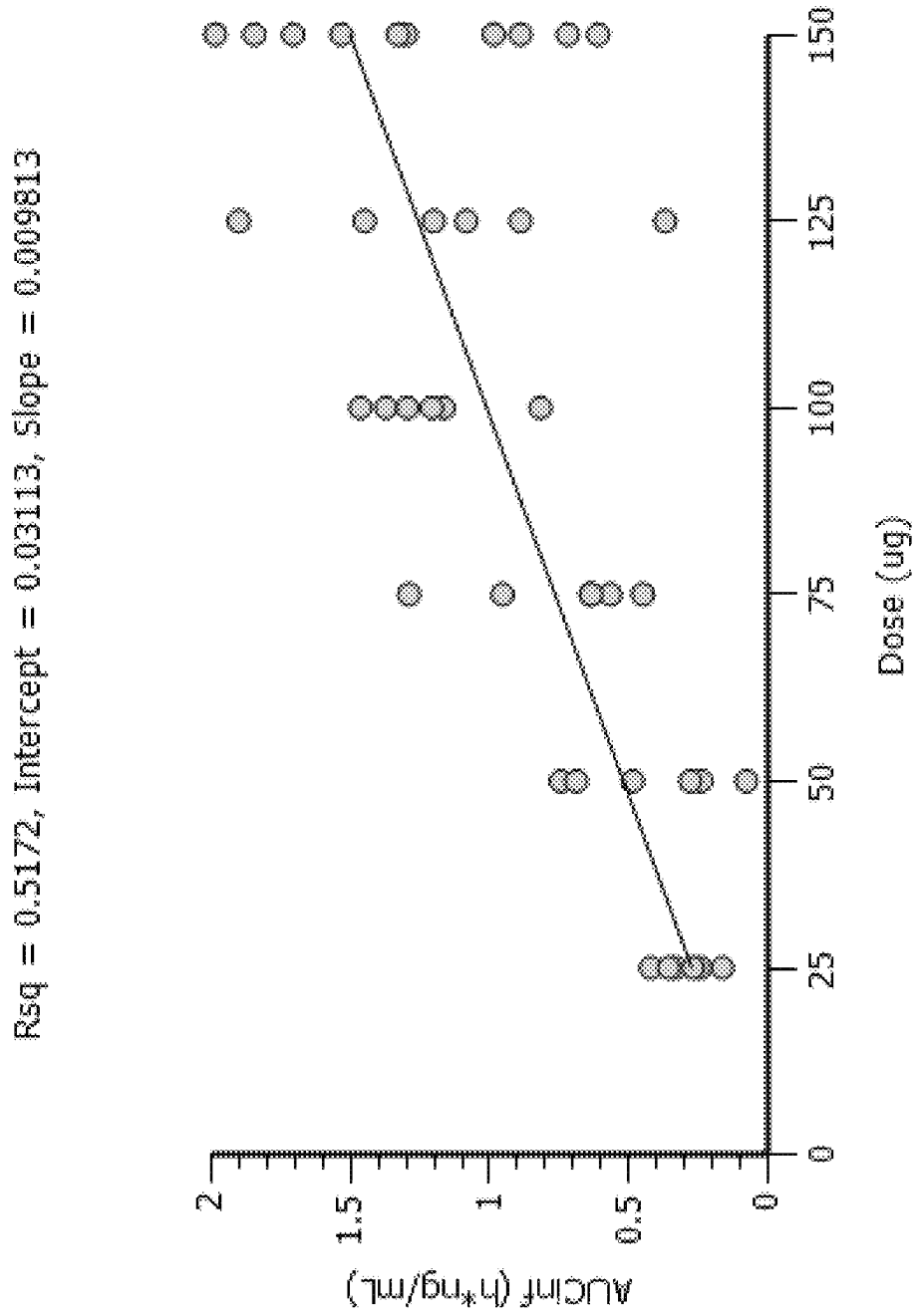

Rsq = 0.5172, Intercept = 0.03113, Slope = 0.009813
AUCinf (h*ng/mL)
2
1.5
1
0.5
0
0
25
50
75
100
125
150
Dose (ug)

