CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214324Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	214324
Supporting document:	2
Applicant's letter date:	4/16/2021
CDER stamp date:	4/16/2021
Product:	Tyvaso DPI™ (treprostinil) Inhalation Powder
Indication:	Pulmonary arterial hypertension (PAH) and pulmonary hypertension associated with interstitial lung disease (PH-ILD)
Applicant:	United Therapeutics Corporation (UTC)
Clinical Review Division:	Division of Cardiology and Nephrology (DCN)
Pharm/Tox Division:	Division of Pharm/Tox for Cardiology, Hematology, Endocrinology, and Nephrology (DPT-CHEN)
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Template Version: September 1, 2010 (Modified by DCRP: June 10, 2013)

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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Treprostinil is a prostacyclin analogue. The major pharmacologic actions of treprostinil are direct vasodilation of pulmonary and systemic arterial vascular beds and inhibition of platelet aggregation. Inhaled treprostinil therapy provides selectivity of the hemodynamic effects to the lung vasculature, thus reducing systemic side effects compared to other routes of administration. Following inhalation of prostacyclin analogs, pulmonary artery pressure decreases, and systemic arterial pressure is stable.

The active pharmaceutical ingredient (API) in Tyvaso DPI™ is identical to the treprostinil drug substance approved in Remodulin (NDA 021272) and Tyvaso (NDA 022387). It is also the same active moiety as the treprostinil diolamine drug substance approved in Orenitram (treprostinil) Extended-Release Tablets (NDA 203496). The safety and efficacy of treprostinil are supported by a comprehensive set of pharmacology, pharmacokinetic (PK), toxicology, and clinical studies conducted for Tyvaso (treprostinil) Inhalation Solution (NDA 022387), Remodulin (treprostinil) Injection (NDA 021272), and Orenitram (treprostinil) Extended-Release Tablets (NDA 203496).

The safety of the excipient fumaryl diketopiperazine (FDKP) is supported by a comprehensive battery of safety pharmacology and toxicology studies conducted for Afrezza (BLA 022472).

1.2 Brief Discussion of Nonclinical Findings

In a rat PK study following intermittently delivery of treprostinil into the tracheal tube, treprostinil Tmax was ≤5 minutes. Treprostinil Inhalation Powder (TrIP) provided much higher treprostinil exposure than the nebulized reference solution. There was no evidence that the excipient FDKP, present in Tyvaso DPI, interfered with the absorption of treprostinil in the lung.

In studies to assess the potential effects of a Tyvaso DPI impurity (b) (4) in vitro, the stimulating activity of (b) (4) at prostanoid receptors IP1, EP2, DP1, and EP1 in tested cell lines were approximately 23-, 10-, 14-, and 25-fold (b) (4) respectively, than treprostinil. (b) (4) (b) (4) (b) (4) in human liver microsomes and hepatocytes, with $T_{1/2}$ < (b) (4) min. In (b) (4), little to no (b) (4) was present at 0 minutes, suggesting that (b) (4) at time zero was also observed ((b) (4) %).

The potential bacterial mutagenicity of 7 impurities identified in Tyvaso DPI was evaluated by (quantitative) structure-activity relationship ((Q)SAR) using Derek Nexus

and Leadscope Model Applier. All 7 impurities ([redacted] and [redacted] were identified as inactive (non-mutagenic) in DEREK Nexus and negative (non-mutagenic) in the Leadscope Model Applier. None of the structures had any structural features which were identified as unclassified/misclassified or out of domain, respectively, in Derek Nexus or Leadscope. As such, all 7 impurities are considered Class 5 impurities and may be treated as non-mutagenic.

[redacted] is identified as an impurity in Tyvaso DPI at a concentration above the qualification threshold of 1% for drug products with a maximum daily dose of drug substance that is <10 mg. [redacted] was qualified at a concentration of up to [redacted]% in the final drug product. [redacted] was shown to have reduced pharmacodynamic effects compared to treprostinil, and it is expected to be [redacted] It is unlikely that the presence of [redacted] in Tyvaso DPI would be a safety concern for patients, if present at up to [redacted]%.

Based on the results of the nonclinical pharmacology, PK, and toxicology studies conducted to support Tyvaso (NDA 022387), Remodulin (NDA 021272), and Orenitram (NDA 203496), and the assessment of impurities present in Tyvaso DPI, it is considered that Tyvaso DPI has an acceptable safety profile and that there are no findings that preclude long-term inhalation administration in humans.

1.3 Recommendations

1.3.1 Approvability

Approvable

1.3.2 Additional Non-Clinical Recommendations

None

1.3.3 Labeling

We suggest following changes (**bold** for insert and cross out for delete):

(1) Under INDICATIONS AND USAGE on page 1

Tyvaso DPI is a ~~prostacyclin mimetic~~ [redacted] indicated for the treatment of

(2) To second paragraph under section 13.1 [redacted]

Oral administration of treprostinil diolamine to Tg.rasH2 mice at 0, 5, 10, and 20 mg/kg/day in males and 0, 3, 7.5, and 15 mg/kg/day in females daily for 26 weeks did not significantly increase the incidence of tumors. [redacted]

