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APPLICATION NUMBER:

217389Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	NDA
Application number(s)	217389
Priority or standard	Standard
Submit date(s)	6/26/2023
Received date(s)	6/26/2023
PDUFA goal date	6/26/2024
Division/office	Division of Pulmonology, Allergy, and Critical Care (DPACC)
Review completion date	6/26/2024
Established/proper name	Ensifentrine inhalation suspension
(Proposed) proprietary name	OHTUVAYRE
Pharmacologic class	Phosphodiesterase 3 (PDE3) inhibitor and phosphodiesterase 4 (PDE4) inhibitor
Other product name(s)	RPL554, VMX554, VRP554
Applicant	Verona Pharma, Inc
Dosage form(s)/formulation(s)	Inhalation suspension: 3 mg ensifentrine per 2.5 mL aqueous suspension in unit-dose ampules
Dosing regimen	One 3 mg ampule (2.5 mL) via inhalation route twice daily. For use with a standard jet nebulizer with a mouthpiece connected to an air compressor
Applicant-proposed indication(s)/ population(s)	Maintenance treatment of chronic obstructive pulmonary disease (COPD) in adult patients
SNOMED CT code for proposed indication disease term(s)[1]	13645005
Regulatory action	Approval
Approved dosage (if applicable)	3 mg (one ampule) twice daily administered by oral inhalation using a standard jet nebulizer with a mouthpiece
Approved indication(s)/ population(s) (if applicable)	Maintenance treatment of chronic obstructive pulmonary disease (COPD) in adult patients
SNOMED CT code for approved indication disease term(s)[1]	13645005

[1] For internal tracking purposes only.
Abbreviations: COPD, chronic obstructive pulmonary disease; DPACC, Division of Pulmonology, Allergy, and Critical Care; PDE, phosphodiesterase; PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

ΔΔHR	placebo-corrected change from baseline in heart rate
ΔΔQTcF	placebo-corrected change from baseline in corrected QT interval using the Fridericia method
AE	adverse event
ALT	alanine aminotransferase
ALP	alkaline phosphatase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BID	twice daily
BLA	biologics license application
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
C_{max}	maximum plasma concentration
COPD	chronic obstructive pulmonary disease
CV	coefficient of variation
DDI	drug-drug interaction
ECG	electrocardiogram
E-RS	Evaluating-Respiratory Symptoms
FDA	U.S. Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
FMQ	FDA medical query
GI	gastrointestinal
GLP	good laboratory practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HI	hepatic impairment
HLM	human liver microsomes
HR	hazard ratio
IC_{50}	half maximal inhibitory concentration
ICH	International Council for Harmonisation
ICS	inhaled corticosteroids
IND	investigational new drug
L	liters
LABA	long-acting $beta_2$-agonists
LAMA	long-active muscarinic antagonists
LLOQ	lower limit of quantitation
LS	least squares
MAR	missing at random
MI	multiple imputation
mITT	modified intent-to-treat
mMRC	modified Medical Research Council
MRHDID	maximum recommended human daily inhalation dose

MS/MS	tandem mass spectrometry
NDA	new drug application
NOAEL	no observed adverse effect level
PD	pharmacodynamic
PDE	phosphodiesterase
PI	prescribing information
PK	pharmacokinetic
PPT	protein precipitation
PT	preferred term
SAE	serious adverse event
SGRQ	St. George's Respiratory Questionnaire
$T_{1/2}$	half-life
TDI	Transitional Dyspnea Index
T_{max}	time to maximum concentration
ULN	upper limit of normal
USPI	United States prescribing information
VzF	apparent volume of distribution

I. Executive Summary

1. Summary of Regulatory Action

The Applicant, Verona Pharma, submitted an NDA for ensifentrine (tradename: OHTUVAYRE), an inhaled small molecule inhibitor of phosphodiesterase (PDE) 3 and PDE4, for the maintenance treatment of chronic obstructive pulmonary disease (COPD) at a dose of 3mg twice daily administered by oral inhalation using a standard jet nebulizer. The NDA was reviewed by a multidisciplinary review team. Each team recommends approval for adult patients with COPD and the signatory authority for this application concurs with these recommendations.