FIGURE 8G

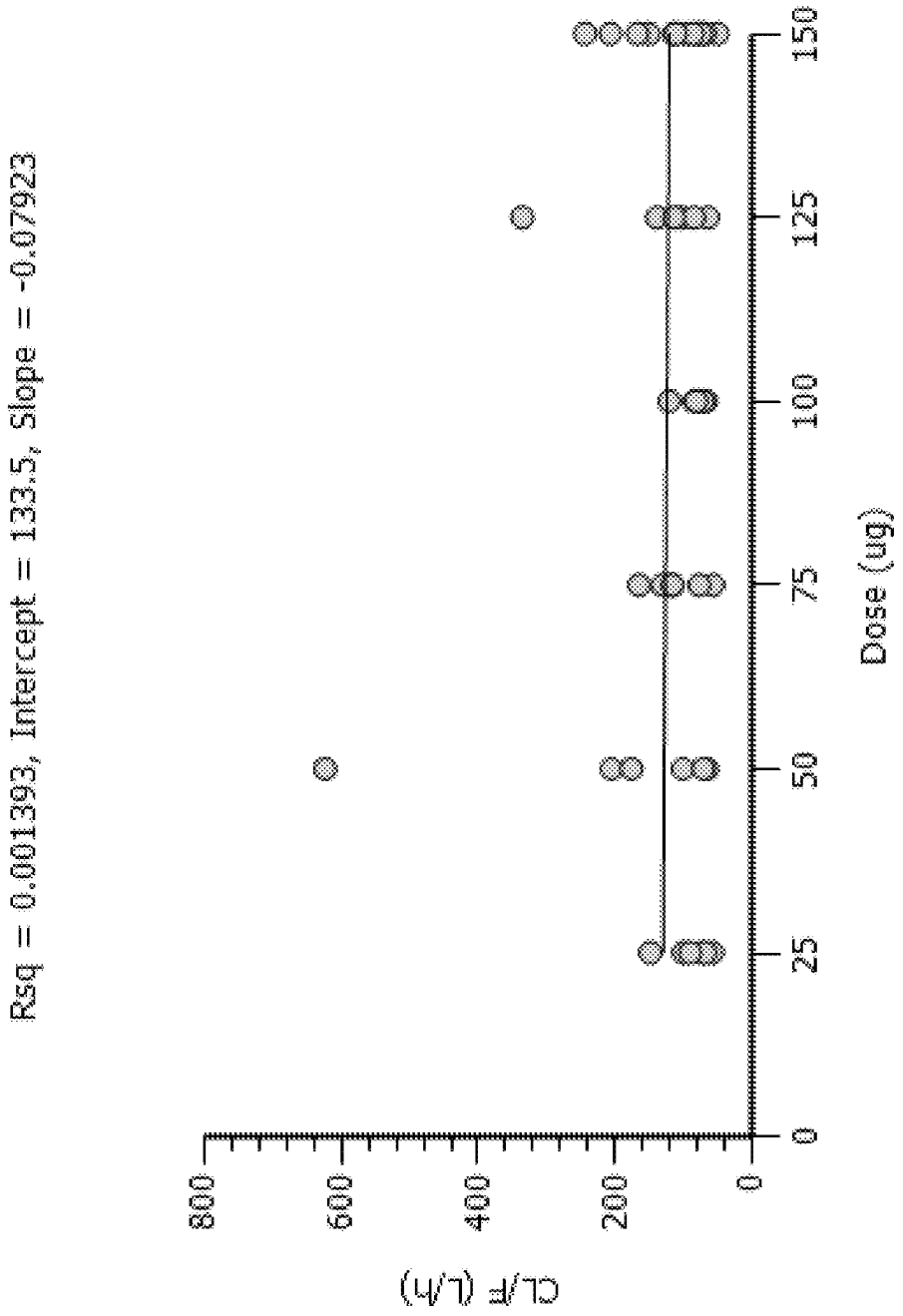

Rsq = 0.001393, Intercept = 133.5, Slope = -0.07923
CL/F (L/h)
800
600
400
200
0
0
25
50
75
100
125
150
Dose (ug)

FIGURE 9

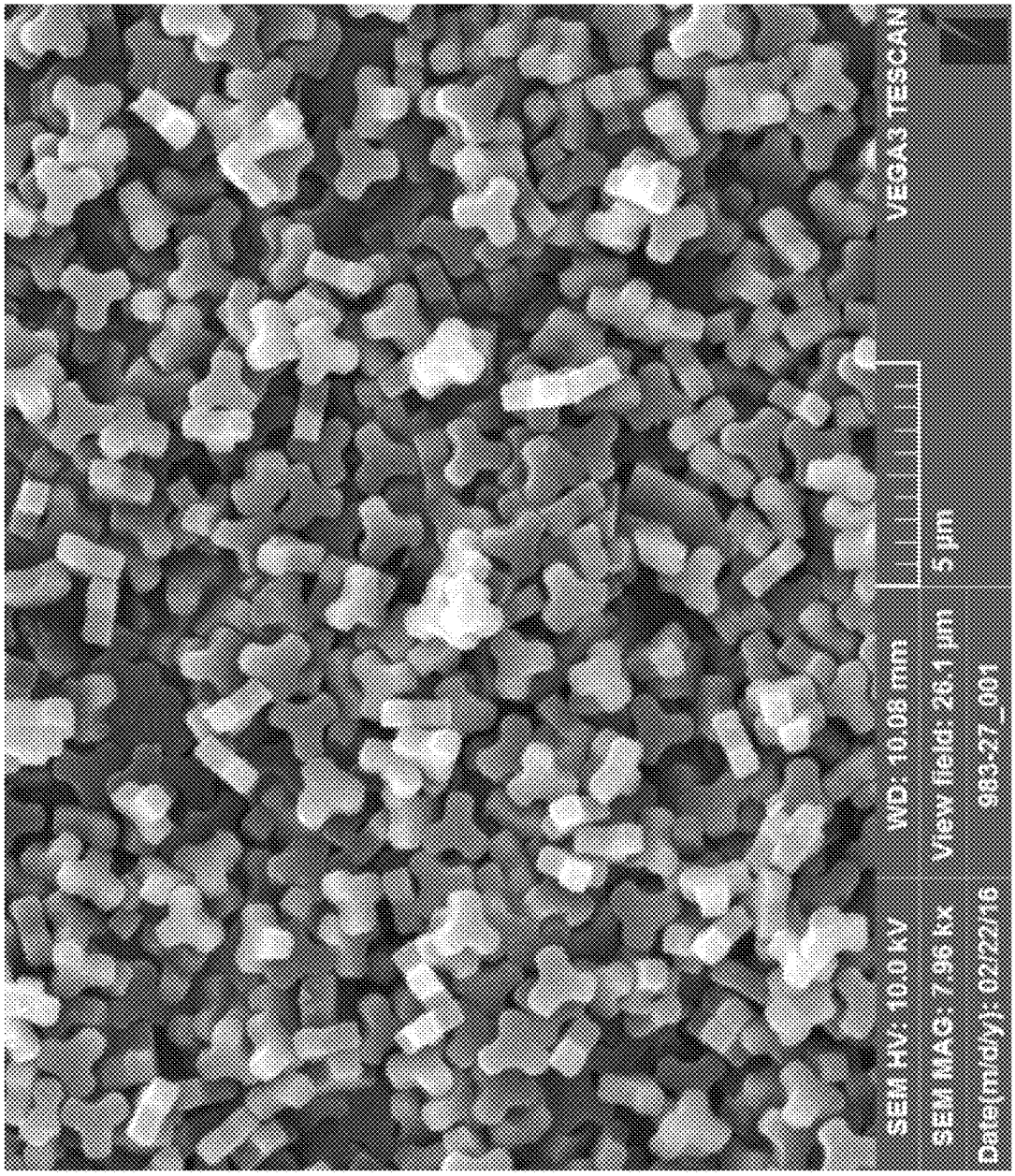

SEM HV: 10.0 kV
WD: 10.08 mm
VEGA3 TESCAN
SEM MAG: 7.06 kx
View field: 26.1 µm
5 µm
Date(m/d/y): 02/22/16
gbc-27_001