2 Drug Information

2.1 Drug

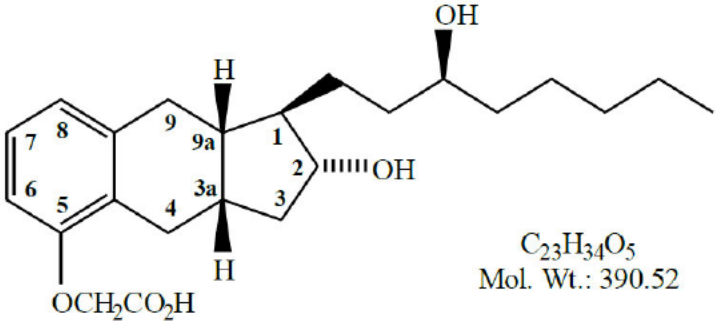
CAS Registry Number: 81846-19-7

Generic Name: Treprostinil

Code Name: UT-15

Chemical Name: [[(1R,2R,3aS,9aS)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]acetic acid

Molecular Formula / Molecular Weight / Structure :



$C_{23}H_{34}O_5$
Mol. Wt.: 390.52

Pharmacologic Class: Prostacyclin vasodilator

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 022387 (Tyvaso), NDA 021272 [Remodulin® (treprostinil) Injection], NDA 203496 [Orenitram® (treprostinil) Extended-Release Tablets], IND 134582 (Tyvaso DPI), BLA 022472 [Afrezza® (insulin human) Inhalation Powder], and DMF (b) (4)

2.3 Drug Formulation

Tyvaso DPI is targeted to contain 10 mcg of treprostinil per mg of powder. Tyvaso DPI is filled into unit-dose cartridges to contain 16 mcg, 32 mcg, 48 mcg, or 64 mcg of treprostinil per cartridge. The 16 mcg, 32 mcg, 48 mcg, and 64 mcg cartridges have nominal fill weights of 1.6 mg, 3.2 mg, 4.8 mg, and 6.4 mg of Tyvaso DPI, respectively. The composition of Tyvaso DPI per cartridge is shown in the table below.

Component	1.6 mg Cartridge	3.2 mg Cartridge	4.8 mg Cartridge	6.4 mg Cartridge
Treprostinil	0.016 mg	0.032 mg	0.048 mg	0.064 mg
Fumaryl Diketopiperazine (FDKP)	(b) (4)			

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Acceptable

2.6 Proposed Clinical Population and Dosing Regimen

Patients with pulmonary arterial hypertension or with pulmonary hypertension associated with interstitial lung disease will use 4 separate treatment sessions each day approximately 4 hours apart, during waking hours. Initial dosage is one 16 mcg cartridge per treatment session. Dosage should be increased by an additional 16 mcg per treatment session at approximately 1- to 2-week intervals, if tolerated.

2.7 Regulatory Background

Written responses for a preNDA meeting were issued on 11/19/2020. The agent stated that “From a clinical perspective and pharmacology/toxicology perspective your planned NDA Table of Contents is acceptable”.

3 Studies Submitted and Cross-referenced

3.1 Studies Reviewed

3515 EVALUATION OF COMPOUNDS AGAINST HUMAN G PROTEIN-COUPLED RECEPTORS

3538 EVALUATION OF COMPOUNDS AGAINST HUMAN G PROTEIN-COUPLED RECEPTORS

MKC-PC-2018-004 Determination of the in vivo pharmacokinetics of an inhaled Treprostinil dry powder and nebulized Treprostinil in Rat

20UNITP1S5 STABILITY IN HUMAN LIVER MICROSOMES STABILITY IN HUMAN HEPATOCYTES

(b) (4) (2020) COMPUTATIONAL EVALUATION OF THE POTENTIAL BACTERIAL MUTAGENICITY OF Impurities Potentially Associated with Treprostinil Inhalation Powder

(b) (4) (2021) Toxicological Profile and Risk Assessment for (b) (4) in Inhaled Treprostinil Drug Product (Tyvaso DPI Inhalation Powder)

3.2 Studies Not Reviewed

None

3.3 Studies Cross-referenced

The safety and efficacy of treprostinil is supported by a comprehensive set of pharmacology, pharmacokinetic (PK), toxicology, and clinical studies conducted for Tyvaso (treprostinil) Inhalation Solution (NDA 022387), Remodulin® (treprostinil) Injection (NDA 021272), and Orenitram® (treprostinil) Extended-Release Tablets (NDA 203496). The active pharmaceutical ingredient (API) in Tyvaso DPI is identical to the treprostinil drug substance approved in Remodulin (NDA 021272) and Tyvaso (NDA 022387). It is also the same active moiety as the treprostinil diolamine drug substance approved in Orenitram (treprostinil) Extended-Release Tablets (NDA 203496). A tabulated list of all nonclinical studies that are cross referenced from Tyvaso (NDA 022387), Remodulin (NDA 021272), and Orenitram (NDA 203496) is presented in Module 1 Section 1.4.4 of this application.

The clinical and toxicology data for the excipient, fumaryl diketopiperazine (FDKP), is cross-referenced from MannKind Corporation's approved drug product, Afrezza® (insulin human) Inhalation Powder (BLA 022472) (Letter of Authorization in Section 1.4.2).

All these nonclinical studies were previously reviewed under the referred NDAs or BLA and are not re-reviewed under current NDA.