The substantial evidence of effectiveness (SEE) of ensifentrine in patients with moderate to severe COPD was established using data from two adequate and well-controlled trials. Each trial demonstrated statistically significant improvements in the pulmonary function in adults with moderate to severe COPD with ensifentrine treatment compared to placebo, as measured by the primary endpoint of change from baseline in the forced expiratory volume in 1 second (FEV_1) area under the concentration-time curve $(AUC)_{0\text{-}12h}$ at Week 12. This was supported by the results of additional secondary spirometry endpoints including the peak FEV_1, FEV_1 $AUC_{0\text{-}4h}$, and morning trough FEV_1.

The available safety data demonstrated that ensifentrine is safe for its intended use as labeled. Common adverse reactions included back pain, hypertension, urinary tract infection, and diarrhea. All safety concerns identified for ensifentrine are similar to other inhaled medications and oral phosphodiesterase inhibitors used in this population and can be adequately managed through labeling and further evaluated during routine pharmacovigilance.

The NDA also included appropriate preapproval nonclinical and clinical pharmacology studies. No additional studies will be conducted as postmarketing requirements.

The review team concludes that the improvement in the pulmonary function observed with ensifentrine treatment compared to placebo outweighs the risks when ensifentrine is used as recommended in the approved labeling. There are no outstanding issues from any review discipline. I concur with the content of the various discipline assessments and their recommendation for approval. The Agency and the Applicant have agreed upon the final labeling language. The submitted clinical program is adequate to support the efficacy and safety of ensifentrine for the maintenance treatment of COPD in adults. While the treatment effect is modest, ensifentrine will be an addition to the treatment armamentarium for bronchodilation in patient with COPD. The action for this application will be **Approval.**

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• COPD is a debilitating respiratory condition that involves significant morbidity, motality, and healthcare utilization. It is a leading cause of death in the U.S. and rates continue to rise. • Common symptoms of COPD include dyspnea, fatigue, cough, sputum production, chest tightness, wheezing, worsened exercise capacity, depression, anxiety, and weight changes. • Treatment involves the use of medications for symptom control and reduction of acute COPD exacerbations in conjunction with other treatment adjuncts (e.g., tobacco cessation, pulmonary rehabilitation, oxygen use).	COPD is a common debilitating respiratory condition causing signficiant morbidity and mortality. The diagnostic and symptom assessment instruments used by the Applicant are reasonable to assess COPD treatments.
Current treatment options	• Several classes of inhaled medications exist for the long-term maintenance treatment of COPD including: anticholinergics, long-acting beta-agonists, and inhaled corticosteroids (ICS; ICS are only recommended in combination). • Roflumilast, an oral PDE4 inhibitor, is indicated as a treatment to reduce the risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations. • There are no currently FDA-approved inhaled PDE inhibitors.	Multiple inhaled (e.g., long-acting bronchodilators) and oral (e.g., a PDE4 inhibitor) medications are currently available for patients with COPD. Ensifentrine, an inhaled small-molecule PDE3 and PDE4 inhibitor, provides another alternative maintenance treatment for patients with COPD.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• The Applicant has demonstrated substantial evidence of effectiveness for ensifentrine in COPD based on the submission of two adequate and well controlled trials (Trials 301 and 302). Both trials demonstrated that the ensifentrine-treated group had a statistically significant increase in the primary endpoint, the change from baseline in the FEV_1 $AUC_{0\text{-}12h}$ compared to placebo. • The ensifentrine-mediated increases in pulmonary function were driven by early bronchodilatory effects; however, the benefit was also supported by a persistent, albeit more modest, increase in the morning trough FEV_1. • The Applicant also submitted data from exploratory COPD exacerbation-based endpoints. The review suggested a potential reduction in the rate of COPD exacerbations and time-to-first COPD exacerbation with ensifentrine treatment compared to placebo; however, the data were insufficient to draw definitive conclusions on a potential exacerbation benefit.	There is sufficient evidence to support that ensifentrine provides a clincally and statistically significant improvement in pulmonary function in patients with COPD as measured by spirometry. Additional data provided by the Applicant suggest a potential trend in the reduction of COPD exacerbations; however, these exploratory endpoints do not provide sufficient support for inclusion in the label.
Risk and risk management	• The safety program for ensifentrine did not reveal any major imbalances in deaths, SAEs, or AEs leading to discontinuation. • Common AEs noted with increased incidence in the ensifentrine group compared to placebo included: back pain, hypertension, urinary tract infection, and diarrhea. • Nonclinical findings of tachycardia, hypotension, and coronary vasculopathy were not observed in the safety review of the phase 3 clinical program. • Labeling of other PDE inhibitors includes psychiatric events (with some including suicidality). There were events of two suicidal behaviors (one suicide in phase 2 and one suicide attempt in phase 3) during the ensifentrine clinical development program, and there were numerically more psychiatric events in the phase 3 safety database. • No REMS is proposed at this time.	Overall, the safety profile for ensifentrine is acceptable for its intended use. The safety database was adequate for the comprehensive safety assessment of ensifentrine for the proposed indication, patient population, and dosage regimen. Given the drug class labeling and numerically increased number of psychiatric events, this risk will be included as a warning and precaution in the USPI, similar to other PDE inhibitors. Safety risks have not been identified that require risk managment beyond routine pharmacovigilance.