FIGURE 10

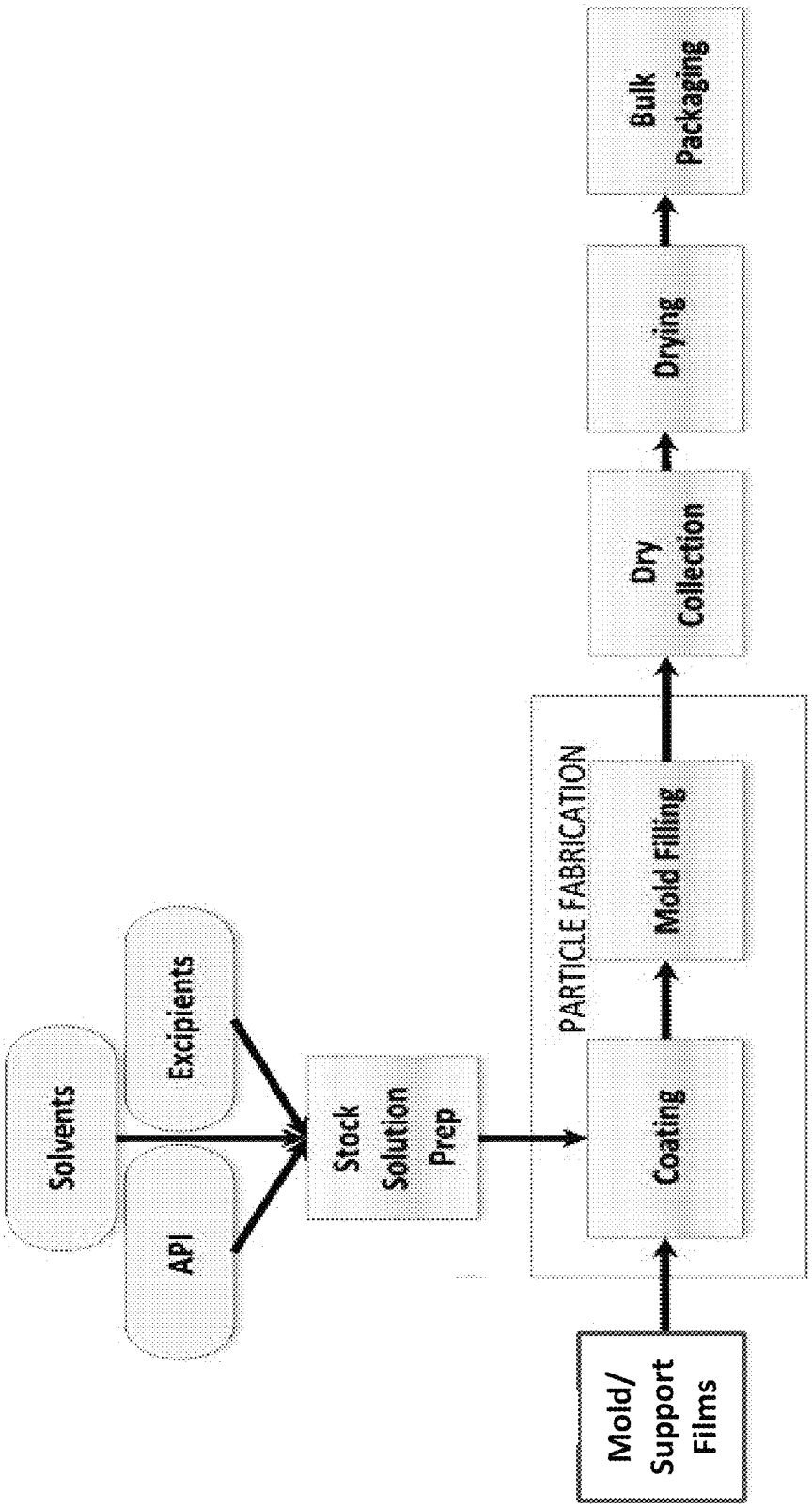
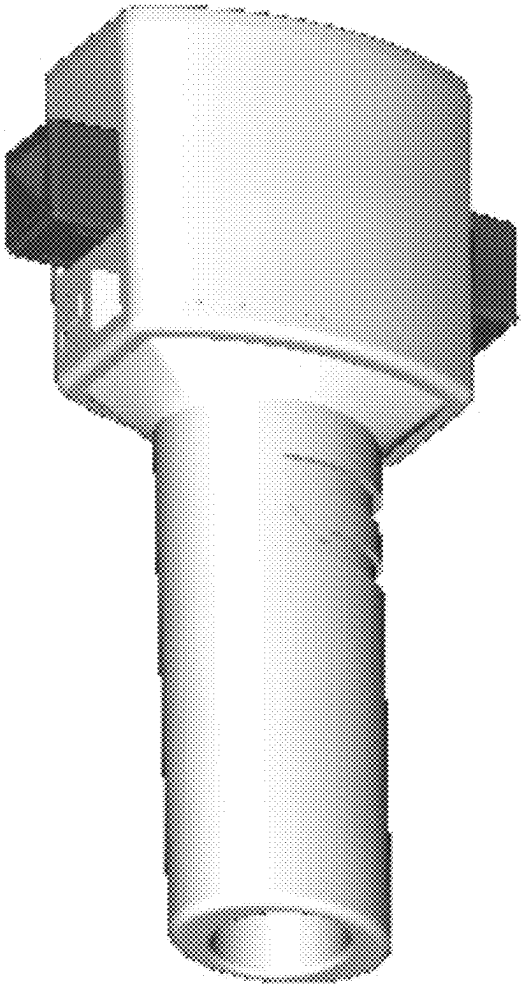

Solvents
Excipients
API
Stock Solution Prep
PARTICLE FABRICATION
Coating
Mold Filling
Mold/ Support Films
Dry Collection
Drying
Bulk Packaging

FIGURE 11



RS00 Model 8 Dry Powder Inhalation Device

DRY POWDER TREPROSTINIL FOR THE TREATMENT OF PULMONARY HYPERTENSION

CROSS-REFERENCE TO RELATED APPLICATIONS

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

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

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 subgroup 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.

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.

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.

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.

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.

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.

Currently Available Treatments

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)

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 REMODU-

LIN® (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).

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

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.

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.

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.

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.

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.

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.

BRIEF DESCRIPTION OF THE FIGURES

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.

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

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).

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.

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.

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.

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.

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.

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.

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.

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

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

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

Drug Substance

The drug substance (DS) according to embodiments of the present invention is treprostinil, which is a synthetic analog of prostacyclin (PGI_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).

Inhalation Powder Drug Product

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.

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.

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.

A detailed description of the LIQ861 formulation, particle composition, particle geometry, packaging, device, delivery, stability, dose, and a description of the use follows.

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.

LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (dihydrate form calculations)

Component	Function	Quantity (mg/g) (Active)	Percent Solids
Treprostinil Sodium	Drug Substance	5.3 (5.0 as treprostinil)	0.53
Trehalose Dihydrate	Bulking Agent	930	92.97
Polysorbate 80	Wetting Agent	20	2.00
L-Leucine	Hydrophobicity Modifier	40	4.00
Sodium Citrate Dihydrate	pH Modifier	2.7	0.27
Sodium Chloride	Buffer Component	2.3	0.23

LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (anhydrous form calculations)

Component	Function	Quantity (mg/g) (Active)	Percent Solids	Normalized mg/g
Treprostinil Sodium	Drug Substance	5.3 (5.0 as treprostinil)	0.581	5.81
Trehalose	Bulking Agent	841	92.32	923.23
Polysorbate 80	Wetting Agent	20	2.19	21.94
L-Leucine	Hydrophobicity Modifier	40	4.39	43.89
Sodium Citrate	pH Modifier	2.4	0.26	2.60
Sodium Chloride	Buffer Component	2.3	0.25	2.52

Inhalation Device

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.

Indication

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.

Chemistry, Manufacturing, and Controls (CMC)

Drug Substance (DS)

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.

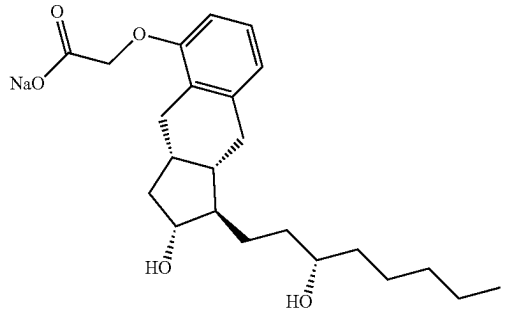
Nomenclature

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].