4 Pharmacology

4.1 Primary Pharmacology

MOA: Treprostinil is a prostacyclin analogue. The major pharmacologic actions of treprostinil are direct vasodilation of pulmonary and systemic arterial vascular beds and inhibition of platelet aggregation. Inhaled treprostinil therapy provides selectivity of the hemodynamic effects to the lung vasculature, thus reducing systemic side effects compared to other routes of administration. Following inhalation of prostacyclin analogs, pulmonary artery pressure decreases, and systemic arterial pressure is stable.

4.1 EVALUATION OF COMPOUNDS AGAINST HUMAN G PROTEIN-COUPLED RECEPTORS (3515)

This study (3515) tested 12 compounds, treprostinil, and 1 control agonist for 3 GPCRs (DP1, EP2, IP1) using cAMP assay and 1 GPCR (EP1) using calcium assay (Table 1).

Table 1. Cell lines, assays, control agonists, and tested compounds

Species	Target	Parental	Catalog #	Assays	Control agonist
Human	DP1	HEK293T	C1200	cAMP	PGD2
Human	EP2	HEK293T	C1202	cAMP	Iloprost
Human	IP1	CHO-K1	C1206-1	cAMP	Iloprost
Human	EP1	HEK293T	C1201a	Calcium	Iloprost

#	Compound ID	Mode
1	Treprostinil	Agonist
	(b) (4)	

Control agonists for all 4 prostanoid receptors (DP1, EP2, IP1, EP1) showed dose-dependent stimulation in the receptor expressing cells with expected EC_{50} values (Figure 1, Figure 2). Treprostinil and some compounds displayed dose-response agonist activity in the receptors tested (Figure 1, Figure 2). Activity of the impurity (b) (4) at the IP1, EP2, DP1, and EP1 receptors were approximately 23-, 10-, 14-, and 25-fold lower, respectively, than treprostinil (Figure 1, Figure 2).

Figure 1. cAMP assay in agonist mode: Dose-dependent stimulation of agonist-induced intracellular cAMP accumulation in human EP2, IP1, or DP1 receptor-expressing cells (modified from the submission)

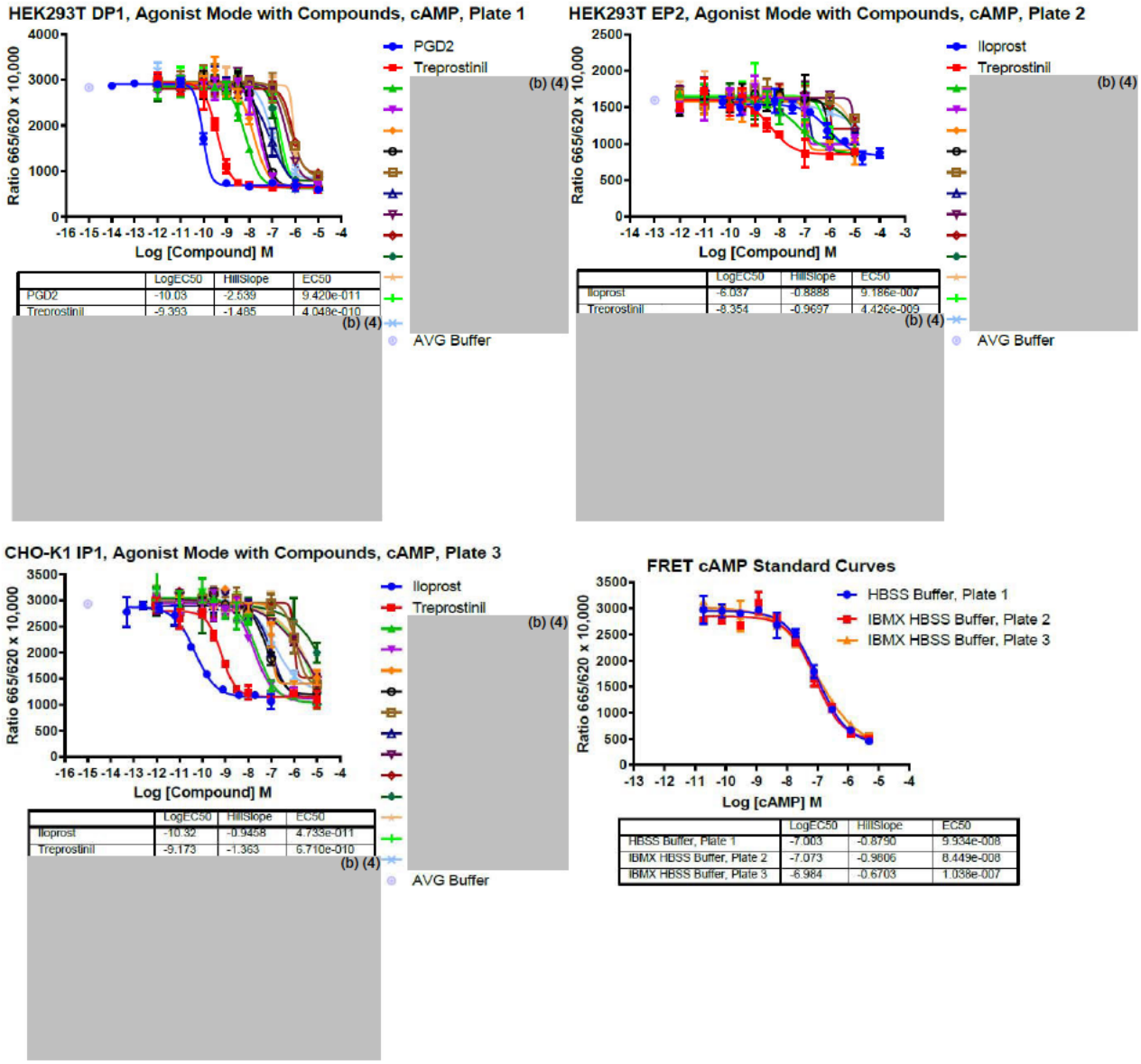
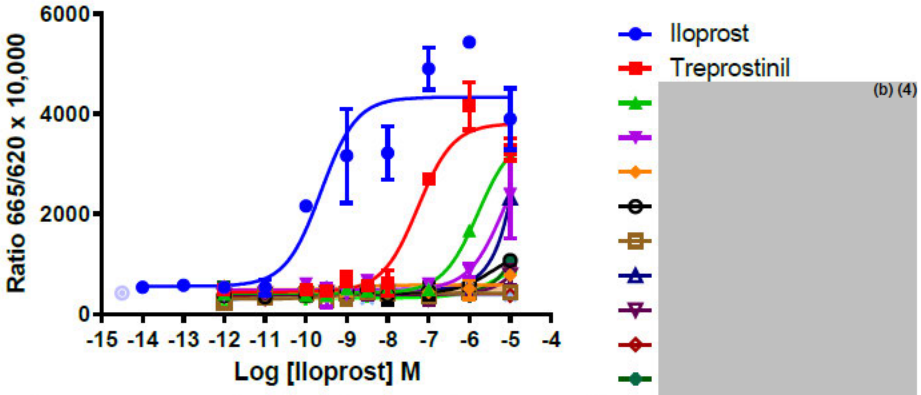