Abbreviations: AE, adverse event; $AUC_{0\text{-}12h}$, area under the concentration-time curve from 0 to 12 hours; COPD, chronic obstructive pulmonary disease; FDA, U.S. Food and Drug Administration; FEV_1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; PDE, phosphodiesterase; REMS, risk evaluation and mitigation strategies; SAEs, serious adverse events; USPI, United States prescribing information

2.2. Conclusions Regarding Benefit-Risk

COPD is a common, progressive, respiratory condition characterized by chronic respiratory symptoms including cough and shortness of breath with persistent and often progressive airflow obstruction. Several classes of medications are currently available for the treatment of COPD including inhaled long-acting bronchodilators (e.g., long-acting beta-agonists and long-acting muscarinic antagonists) and an oral PDE4 inhibitor. Despite this, there remains significant morbidity and mortality associated with COPD. Ensifentrine, an inhaled small molecule PDE3 and PDE4 inhibitor, provides a potential alternative treatment for patients with COPD.

To support the NDA for ensifentrine, the Applicant submitted the results from two phase 3, placebo-controlled trials. Both trials, RPL554-CO-301 (Trial 301) and RPL554-CO-302 (Trial 302), evaluated adults with moderate to severe COPD over 24 weeks. Trial 301 also included a cohort of subjects evaluated for 48 weeks. These trials randomized subjects to either placebo or ensifentrine 3 mg twice daily delivered via a standard jet nebulizer. Both trials demonstrated a statistically significant increase in pulmonary function in adults with moderate to severe COPD as measured by the change from baseline of the FEV_1 $AUC_{0\text{-}12h}$. The benefit of ensifentrine was largely driven by bronchodilation early in the dosing interval, as supported by improvements in the secondary endpoints of peak FEV_1 and the FEV_1 $AUC_{0\text{-}4h}$. In addition, there was a persistent, albeit more modest, improvement with ensifentrine treatment compared to placebo at the end of the dosing interval, as measured by the morning trough FEV_1. Note that in Trial 302, the morning trough FEV_1 and FEV_1 $AUC_{0\text{-}4h}$ were not statistically significant due to losses earlier in the statistical hierarchy; however, these data were considered supportive of the primary endpoint given favorable point estimates and 95% confidence intervals that excluded the null.

The Applicant also submitted data regarding the potential impact of ensifentrine on COPD exacerbations, although neither trial was designed as a formal exacerbation trial and did not enrich for subjects with COPD who had frequent exacerbations. Overall, these endpoints were considered exploratory as they were analyzed outside of the statistical hierarchy for multiplicity control. There was a numerical reduction in the rate of COPD exacerbations and time-to-first COPD exacerbation with ensifentrine treatment compared to placebo, however the clinical impact of this reduction in a population without frequent COPD exacerbations is not clear and, (b) (4)

The safety assessment of ensifentrine did not demonstrate large imbalances in terms of death, serious adverse events, adverse events leading to discontinuation, or common adverse events with ensifentrine treatment compared to placebo. Observed events were generally consistent with other inhaled medications and oral PDE inhibitors in the treated population. There were no safety concerns identified that would preclude approval.

The overall risk-benefit assessment supports approval of ensifentrine for the maintenance treatment of COPD at a dose of 3 mg nebulized twice daily.

II. Interdisciplinary Assessment

3. Introduction

Ensifentrine is a small molecule inhibitor of phosphodiesterase (PDE) 3 and PDE4 developed for the maintenance treatment of chronic obstructive pulmonary disease (COPD). The proposed dose is one 3 mg unit-dose ampule administered twice daily by standard jet nebulizer using a mouthpiece.