Structure

The structure of treprostinil sodium is depicted herein below. The molecular formula is $C_{23}H_{33}NaO_5$ and it has a molecular weight of 412.49 daltons.

Chemical Structure of Treprostinil Sodium



General Properties

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 $[\alpha]_D^{20}$=+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.

Inhalation Particle Drug Product—LIQ861

Description and Composition of the Drug Product Particle

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.

LIQ861 Drug Product-Intermediate Description for Active (LIQ861) and Placebo Formulations (dihydrate)

Component	Function	Quantity (mg/g) (Active)	Percent Solids
Treprostinil Sodium	Drug Substance	5.3 (5.0 as treprostinil)	0.53
Trehalose Dihydrate	Bulking Agent	930	92.97
Polysorbate 80	Wetting Agent/ Process Aide	20	20
L-Leucine	Hydrophobicity Modifier	40	40
Sodium Citrate Dihydrate	pH Modifier	2.7	0.27
Sodium Chloride	Buffer Component	2.3	0.23

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 per Capsule (mg)	15	5	10	15

*Excipients only (no treprostinil).
Abbreviations: HPMC, hydroxypropyl methylcellulose

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.

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.

Excipients

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.

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.

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.

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

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.

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.

Physiochemical and Biological Properties

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.

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.

Representative Aerosol Data (NGI) for Active Particles

Sample	MMAD (μm)	GSD	ED (% nominal)	FPF (% ED)
Treprostinil Sodium: Trehalose: Leucine: Polysorbate 80: Sodium Citrate: Sodium Chloride ("pollen-shaped")	1.88	1.99	64	83

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

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**.

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.

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.

Particle Fabrication

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.

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.

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

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

Stock component	Target Solution Concentration (Active)	Target Solution Concentration (Placebo)	Target
Trehalose	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

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.

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.

Drying and Bulk Packaging

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.

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.

Batch-to-Batch Uniformity of Drug Particles

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**.

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

Uniformity: Sample aerosol data (NGI) for active particles (PAH-1R-0974-010)					
Sample	MMAD	GSD	ED (% rec)	FPF (% ED)	F345 (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

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.

Stability studies

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.

Prototype Stability Study

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.

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

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

Data for the 25 μg dose drug particles stored at 40° C./75% RH is shown in the table below.

			Time Points			
Test	Specifications		Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.489	0.512	0.502	0.492
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	1.92	2.09	2.1	2.1
		GSD (μm)	1.72	1.65	1.6	1.6
		FPF (%)	87	85	84.8	84.7
Delivered Dose Uniformity	Report Results	Average (μg)	19.9	21.2	18.86	19.28

Data for the 75 μg dose drug particles stored at 25° C./60% RH is shown in the table below.

			Time Points				
Test	Specifications		Initial	1 Month	3 Months	6 Months	9 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.489	0.500	0.496	0.494	0.487
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.13	2.17	2.2	2.2	2.2
		GSD (μm)	1.60	1.61	1.6	1.6	1.6
		FPF (%)	85	84	84.2	83.7	82.3
Delivered Dose Uniformity	Report Results	Average (μg)	63.6	63.0	60.76	59.62	60.01

Data for the 75 μg dose drug particles stored at 40° C./75% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.489	0.509	0.506	0.491
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.13	2.14	2.2	2.1
		GSD (μm)	1.60	1.61	1.6	1.6
		FPF (%)	85	85	84.8	85.3
Delivered Dose Uniformity	Report Results	Average (μg)	63.6	61.0	59.86	59.42

Clinical Trial Material Stability Study

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.

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

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.520	0.505	0.504	0.504
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.3	2.3	2.2	2.1
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	84.1	82.7	83.6	85.0
Delivered Dose Uniformity	Report Results (μg)	Average (μg)	19.784	20.48	19.24	19.47

Data for the 25 μg dose drug particles stored at 40° C./75% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.520	0.518	0.501	0.506
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.3	2.2	2.2	2.2
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	84.1	84.1	85.8	84.7
Delivered Dose Uniformity	Report Results	Average (μg)	19.784	20.73	18.89	18.80

Data for the 50 μg dose drug particles stored at 25° C./60% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.515	0.509	0.509	0.506
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.2	2.2	2.2	2.2
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	86.2	85.6	85.7	85.3
Delivered Dose Uniformity	Report Results (μg)	Average (μg)	40.417	40.75	39.14	40.05

Data for the 50 μg dose drug particles stored at 40° C./75% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.515	0.520	0.505	0.501
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.2	2.2	2.2	2.2
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	86.2	86.5	86.2	84.1
Delivered Dose Uniformity	Report Results	Average (μg)	40.417	39.55	38.96	37.50

Data for the 75 μg dose drug particles stored at 25° C./60% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.517	0.512	0.509	0.508
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.3	2.3	2.3	2.2
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	84.8	84.1	84.5	85.1
Delivered Dose Uniformity	Report Results (μg)	Average (μg)	61.851	63.91	59.88	60.25

Data for the 75 μg dose drug particles stored at 40° C./75% RH is shown in the table below.

Test	Specifications		Time Points			
			Initial	1 Month	3 Months	6 Months
Assay	0.450-0.550% w/w Treprostinil as free acid (%)		0.517	0.513	0.503	0.495
Aerodynamic Particle Size Distribution	Report Results	MMAD (μm)	2.3	2.3	2.2	2.2
		GSD (μm)	1.6	1.6	1.6	1.6
		FPF (%)	84.8	85.1	85.8	84.9
Delivered Dose Uniformity	Report Results	Average (μg)	61.851	61.94	58.61	58.17

Dry Powder Inhalation Device

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**.

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.

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.

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.

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.

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.

Nonclinical Studies

Treprostinil is a tricyclic benzidine analogue of endogenous PGI_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_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_2 but, unlike PGI_2, treprostinil is chemically stable.

A series of in vivo studies were conducted to evaluate pharmokinetics (PK) and toxicology of the present invention dry powder treprostinil formulation.

Pilot, non-GLP, single-dose, inhalation PK study of treprostinil in dogs (Study 19073)

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.

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.

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.

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.

Pilot, Non-GLP, Single-Dose, Inhalation PK Study of LIQ861 in Male Rats (Study 75670)

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

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.

The test item was administered once by inhalation to 3 male rats per group as described in the table below:

Group No.	Group Designation	Achieved Mean Total Inhaled Dose Level of Treprostinil (mg/kg/day)	Achieved Aerosol Concentration of Treprostinil (μg/L)	Achieved Aerosol Concentration of Trehalose (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

Assessments of mortality, clinical signs and body weights were performed. Blood samples were collected and analyzed for treprostinil content.

No mortality occurred. No clinical signs were observed and body weights were unaffected.

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.

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:

Group		$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (hr*ng/mL)	AUC_{INF} (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

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

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.

The study was not performed in compliance with GLP regulations but followed appropriate Standard Operating Procedures (SOPs).