Figure 2. Calcium assay in agonist mode: Dose-dependent stimulation of Calcium flux in human EP1 receptor-expressing cells (from the submission).

HEK293T EP1, Agonist Mode with Compounds, Calcium, Plate 4



	LogEC50	HillSlope	EC50
Iloprost	-9.633	= 1.000	2.327e-010
Treprostinil	-7.260	= 1.000	5.490e-008

(b) (4)

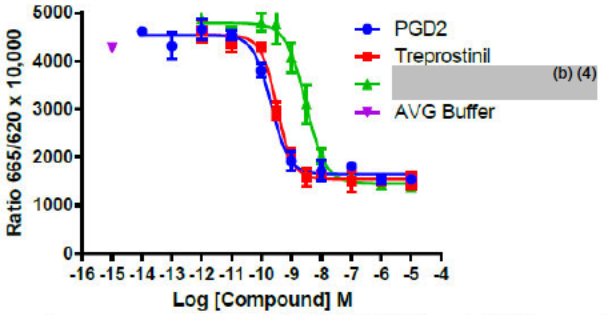
4.2 EVALUATION OF COMPOUNDS AGAINST HUMAN G PROTEIN-COUPLED RECEPTORS (3538)

This study (3538) tested (b) (4) treprostinil, and 1 control agonist for 3 GPCRs (DP1, EP2, IP1) using cAMP assay and 1 GPCR (EP1) using calcium assay. Cell lines, assays, and control agonists are list in Table 1.

Control agonists for all 4 prostanoid receptors (DP1, EP2, IP1, EP1) showed dose-dependent stimulation in the receptor expressing cells with expected EC_{50} values (Figure 3, Figure 4). Both treprostinil and (b) (4) displayed dose-response agonist activity in the receptors tested, and activity of (b) (4) at the IP1, EP2, DP1, and EP1 receptors were approximately 23-, 10-, 14-, and 25-fold lower, respectively (Figure 3, Figure 4). Similar to the results in study 3515, activity of (b) (4) at the IP1, EP2, DP1, and EP1 receptors were lower than that of treprostinil (Figure 3, Figure 4).

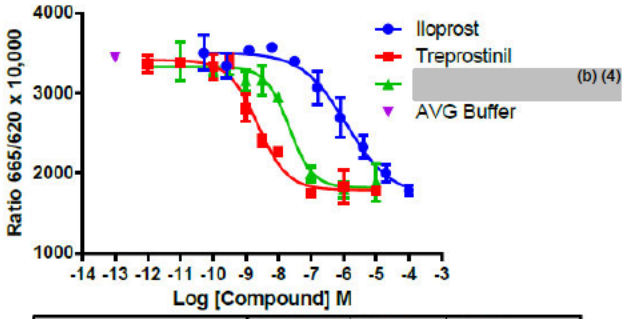
Figure 3. cAMP assay in agonist mode: Dose-dependent stimulation of agonist-induced intracellular cAMP accumulation in human EP2, IP1, or DP1 receptor-expressing cells (modified from the submission)

HEK293T DP1, Agonist Mode with Compounds, cAMP



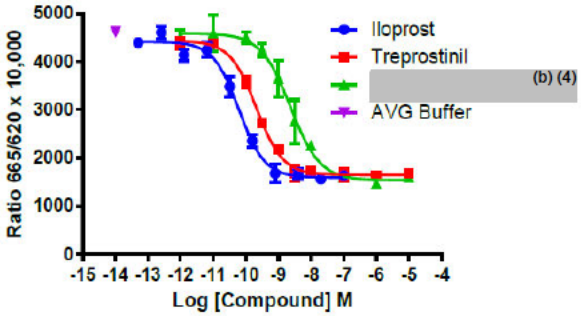
	LogEC50	HillSlope	EC50
PGD2	-9.679	-1.455	2.095e-010
Treprostinil	-9.483	-1.606	3.292e-010
(b) (4)			