COPD is a common progressive respiratory condition that impacts almost 6% of adults in the US (Sullivan et al. 2018) and was the third leading cause of death worldwide in 2019 (WHO 2023). COPD is characterized by chronic respiratory symptoms including cough and shortness of breath with persistent and often progressive airflow obstruction (GOLD 2023). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) staging and grading system of COPD takes into account the severity of airflow obstruction (based on the percent predicted post-bronchodilator forced expiratory volume in 1 second [FEV_1]) and the combination of exacerbation history and overall symptom burden as measured by the modified Medical Research Council (mMRC) dyspnea score and/or the COPD Assessment Test (GOLD 2023).

Pharmacological therapy in COPD is used primarily to reduce symptom burden, reduce the frequency and severity of exacerbations, and improve exercise tolerance. The common medication classes currently used for patients with COPD include bronchodilators such as short-acting $beta_2$-agonists (SABA), short-acting muscarinic antagonists, long-acting $beta_2$-agonists (LABA), and long-active muscarinic antagonists (LAMA) and inhaled corticosteroids (GOLD 2023).

Ensifentrine, through the inhibition of PDE3 and PDE4, leads to relaxation of airway smooth muscle with bronchodilation. There currently are no other approved inhaled small molecule PDE3 and PDE4 inhibitors for the maintenance treatment of COPD, although roflumilast, an oral PDE4 inhibitor, is approved for the reduction of risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations. Additionally, theophylline, a non-specific PDE inhibitor, is approved for the treatment of the symptoms and reversible airflow obstruction associated with chronic asthma and other chronic lung diseases (e.g., emphysema and chronic bronchitis), although its use has fallen out of favor.

To support substantial evidence of effectiveness (SEE) and safety for ensifentrine in moderate to severe COPD, the Applicant submitted data from two phase 3, multicenter, international, randomized, double-blind, placebo-controlled trials evaluating adult subjects ages 40-80 years who are current or former smokers with moderate to severe COPD over 24 weeks: Trial RPL554-CO-301 (Trial 301) and Trial RPL554-CO-302 (Trial 302). In addition, Trial 301 included a placebo-controlled safety cohort evaluated over 48 weeks. In these trials, subjects received placebo or ensifentrine 3 mg twice daily delivered via a standard jet nebulizer. This phase 3 clinical development program is reasonable and similar to other inhaled products evaluated for the maintenance treatment of COPD including in the overall design and endpoints evaluated.

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Spirometric Effects

3.1.1.2. COPD Exacerbations

3.1.2. Key Safety Review Issues

3.1.2.1. Cardiac and Vascular Disorders

3.1.2.2. Psychiatric Disorders

3.2. Approach to the Clinical Review

The Applicant submitted data from two phase 3 clinical trials, Trial RPL554-CO-301 (Trial 301) and Trial RPL554-CO-302 (Trial 302), as the primary support for the safety and efficacy of ensifentrine in COPD. These trials were both international, multicenter, randomized, double-blind, placebo-controlled trials evaluating the efficacy and safety of ensifentrine over 24 weeks with an additional 48-week safety subset in Trial 301. The in-depth review of the clinical protocols is included in Section 15.

Efficacy analyses were performed by FDA biostatisticians to confirm the Applicant's results with respect to the primary and secondary endpoints. The review of the efficacy of ensifentrine is included in Section 6 and Section 16. The safety review, included in Section 7 and Section 17, was performed by the clinical reviewer and an FDA clinical data scientist. Dose ranging trials were reviewed by the clinical pharmacology review team and are included in Section 5.2 and Section 6.1.

A summary of the clinical trials submitted by the Applicant in support of ensifentrine is reviewed below in Table 3.