Experimental Design

The test item was administered to groups of rats by inhalation administration for one day as described in the table below:

Group No.	Group Designation	Targeted Total Inhaled Dose Level of Treprostinil (mg/kg/day)[a]	Targeted Aerosol Concentration of Treprostinil (μg/L)	Targeted Aerosol Concentration of Trehalose (μg/L)	No. of Animals 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

[a]= Targeted aerosol concentrations were calculated based on an estimated body weight of 0.250 kg

Following dosing, a series of 6 blood samples for pharmacokinetic evaluation were taken.

Characterization of Test Item

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

Acclimatization to Exposure System

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.

Animal Exposure

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

The target aerosol concentrations and dose levels were as follows:

Group No.	Group Designation	Targeted Dose Level of Treprostinil (mg/kg/day)[a]	Targeted Aerosol Concentration of Treprostinil (μg/L)	Targeted Aerosol Concentration of Trehalose (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

[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:

$$D_T = \frac{E_c \times RMV \times T}{BW}$$

D_L=Achieved dose levels (mg/kg/day)=

E_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 $(kg)^{0.809}$

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

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.

Inhalation System Monitoring

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.

Oxygen Concentration

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%.

Relative Humidity/Temperature

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.

Determination of Aerosol Concentration

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).

Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)

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.

In-Life Observations

Mortality

Mortality checks were performed at least once a day during all phases of the study.

Clinical Observations

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.

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.

Animal whose health status was judged to warrant additional evaluation was examined by a Clinical Veterinarian.

Body Weights

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).

Pharmacokinetics

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.

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.

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.

Non-compartmental analysis of treprostinil concentrations in plasma were performed by using the Phoenix WinNonlin 6.3 software.

The following configuration was used for the analysis:

Sampling Method: Sparse

AUC Calculation Method: Linear Trapezoidal with Linear Interpolation

Lambda Z (λ_z) Method: Best fit for λ_z, Log regression

Weighting (λ_z calculation): Uniform

Pharmacokinetic parameters (including abbreviation and description for each parameter) are described in the following table:

Parameters	Abbreviation	Unit*
Area under the plasma drug concentration-time curve from the time of dosing to the last quantifiable concentration	$AUC_{0\text{-}Tlast}$	μg*hr/mL
Area under the plasma drug concentration-time curve from the time of dosing extrapolated to infinity	AUC_{INF}	μg*hr/mL
Terminal elimination half-life	$T_{1/2}$	hr
The maximum plasma concentration	C_{max}	μg/mL
Time to maximum plasma concentration	T_{max}	hr

*Different units may be presented in the study report

Data Evaluation and Statistics

Numeric and non-numeric data obtained during the study were reported only as individual values.

RESULTS

Aerosol Concentrations

Achieved gravimetric test atmosphere concentrations were as follows:

Group No.	Targeted Aerosol Concentration (mg\L)	Achieved Mean Aerosol Concentration (mg\L)	Coefficient of Variation (%)	% of 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

Achieved analytical test atmosphere concentrations for treprostinil were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

Achieved analytical test atmosphere concentrations for trehalose were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

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.

Dose Levels

Overall achieved doses for treprostinil are presented below:

Group No.	Targeted Dose Levels (mg/kg/day)	Duration of Exposure (min)	Animal	Body Weight (kg)	Estimated Achieved Doses (mg/kg/day)	% from Targeted Dose 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

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.

Particle Size Distribution

The average gravimetric particle size distribution measurement data were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The average chemical determination of particle size distribution for treprostinil were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The average chemical determination of particle size distribution for trehalose were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

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.

Exposure Chamber Conditions

Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.

Group No.	Humidity (% RH)	Temperature (° C.)	Oxygen Concentration (%)
1	31.4	21.2	20.9
2	34.2	21.7	20.9
3	26.2	20.7	20.9

Exposure atmosphere oxygen concentrations, temperature and relative humidity ranges were considered acceptable on all occasions.

Mortality

There were no mortalities during the study.

Clinical Signs

There were no adverse clinical signs observed during the study.

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.

Body Weight

Body weights were performed for dose level calculation purposes.

Pharmacokinetics

Following the administration of PRINT-Tre at all achieved dose levels, mean C_{max} ranged from 6.800 to 46.133 ng/mL. The mean maximum plasma concentration (T_{max}) was reached at 3.75 hour (15 minutes before end of dosing) for all groups. The mean $AUC_{0\text{-}Tlast}$ (AUCINF)

ranged from 17.320 (18.335) to 121.258 (137.512) hr*ng/mL. Following T_{max}, the treprostinil plasma concentrations declined gradually with an estimated mean $T_{1/2}$ ranging from 1.01 to 1.68 hours.

Over the dose range, exposure to treprostinil (based on C., $AUC_{0\text{-}Tlast}$ 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_{max} and $AUC_{0\text{-}Tlast}$ 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_{max}) to 1.5-fold ($AUC_{0\text{-}Tlast}$) 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

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.

Non-GLP, Single-Dose, Inhalation, Comparative PK Study of LIQ861 and Nebulized Treprostinil in Rats (Study 75658)

This study evaluated and compared the PK profile of LIQ861 to treprostinil (nebulized) to establish a bridge between the two formulations.

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.

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.

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.

Summary of Mean Noncompartmental PK Parameters by Treatment and Sex for Study 75658

Type of inhalation	Group	Achieved Mean Dose Level (mg/kg)	Sex	R^2	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}Tlast}$ (hr*ng/mL)	AUC_{INF} (hr*ng/mL)
Treprostinil Sodium (Nebulized)	1	0.785	Female	0.99	0.59	4.00	16.2	62.4	63.5
			Male	0.97	0.77	0.50	16.5	59.3	60.6
Dry Powder (PRINT treprostinil)	2	0.273	Female	0.92	1.77	4.00	5.38	22.2	24.2
			Male	1.00	0.73	1.00	7.18	26.9	27.4
	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; $t^{1/2}$, half-life; AUCinf, area under the concentration-time curve extrapolated to time infinity. PRINT treprostinil = LIQ861 DP-intermediate.

Calculated Relative Bioavailability (Combined Genders) Based on AUCinf (Dose Corrected) from Study 75658

Group	Treatment	Dose Level (mg/kg)	Mean AUCinf (hr * ng/mL)	Frel (PRINT/ Treprostinil)
1	Treprostinil	0.785	62.1	NA
2	PRINT-treprostinil Low	0.273	25.8	1.20
3	PRINT-treprostinil Mid	0.762	129	2.15
4	PRINT-treprostinil High	1.498	165	1.39

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

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.

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:

Group No.	Group Designation	Achieved Mean Total Inhaled Dose Level of Treprostinil (mg/kg/day)	Achieved Aerosol Concentration of Treprostinil (μg/L)	Achieved Aerosol Concentration of Trehalose (μg/L)
1	Tre Solution	0.785	5.07	0
2	PRINT-Tre (Low Dose)	0.273	1.76	254.84
3	PRINT-Tre (Mid Dose)	0.762	4.95	719.53
4	PRINT-Tre (High Dose)	1.498	9.73	1394.33

Assessments of mortality, clinical signs and body weights were performed. Pharmacokinetic samples were collected and the analysis of these samples was performed.