HEK293T EP2, Agonist Mode with Compounds, cAMP



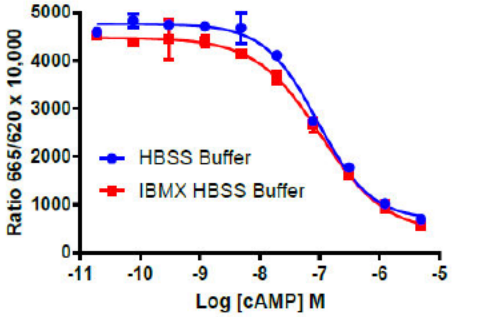
	LogEC50	HillSlope	EC50
Iloprost	-5.915	-0.6378	1.215e-006
Treprostinil	-8.629	-0.9616	2.352e-009
(b) (4)			

CHO-K1 IP1, Agonist Mode with Compounds, cAMP



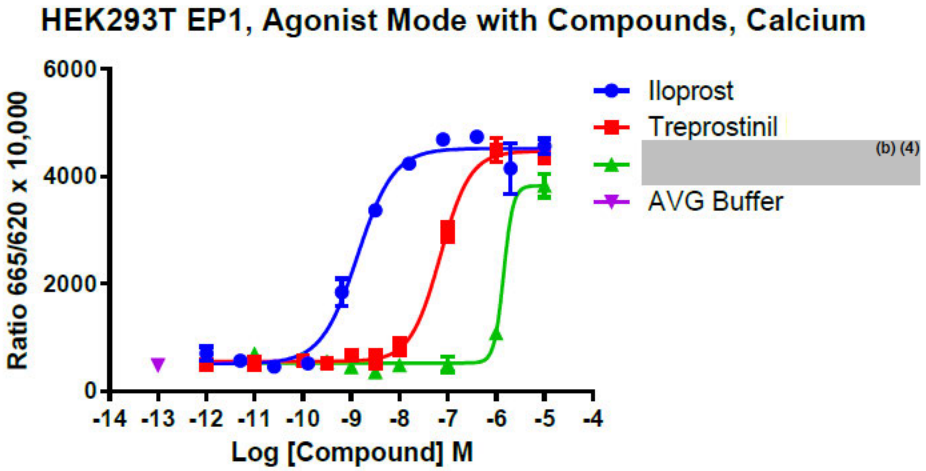
	LogEC50	HillSlope	EC50
Iloprost	-10.21	-1.095	6.195e-011
Treprostinil	-9.672	-1.073	2.128e-010
(b) (4)			

FRET cAMP Standard Curves



	LogEC50	HillSlope	EC50
HBSS Buffer	-7.030	-0.9740	9.334e-008
IBMX HBSS Buffer	-6.981	-0.7968	1.046e-007

Figure 4. Calcium assay in agonist mode: Dose-dependent stimulation of Calcium flux in human EP1 receptor-expressing cells (from the submission).



	LogEC50	HillSlope	EC50
Iloprost	-8.868	1.161	1.354e-009
Treprostinil	-7.155	1.404	7.000e-008
(b) (4)			

5 Pharmacokinetics

5.1 Determination of the in vivo pharmacokinetics of an inhaled Treprostinil dry powder and nebulized Treprostinil in Rat (MKC-PC-2018-004)

This study (MKC-PC-2018-004) sponsored by MannKind Corporation, Danbury, CT was done in (b) (4) (The sponsorship under the associated IND-134582 was changed from MannKind Corporation to United Therapeutics Inc. on Oct 18, 2018). This study evaluated the pharmacokinetics (PK) of two dry powder formulations of treprostinil and a reference compound nebulized treprostinil.

Male Sprague Dawley rats (body weight 200-250g) were anesthetized with isoflurane, then were intubated and Treprostinil Inhalation Powder (TrIP) was delivered intermittently into the tracheal tube (Table 2). After dosing, blood samples were collected at specific time points over a period of 6 hours. Nebulized treprostinil was used as a reference compound. The selected doses reflect a range believed to exceed the therapeutic dose in humans.

Table 2. The study design for treprostinil administration (from the submission)

Group	Test article	Amount of TA	Target amount of inhalation powder per rat (mg)	Treprostinil content	Target dose of Treprostinil per rat* (mg/kg)	n
1	TrIP1	(b) (4)	1	1.02 (wt%)	0.041	3
2	TrIP1		0.45	1.02 (wt%)	0.018	3
3	TrIP2		1.2	0.39 (wt%)	0.019	3
4	TrIP2		0.4	0.39 (wt%)	0.006	3
5	Treprostinil in PBS		N/A	102.5 µg/mL	0.041**	3

*Based on 250 g rat.

(b) (4)

**The total nebulized dose was 0.041 mg/kg. No more than 25% (0.010 mg/kg) was deposited.

The lower dose of TrIP formulation #2 (target 0.006 mg/rat,) could not be detected in plasma. For all detectable test compounds, the maximal plasma concentration was detected 5 minutes post-dosing (first time point of blood collection). TrIP provided much higher treprostinil exposure than the nebulized reference solution (Figure 5, Table 3). It was stated that there was no evidence that the excipient FDKP, present in TrIP formulations, interfered with the absorption of treprostinil into the lung.