No mortality occurred and no clinical signs were observed.

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.

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.

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.

Based on $AUC_{0\text{-}Tlast}$, AUCINF and C_{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_{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.

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 $T_{1/2}$ 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.

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_{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 $T_{1/2}$ values of 0.6 hours in males and 0.8 hours in females.

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 $AUC_{0\text{-}Tlast}$ was approximately twice as high (126 versus 61 h*ng/mL, respectively) and mean C_{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 $T_{1/2}$ values of 0.7 to 1.0 hours for PRINT-Tre and 0.6 to 0.8 hours for Tre solution.

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 $AUC_{0\text{-}Tlast}$ was approximately twice as great and mean C_{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.

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

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

Group No.	Group Designation	Targeted Total Inhaled Dose Level of Treprostinil (mg/kg/day)	Targeted Aerosol Concentration of Treprostinil (μg/L) [a]	Targeted Aerosol Concentration of Trehalose (μg/L) [a]	No. of Animals Males	No. of Animals 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

[a] = Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg

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

Justification for Selection of Route of Administration, Species and Dose Levels

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

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.

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

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).

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.

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

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.

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.

Treatment

Acclimatization to Exposure System

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.

Animal Exposure

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

The target aerosol concentrations and dose levels were as follows:

Group No.	Group Designation	Targeted Dose Level of Treprostinil (mg/kg/day)	Targeted Aerosol Concentration of Treprostinil (μg/L) [a]	Targeted Aerosol Concentration of Trehalose (μg/L)
1	Tre solution	0.75	5	0
2	PRINT-Tre (Low Dose)	0.15	1	130.7
3	PRINT-Tre (Mid Dose)	0.75	5	653.5
4	PRINT-Tre (High Dose)	1.5	10	1306.9

[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:

$$D_T = \frac{E_c \times RMV \times T}{BW}$$

D_L=Achieved dose levels (mg/kg/day)

E_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

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.

Inhalation System Monitoring

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.

Oxygen Concentration

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%.

Relative Humidity/Temperature

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.

Determination of Aerosol Concentration

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).

Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)

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).

The MMAD and the Geometric Standard Deviation (GSD) were calculated based on the results obtained from the impactor using a log-probit transformation.

Reporting of Analytical Results

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.

In-Life Observations

Mortality

Mortality checks were performed at least once a day during all phases of the study.

Clinical Observations

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.

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.

Body Weights

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).

Pharmacokinetics

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.

		Toxicokinetic time point							
Group Number	Number of animals/sex	30 min post start	1 hour post start	IPE	5 min post end	15 min post end	30 min post end	75 min post end	105 min 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

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.

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.

The plasma analysis was performed and the bioanalytical data was prepared for inclusion in the final report.

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.

The following configuration was used for the analysis:

Sampling Method: Sparse

AUC Calculation Method: Linear Trapezoidal with Linear Interpolation

Lambda Z (λ_z) Method: Best fit for λ_z, Log regression

Weighting (λ_z calculation): Uniform

Pharmacokinetic parameters (including abbreviation and description for each parameter) were described in the following table:

Parameters	Abbreviation	Unit*
Area under the plasma drug concentration-time curve from the time of dosing to the last quantifiable concentration	$AUC_{0\text{-}Tlast}$	μg*hr/mL
Area under the plasma drug concentration-time curve from the time of dosing extrapolated to infinity	AUC_{INF}	μg*hr/mL
Terminal elimination half-life	$T_{1/2}$	hr
The maximum plasma concentration	C_{max}	μg/mL
Time to maximum plasma concentration	T_{max}	hr

Data Evaluation and Statistics

Numeric and non-numeric data obtained during the study were reported only as individual values.

RESULTS

Formulation Analysis

Formulation concentration for Group 1 was as follows:

Group No.	Average Targeted Concentration (mg/mL)	Average Measured Concentration (mg/mL)	% of Targeted Concentration
1	0.50	0.492	98.4

The formulation concentration for Group 1 was within 2% of the targeted concentration therefore the formulation concentration was considered acceptable for the study.

Aerosol Concentrations

Achieved gravimetric test atmosphere concentrations were as follows:

Group No.	Targeted Aerosol Concentration (mg\L)	Achieved Mean Aerosol Concentration (mg\L)	Coefficient of Variation (%)	% of 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

Achieved chemical test atmosphere concentrations for treprostinil were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

Achieved chemical test atmosphere concentrations for trehalose were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

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.

Achieved Dose Levels

Overall achieved doses for treprostinil are presented below:

Group No.	Targeted Dose Level (mg/kg/day)	Duration of Exposure (min)	Sex	Body Weight (kg)	Estimated Achieved Doses (mg/kg/day)	% from Targeted Dose 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

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.

Particle Size Distribution

The average gravimetric particle size distribution measurement data were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The average chemical determination of particle size distribution for treprostinil were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The average chemical determination of particle size distribution for trehalose were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

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.

Exposure Chamber Conditions

Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.

Group No.	Humidity (% RH)	Temperature (° C.)	Oxygen Concentration (%)
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

Exposure atmosphere oxygen concentrations, temperature and relative humidity ranges were considered acceptable on all occasions.

Mortality

There were no mortalities during the study.

Clinical Signs

There were no clinical signs observed during the study.

Body Weight

Body weights were performed for dose level calculation purposes.

Pharmacokinetics

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.

Based on $AUC_{0\text{-}Tlast}$, AUCINF and C_{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_{max}) was at the end of inhalation for both sexes, except for low-dose males and high-dose females, where mean T_{max} was at 1 and 0.5 hours after inhalation began, respectively.

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.

	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^	5.4	33**	44

*Individual values were 33, 63, and 67 ng/mL

^Individual values were 34, 48, and 50 ng/mL

**Individual values were 22, 27, and 49 ng/mL

When inhalation ended, treprostinil plasma concentrations declined gradually. Given the degree of individual variation, the estimated mean $T_{1/2}$ 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.

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_{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.

	Males	Females
0.5 hours of inhalation	17	12
1 hour of inhalation	11	14
4 hours of inhalation	16	16

When inhalation ended, treprostinil plasma concentrations declined gradually, with estimated mean $T_{1/2}$ values of 0.6 hours in males and 0.8 hours in females.

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 $AUC_{0\text{-}Tlast}$ 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 $T_{1/2}$ values of 0.7 to 1.0 hours for PRINT-Tre and 0.6 to 0.8 hours for Tre solution.

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 $AUC_{0\text{-}Tlast}$ was approximately twice as great and mean C_{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.

Non-GLP, 7-Day, Repeat-Dose, Range-Finding (DRF), Inhalation Study with LIQ861 in Rats (Study 75654)

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.

Summary: Study 75654

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.

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:

		Mean Dose Levels and Concentrations[a]				
		Treprostinil		Trehalose		Leucine
Group No.	Group Designation	Dose Level (μg/kg/day)	Aerosol Conc. (μg/L)	Dose Level (mg/kg/day)	Aerosol Conc. (μg/L)	Dose Level (mg/kg/day)[b]
1	Air Control	0	0	0	0	0
2	Placebo Control[b]	0	0	281.2	1832.13	12.0
3	PRINT-Tre (Low Dose)	170	1.10	33.1	216.30	1.3
4	PRINT-Tre (Mid Dose)	680	4.44	133.5	869.99	5.1
5	PRINT-Tre (High Dose)	1370	8.94	266.6	1735.84	10.3

[a]= Based on the mean body weight of each group during the dosing period.

[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.

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.

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

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

Synopsis

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.

Group		Targeted Aerosol Concentration (μg/L)		Targeted Dose Level (mg/kg/day)		Leucine Dose Level
No.	Test Material	Treprostinil	Trehalose	Treprostinil[a]	Trehalose[b]	(mg/kg/day)[c]
1	Air Control	0	0	0	0	0
2	PRINT Placebo[b]	0	1684.6	0	262.9	11.2
3	PRINT-Tre (Low Dose)	1	175.2	0.15	26.3	1.1
4	PRINT-Tre (Mid Dose)	5	876.2	0.75	131.4	5.7
5	PRINT-Tre (High Dose)	10	1752.5	1.5	262.9	11.3

[a]= Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg

[b]= The target dose level for the placebo control was the same dose level as the high dose group (Group 5)

[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)

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

The low- and mid-dose levels for PRINT-Tre were selected on the basis of a previous PK study in rats (Study No. 75658).

Test and Control Item Information

Test Item Action

Treprostinil, the active ingredient in PRINT-Tre, is a prostacyclin compound approved for treatment of pulmonary arterial hypertension.

Characterization of Test Item

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

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.

Treatment

Acclimatization to Exposure System

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.

Animal Exposure

Exposure system used: Flow-past rodent inhalation exposure system

Exposure method: Inhalation by nose-only exposure

Test and Control Item type: Air (Group 1), Dry Powder (Groups 2 to 5)

Generation method: Piston feed/rotating brush generator (Groups 2 to 5)

Duration of exposure: 240 minutes

The target aerosol concentrations and dose levels were as follows:

Group No.	Group Designation	Targeted Total Inhaled Dose Level of Treprostinil (mg/kg/day)[a]	Targeted Aerosol Concentration of Treprostinil (μg/L)	Targeted Total Inhaled Dose Level of Trehalose (mg/kg/day)[a]	Targeted Aerosol Concentration of Trehalose (μg/L)	Estimated Total Inhaled Dose Level of Leucine (mg/kg/day)[c]
1	Air Control	0	0	0	0	0
2	Placebo Control[b]	0	0	262.9	1684.6	11.2
3	PRINT-Tre (Low Dose)	0.15	1	26.3	175.2	1.1
4	PRINT-Tre (Mid Dose)	0.75	5	131.4	876.2	5.7
5	PRINT-Tre (High Dose)	1.5	10	262.9	1752.5	11.3

[a]= Target aerosol concentrations were calculated based on an estimated body weight of 0.250 kg

[b]= The target dose level for the placebo control was the dose level as the high dose group (Group 5)

[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)

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

$$D_T = \frac{E_c \times RMV \times T}{BW}$$

D_L=Achieved dose levels (mg/kg/day)=

E_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 $(kg)^{0.809}$

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

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.

Inhalation System Monitoring

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.

Oxygen Concentration

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%.

Relative Humidity/Temperature

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.

Determination of Aerosol Concentration

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).

Determination of Aerosol Homogeneity

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.

Determination of the Particle Size Distribution and Mass Median Aerodynamic Diameter (MMAD)

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).

The MMAD and the Geometric Standard Deviation (GSD) were calculated based on the results obtained from the impactor using a log-probit transformation.

Reporting of Analytical Results

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.

Standard Operating Procedures

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

Inhalation System Monitoring

Oxygen Concentration, Temperature, and Relative Humidity

Exposure chamber conditions from the reported aerosol concentration exposures are summarized below.

Group Number	Humidity (% RH) Average	Temperature (° C.) Average	Oxygen Concentration (%) 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

Exposure atmosphere oxygen concentration, temperature and relative humidity were considered acceptable throughout the study.

Aerosol Concentrations

Achieved gravimetric test atmosphere concentrations were as follows:

Group No.	Targeted Aerosol Concentration (mg\L)	Achieved Mean Aerosol Concentration (mg\L)	Coefficient of Variation (%)	% of 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.

Achieved test atmosphere concentrations for treprostinil were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

Achieved test atmosphere concentrations for trehalose were as follows:

Group No.	Targeted Aerosol Concentration (μg\L)	Achieved Mean Aerosol Concentration (μg\L)	Coefficient of Variation (%)	% of 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

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.

Aerosol Homogeneity

Achieved gravimetric test atmosphere homogeneity concentrations were as follows:

Group No.	Aerosol Concentration of Top Tier (mg/L)	Aerosol Concentration of Middle Tier (mg/L)	Aerosol Concentration of Bottom Tier (mg/L)	CV (%)
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

Achieved test atmosphere homogeneity concentrations for treprostinil were as follows:

Group No.	Aerosol Concentration of Top Tier (μg/L)	Aerosol Concentration of Middle Tier (μg/L)	Aerosol Concentration of Bottom Tier (μg/L)	CV (%)
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

Achieved test atmosphere homogeneity concentrations for trehalose were as follows:

Group No.	Aerosol Concentration of Top Tier (μg/L)	Aerosol Concentration of Middle Tier (μg/L)	Aerosol Concentration of Bottom Tier (μg/L)	CV (%)
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

Chamber homogeneity of the aerosol concentrations were considered acceptable since the coefficient of variance of aerosol concentration between samples was not greater than 20%.

Particle Size Distribution

The average gravimetric particle size distribution measurement data were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The chemical determinations of particle size distribution for treprostinil were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

The chemical determinations of particle size distribution for trehalose were as follows:

Group	Cumulative % Less Than Stated Effective Cut-Off Diameter (μm)								Mean 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.

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.

Estimation of Achieved Dose Levels

Overall achieved doses for treprostinil are presented below:

Group No.	Targeted Dose Level (mg/kg/day)	Duration of Exposure (min)	Sex	Body Weight (kg)	Estimated Achieved Doses (mg/kg/day)	% of Targeted 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

Overall achieved doses for trehalose are presented below:

Group No.	Targeted Dose Level (mg/kg/day)	Duration of Exposure (min)	Sex	Body Weight (kg)	Estimated Achieved Doses (mg/kg/day)	% of Targeted 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

Overall achieved doses for leucine are presented below:

Group No.	Targeted Dose Level (mg/kg/day)	Duration of Exposure (min)	Sex	Body Weight (kg)	Estimated Achieved Doses[a] (mg/kg/day)	% of Targeted 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

[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)

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.

Mortality

There were no mortalities during the study.