Figure 5. Concentrations of Treprostinil in Rat Plasma Samples (from the submission)

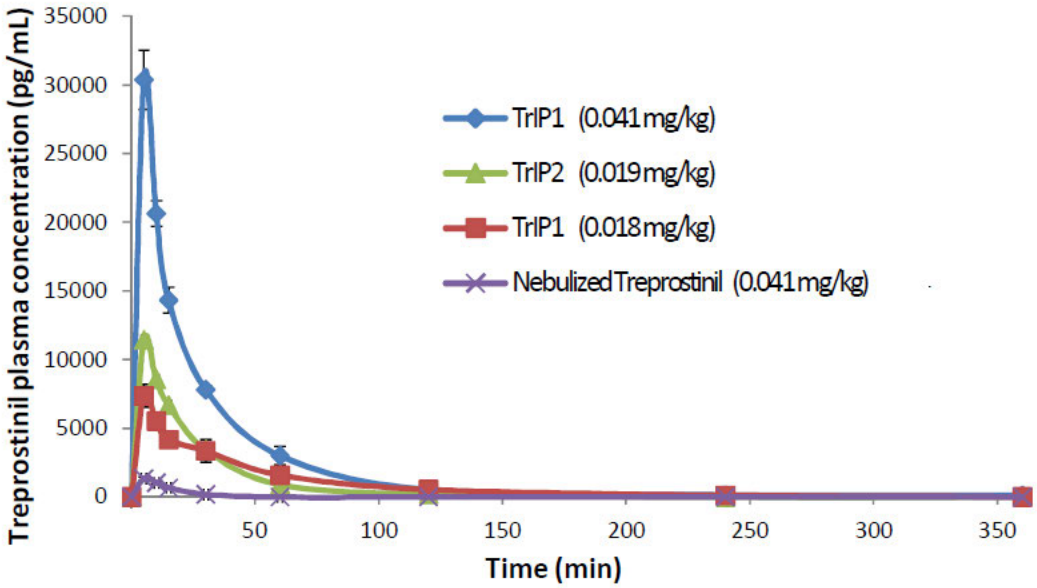


Table 3. Non-compartmental analysis of plasma data after insufflation of TrIP1/2 or nebulization of treprostinil (from the submission)

Parameter	Unit	Group 1	Group 2	Group 3	Group 4	Group 5
Dose	*mg/kg*	0.041	0.018	0.019	0.006	0.041*
Elimination rate	*L/min*	0.015	0.014	0.035	BLOQ	0.082
Half-life	*min*	44.36	48.97	19.71	BLOQ	8.38
Tmax	*min*	5	5	5	BLOQ	5
Cmax	*pg/mL*	30375.3	7364.6	11449.3	BLOQ	1330.7
Total drug exposure	*pg/mL*min*	792886.7	317547.3	296854.6	BLOQ	22542.1
CL/F	*mL/min/kg*	<52	<57	<64	BLOQ	<444

*The total nebulized dose was 0.041 mg/kg. No more than 25% (0.010 mg/kg) was deposited. CL/F was calculated based on a dose of 0.010 mg/kg.

5.2 STABILITY IN HUMAN LIVER MICROSOMES STABILITY IN HUMAN HEPATOCYTES (20UNITP1S5)

This study (20UNITP1S5) was to determine the stability of (b) (4) in human liver microsomes and human hepatocytes.

(b) (4) was added into the reaction mixture (containing mixed-gender human liver microsomes) or mixed-gender human cryopreserved hepatocytes suspension (1.5 × 106 cells/mL) at a final concentration of 1 µM (in duplicate). The mixture was incubated in a shaking water bath at 37°C. Aliquots (200 µL) were withdrawn at 0, 10, 20, 30, and 60 minutes for liver microsome reaction and 0, 15, 30, 60, and 120 minutes for hepatocyte assay, and analyzed using LC-MS/MS. Positive controls, testosterone (1 µM) and 7-hydroxycoumarin (7-HC) (100 µM), were performed in parallel.

(b) (4) (b) (4) in human liver microsomes (Table 4) and hepatocytes (Table 5), with T1/2 < (b) (4) min.

Table 4. Stability of [(b) (4)] [(b) (4)] in Human Liver Microsomes (modified from the submission)

Test Article	Species	[(b) (4)] Percent Remaining (AVG, n=2)					Half-life[a] (min)	CL_{int}[b] (mL/min/ mg protein)
		0 min	10 min	20 min	30 min	60 min		
[(b) (4)]	Human	[(b) (4)]						

Test Article Dosed	Species	Treprostinil Concentration (µM) (AVG, n=2)				
		0 min	10 min	20 min	30 min	60 min
[(b) (4)]	Human	[(b) (4)]				

[a] When the calculated half-life is < the first non-zero timepoint, the half-life is listed as < [(b) (4)] with the calculated half-life also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the protein concentration in the incubation.
* Little to no [(b) (4)] was present in the time zero sample. [(b) (4)] [(b) (4)] Stability results should be interpreted with caution for these experiments.