Clinical Signs

There were no clinical signs observed during the study.

Body Weight

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.

These data are summarized in the table below, with differences potentially related to treprostinil in bold.

	Test Material =				
	Air	PRINT 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

-continued

	Test Material =				
	Air	PRINT 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
Change					
Absolute (g)*	−15	−14	−16	−14	−15
Relative to Air Control	—	+1	−1	−1	0

*All animals were fasted overnight prior to necropsy.

Remaining differences were considered incidental and of no biological significance.

Hematology

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.

These data are summarized in the table below, with differences potentially related to treprostinil in bold.

	Test Material =				
	Air	PRINT 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 ($\times 10^{12}$/L)	0.226	0.228	0.309	0.333	0.337
Relative to Air Control	—	+1%	**+37%**	**+47%**	**+49%**
Females					
Mean ($\times 10^{12}$/L)	0.179	0.176	0.214	0.290	0.283
Relative to Air Control	—	+1%	**+20%**	**+62%**	**+58%**

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.

Remaining differences among mean hematology parameters were considered incidental and of no biological significance.

Coagulation

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.

Clinical Chemistry

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.

Urinalysis

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.

Organ Weights

Differences in mean organ weight potentially related to administration of the test or control item were noted for lungs, adrenal glands, thymus, and testes.

Remaining differences in mean organ weight were considered incidental and of no biological significance.

Lungs

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.

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.

Lung weight data are summarized in the table below, with differences potentially related to PRINT Placebo and PRINT-Tre in bold.

Mean Lung Weight Data					
	Test Material =				
	Air	PRINT 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

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.

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.

Thymus weight data are summarized in the table below, with differences potentially related to treprostinil in bold.

Mean Thymus Weight Data					
	Test Material =				
	Air	PRINT 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 weight)	0.168	0.198	0.173	0.128	0.143
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 weight)	0.229	0.210	0.228	0.202	0.166
Relative to Air Control	—	−8%	±0%	−12%	**−28%**

Adrenal Glands

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 trepro-

stinil, 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.

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.

Adrenal glands weight data are summarized in the table below, with differences potentially related to treprostinil in bold.

Mean Adrenal Glands Weight Data					
	Test Material =				
	Air	PRINT 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 weight)	0.021	0.023	0.025	0.023	0.027
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 weight)	0.036	0.035	0.035	0.036	0.042
Relative to Air Control	—	−3%	−3%	±0%	**+17%**

Testes

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.

Testes weight data are summarized in the table below, with differences potentially related to treprostinil in bold.

Mean Testes Weight Data					
	Test Material =				
	Air	PRINT 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 weight)	1.17	1.13	1.10	1.08	1.06
Relative to Air Control	—	−3%	−6%	**−8%**	**−9%**

Macroscopic Findings

There was no evidence of test item-related macroscopic findings at necropsy.

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.

Microscopic Findings

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.

Lungs

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.

These data are summarized in the table below, with differences potentially related to test or control item in bold.

Incidence and Grade of Increased Alveolar Macrophages

	Test Material =				
	Air	PRINT Placebo	PRINT-Tre		
	Treprostinil 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

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.

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.

These data are summarized in the table below, with differences potentially related to test or control item in bold.

Incidence and Grade of Goblet-cell Hypertrophy/Hyperplasia

	Test Material =				
	Air	PRINT Placebo	PRINT-Tre		
	Treprostinil 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

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.

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.

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.

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.

Clinical Study: LIQ861

Randomized, Placebo-controlled, Single-ascending Dose Study Evaluating Pharmacokinetics (PK) and Safety in Healthy Male and Female Volunteers

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.

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).

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.

Cohort 1

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.

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.

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_{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.

The C_{max} averaged 0.364 ng/mL and the most frequent T_{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%.

Cohort 2

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.

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.

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.

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.

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%.

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.

Cohort 3

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.

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.

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.

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.

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%.

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_{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.

Cohort 4

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.

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.

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.

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%.

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.

Cohort 5

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.

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.

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.

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%.

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.

Cohort 6

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.

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.

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.

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%.

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

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.

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 Powder in mg	5	10	15	Combination of two 50 mcg capsules or one 25 mcg and one 75 mcg	Combination of, ex., 1 at 50 mcg and 1 at 75 mcg	Combination of, ex., two capsules at 75 mcg
Active Agent Load in mcg	25	50	75	Varies, see above	Varies, see above	Varies, see above
Breaths to Deliver	1 to 2	1 to 2	1 to 2	1 to 2 per capsule	1 to 2 per capsule	1 to 2 per capsule
Particle Powder per Breath in mg	2.5-5	5-10	7.5-15	Varies, up to 15	Varies, up to 15	Varies, up to 15
Active Agent per Breath in mcg	12.5-25	25-50	37.5-75	Varies, up to 75	Varies, up to 75	Varies, up to 75

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.

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.

LIQ861 Clinical Outcomes	C_{max} (ng/mL)	T_{max}[a] (h)	$t_{1/2}$ (h)	AUC_{last} (h * ng/mL)	AUC_{inf} (h * ng/mL)	CL/F (L/h)	Vz/F (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_{max} = maximum observed plasma concentration; T_{max} = time to C_{max}; $t_{1/2}$ = half-life; AUC = area under the curve; CL/F = apparent clearance; Vz/F = apparent volume of distribution
All values except for T_{max} are reported as arithmetic means with SD in parentheses.
[a]T_{max} reports median values with minimum and maximum values in parentheses

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.

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.

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.

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 Powder in mg	2.5	5	7.5	10	12.5	15
Active Agent Load in mcg	25	50	75	100	125	150
Breaths to Deliver	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2	1 to 2
Particle Powder per Breath in mg	1.25-2.5	2.5-5	3.75-7.5	5-10	6.25-12.5	7.5-15
Active Agent per Breath in mcg	12.5-25	25-50	37.5-75	50-100	62.5-125	75-150

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.

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.

Kits

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.

Abbreviations and Nomenclature Cross-references

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

-continued

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

-continued

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_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

Product Nomenclature and Reference Table

Term	Designation	Exemplary Embodiment
Drug Substance	DS or treprostinil	Treprostinil, supplied as treprostinil sodium for manufacture of the LIQ861 drug product-intermediate
Drug Product-Intermediate	DP-intermediate or LIQ861 DP-intermediate	Dry powder particles of precise size and 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-Intermediate	Placebo DP-intermediate	Identical formulation as the DP-Intermediate, but treprostinil is replaced with an equal mass of trehalose
Inhalation Powder Drug Product	DP or LIQ861 Drug Product or LIQ861	LIQ861 DP-intermediate filled into Size 3 HPMC capsules for oral 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

-continued

Product Nomenclature and Reference Table		
Term	Designation	Exemplary Embodiment
Drug Product Strength or Dose	Treprostinil in LIQ861	Amount of treprostinil in drug product
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 Inhaler	DPI	Drug Product integrated with the Inhalation Device; i.e., the final product for patient use

We claim:

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.

* * * * *