Table 5. Stability of [(b) (4)] [(b) (4)] in Human hepatocytes (modified from the submission)

Test Article	Species	[(b) (4)] Percent Remaining (AVG, n=2)					Half-life[a] (min)	CL_{int}[b] (mL/min/ mg protein)
		0 min	15 min	30 min	60 min	120 min		
[(b) (4)]	Human	[(b) (4)]						

Test Article	Species	Treprostinil Concentration (µM) (AVG, n=2)				
		0 min	15 min	30 min	60 min	120 min
[(b) (4)]	Human	[(b) (4)]				

[a] When the calculated half-life is < the first non-zero timepoint, the half-life is listed as [(b) (4)] with the calculated half-life also listed in parentheses.
[b] Intrinsic clearance (CL_{int}) was calculated based on $CL_{int} = k/P$, where k is the elimination rate constant and P is the cell concentration in the incubation.

10 Special Toxicology Studies

10.1 [(b) (4)] (2020) COMPUTATIONAL EVALUATION OF THE POTENTIAL BACTERIAL MUTAGENICITY OF Impurities Potentially Associated with Treprostinil Inhalation Powder

This is an in-silico assessment of impurities in treprostinil prepared by [(b) (4)]

The potential bacterial mutagenicity of seven impurities ([(b) (4)] [(b) (4)] and [(b) (4)] associated with treprostinil inhalation powder, a dry powder formulation of the active pharmaceutical ingredient (API) treprostinil and the excipient fumaryl diketopiperazine (FDKP), was evaluated by (quantitative) structure-activity relationship

[(Q)SAR] using the Derek Nexus [Nexus v. 2.2.1 (Build 91, January 2018), DEREK Nexus v. 6.0.1] and Leadscope Model Applier (v. 2.4.2-1) systems.

Chemical structures were entered as .mol files into ChemIDplus (https://chem.nlm.nih.gov/chemidplus/) to ascertain if a Chemical Abstracts Service Registry Number (CAS RN) was assigned. If available, this identifier would be used to search for any available results of experimental bacterial mutagenicity assessment. In the case of the seven impurities ((b) (4) (b) (4) (b) (4) and (b) (4) identified by United Therapeutics, no matching CAS RN was identified for any of the substances.

The chemical structures of each of the impurities as well as that of Treprostinil were submitted to DEREK Nexus and Leadscope Model Applier for evaluation of potential bacterial mutagenicity. All seven impurities ((b) (4) (b) (4) (b) (4) and (b) (4) were identified as inactive (i.e., non-mutagenic) in DEREK Nexus and negative (non-mutagenic) in the Leadscope Model Applier. DEREK Nexus and Leadscope did not identify any of the structural features of these substances as unclassified / misclassified or out of domain, respectively. Furthermore, evaluation of the structure of treprostinil suggested that this substance was not mutagenic, and this result was confirmed by Ames assay test data. Each of the treprostinil impurities assessed in this report bears structural resemblance to treprostinil and, thus, the Ames assay data for treprostinil help to substantiate the in-silico mutagenicity predictions for the seven impurities.

Based on the in-silico assessments and expert judgement[1] and per the ICH M7 guideline[2], (b) (4) (b) (4) (b) (4) and (b) (4) are considered as Class 5 impurities and may be treated as non-mutagenic. Treprostinil, an API, was considered non-mutagenic based on test results in a GLP 5-test strain Ames assay[3].

The chemical structures used for the predictions, as well as that of the API, and results of in silico assessment are shown in Table 6.

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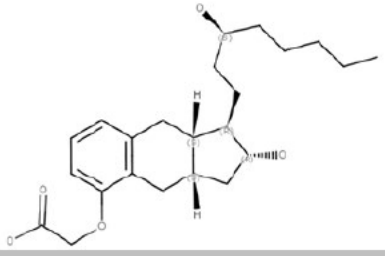
[1] Powley, M.W. (2015). (Q)SAR Assessments of Potentially Mutagenic Impurities: a Regulatory Perspective on the Utility of Expert Knowledge and Data Submission. Regul Toxicol Pharm, 71: 295–300.

[2] ICH – International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2017). ICH Harmonized Tripartite Guideline: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7 (R1). Step 4 – 31 March 2017.

[3] [UT] United Therapeutics Corporation. (2013). Pharmacology Review(s). NDA 203496. Approval Date 20 December 2013. Submitted to US FDA Center for Drug Evaluation and Research (CDER). [online]. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/203496Orig1s000TOC.cfm .

Table 6. Summary of in silico assessment (from the submission)

Compound	Structure	Derek Analysis	Leadscope Analysis	Expert Review
Treprostinil (CAS RN 81846-19-7)		Inactive in *Salmonella* and in *E. Coli*	Negative in *Salmonella* and in *E. Coli*	Negative for mutagenicity based on experimental data (UT, 2013)

(b) (4)

10.2 [redacted] (2021) Toxicological Profile and Risk Assessment for [redacted] in Inhaled Treprostinil Drug Product (Tyvaso DPI Inhalation Powder)

This assessment for treprostinil impurity [redacted] was prepared by [redacted]

[redacted] is identified as an impurity in Tyvaso DPI at a concentration above the qualification threshold of 1% for drug products with a maximum daily dose of drug substance that is <10 mg (For Tyvaso DPI Inhalation Powder, the maximum daily dose being administered is 16 µg 4 times daily for a total daily dose of 64 µg treprostinil and may increase by 16 µg/time every 1-2 weeks). A hazard characterization was conducted for [redacted] to qualify that impurity to a concentration of not more than [redacted]%.

In cell-based assays compared to treprostinil, the activity of [redacted] on the prostacyclin receptor (IP), prostaglandin E2 (PGE2) receptor 2 (EP2), prostaglandin D2 (PGD2) receptor 1 (DP1), and PGE2 receptor 1 (EP1) receptors was approximately 23-, 10-, 14-, and 25-fold lower, respectively. [redacted] In microsomes, little to no [redacted] was present at 0 minutes, suggesting that [redacted] during the 5-minute pre-incubation period.

[redacted] is not predicted to be mutagenic. No other nonclinical toxicology studies have been conducted with [redacted] either neat or present in treprostinil drug products.

[redacted] Impurity [redacted] is expected to have reduced pharmacological activity compared to treprostinil and to be [redacted] If any [redacted] remains intact following absorption in the lung, it is not expected to interfere with the normal CYP-mediated metabolism of treprostinil and is unlikely to cause unforthcoming toxicity.

Following patient use of Tyvaso DPI, the small quantity of [redacted] present in the drug product or treprostinil released from [redacted] is unlikely to substantially impact the pharmacological effects of treprostinil. Also, because the daily dose of inhaled treprostinil is individually titrated to tolerability and effect for each patient, it is unlikely that the presence of [redacted] in Tyvaso DPI would be a safety concern for patients.

Using structurally similar surrogate treprostinil, a Permitted Daily Exposure (PDE) of [redacted] was estimated. Briefly, adjusting [redacted]-fold for inter-individual variability and [redacted]-fold to extrapolate from a therapeutically efficacious dose to a No-

Observed-Effect-Level (NOEL), which is consistent with ICH guidelines and current industry standards for calculating a PDE, there are no additional safety concerns if [redacted] is present at approximately [redacted]% of the dose of Tyvaso DPI.

$$\frac{\text{Dose (mg/day)}}{\text{[redacted]}} = \text{[redacted]} \times \text{Dose (mg/day)}$$

11 Integrated Summary and Safety Evaluation

Brief Background / Introduction

The API in Tyvaso DPI is identical to the treprostinil drug substance approved in Remodulin (NDA 021272) and Tyvaso (NDA 022387). It is also the same active moiety as the treprostinil diolamine drug substance approved in Orenitram (treprostinil) Extended-Release Tablets (NDA 203496). The safety and efficacy of treprostinil are supported by a comprehensive set of pharmacology, pharmacokinetic (PK), toxicology, and clinical studies conducted for Tyvaso (treprostinil) Inhalation Solution (NDA 022387), Remodulin (treprostinil) Injection (NDA 021272), and Orenitram (treprostinil) Extended-Release Tablets (NDA 203496).

The safety of the excipient FDKP is supported by a comprehensive battery of safety pharmacology and toxicology studies conducted for Afrezza (BLA 022472).

No additional pharmacology studies nor in vivo nonclinical toxicology studies were conducted to support the NDA for Tyvaso DPI.

Pharmacology

No additional nonclinical pharmacology studies were conducted to support the NDA for Tyvaso DPI. Following intermittently delivery of treprostinil into the rat tracheal tube, treprostinil Tmax was ≤5 minutes. Treprostinil Inhalation Powder (TrIP) provided much higher treprostinil exposure than the nebulized reference solution. There was no evidence that the excipient FDKP, present in Tyvaso DPI, interfered with the absorption of treprostinil in the lung.

The stimulating activity of the impurity [redacted] at the IP1, EP2, DP1, and EP1 receptors in tested cell lines were approximately 23-, 10-, 14-, and 25-fold lower, respectively, than treprostinil. [redacted] was [redacted] in human liver microsomes and hepatocytes, with T1/2 <[redacted] min. In microsomes, little to no [redacted] was present at 0 minutes, suggesting that [redacted] In hepatocytes, [redacted] at time zero was also observed ([redacted]%).

Toxicology

No additional in vivo nonclinical toxicology studies were conducted to support the NDA for Tyvaso DPI. The potential bacterial mutagenicity of 7 impurities identified in Tyvaso DPI was evaluated by (quantitative) structure-activity relationship ((Q)SAR) using Derek Nexus and Leadscope Model Applier. All 7 impurities ([redacted] and [redacted] were identified as inactive (non-mutagenic) in DEREK Nexus and negative (non-mutagenic) in the Leadscope Model Applier. None of the structures had any structural features which were identified as unclassified/misclassified or out of domain, respectively, in Derek Nexus or Leadscope. As such, all 7 impurities are considered Class 5 impurities and may be treated as non-mutagenic.

[redacted] is identified as an impurity in Tyvaso DPI at a concentration above the qualification threshold of 1% for drug products with a maximum daily dose of drug substance that is <10 mg. [redacted] was qualified at a concentration of up to [redacted]% in the final drug product. [redacted] was shown to have reduced pharmacodynamic effects compared to treprostinil, and it is expected to be [redacted] It is unlikely that the presence of [redacted] in Tyvaso DPI would be a safety concern for patients, if present at up to [redacted]%.

Conclusions

Based on the results of the nonclinical pharmacology, PK, and toxicology studies conducted to support Tyvaso (NDA 022387), Remodulin (NDA 021272), and Orenitram (NDA 203496), and the assessment of impurities present in Tyvaso DPI, it is considered that Tyvaso DPI has an acceptable safety profile and that there are no findings that preclude long-term inhalation administration in humans.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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