Table 3. Clinical Trials Submitted in Support of Efficacy and Safety Determinations for Ensifentrine

Study (Trial Identifier) NCT#	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized	Number of Centers and Countries
RPL554-CO-301 (Trial 301) NCT 04535986	Phase 3, moderate to severe COPD	Control type: placebo-controlled; Randomization: 5:3 (24-wk cohort-1:1; 48-wk cohort-3:1); Blinding: double-blind	Drug: ensifentrine inhalation suspension; Dosage: 3 mg BID Number treated: ensifentrine (N=477); PBO (N=283); Duration (quantity and units): 24 wk (48 wk)	Primary: FEV_1 AUC_{0-12h} at Week 12; Key secondary: morning trough FEV_1 at Week 12	Number planned: 800 subjects; Number randomized: 763 subjects	120 centers in 12 countries (U.S. and outside U.S.)
RPL554-CO-302 (Trial 302) NCT 04542057	Phase 3, moderate to severe COPD	Control type: placebo-controlled; Randomization: 5:3; Blinding: double-blind	Drug: ensifentrine inhalation suspension Dosage: 3 mg BID Number treated: ensifentrine (N=498); PBO (N=291) Duration (quantity and units): 24 wk	Primary: FEV_1 AUC_{0-12h}; Key secondary: morning trough FEV_1 at Week 12	Number planned: 800 subjects; Number randomized: 790 subjects	130 centers in 10 countries (U.S. and outside U.S.).
RPL554-CO-203 (Trial 203) NCT 03443414	Phase 2b, dose-ranging, moderate to severe COPD	Control type: placebo-controlled; Randomization: 1:1:1:1:1; Blinding: double-blind	Drug: ensifentrine inhalation suspension; Dosage: 0.75 mg, 1.5 mg, 3 mg, 6 mg BID; Number treated: ensifentrine 0.75 mg (N=82), 1.5 mg (N=81), 3 mg (N=82), 6 mg (N=80), PBO (N=79); Duration (quantity and units): 4 wk	Primary: peak FEV_1 (over 3 h) at Week 4; Key secondary: morning trough FEV_1 at Weeks 1, 2, 3, and 4	Number planned: 400 subjects; Number randomized: 403 subjects	47 centers in 6 countries (all outside U.S.)
RPL554-CO-205 (Trial 205) NCT 03937479	2b, dose-ranging, moderate to severe COPD (added on to tiotropium)	Control type: placebo-controlled; Randomization: 1:1:1:1:1; Blinding: double-blind	Drug: ensifentrine inhalation suspension; Dosage: 0.375 mg, 0.75 mg, 1.5 mg, 3 mg BID; Number treated: ensifentrine 0.375 mg (N=82), 0.75 mg (N=83), 1.5 mg (N=81), 3 mg (N=83), PBO (N=84); Duration (quantity and units): 4 wk	Primary: peak FEV_1 (max during hours 0-3) at Week 4; Key secondary: Morning trough FEV_1 at Weeks 1 to 4	Number planned: 40 subjects; Number randomized: 416 subjects	49 centers, all in U.S.

Source: Reviewer summary
Abbreviations: AUC_{0-12h}, area under the concentration-time curve from 0 to 12 hours; BID, twice daily; COPD, chronic obstructive pulmonary disease, FEV_1, forced expiratory volume in 1 second; h, hours; max, maximum; N, number of subjects in treatment group; NCT, national clinical trial; PBO, placebo; U.S., United States; wk, week(s)

4. Patient Experience Data

The Applicant included multiple forms of patient experience data to support use of ensifentrine in COPD including the St. George's Respiratory Questionnaire (SGRQ), Evaluating-Respiratory Symptoms, and Baseline Dyspnea Index/Transitional Dyspnea Index.

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical Outcome Assessment Data Submitted in the Application		
☒	Patient-reported outcome	Section 6.2.2.4, Section 6.2.3.1, Section 16.2
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

Ensifentrine is a small molecule inhibitor of both PDE3 and PDE4. PDE enzymes hydrolyze cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to inactive 5'-nucleotide products. PDE3 can hydrolyze both cAMP and cGMP while PDE4 only hydrolyzes cAMP. PDE3 isozymes are expressed in vascular smooth muscle cells, cardiac myocytes, and multiple other tissues. The various PDE4 isozymes are expressed throughout various tissues and play notable roles in the lung and inflammatory cells. When PDE enzyme activity is inhibited, intracellular cAMP and cGMP increase, resulting in signaling cascades that can lead to relaxation of airway smooth muscles and inhibition of inflammatory cell activity, among many potential effects. Overall, the submitted pharmacology studies suggest that ensifentrine inhibits PDE3 and PDE4 and alters bronchoconstriction and inflammatory activities.

In *in vitro* studies, ensifentrine inhibited recombinant enzyme PDE3 activity with subnanomolar potency. The inhibitory potency at PDE4 ranged from approximately 50 to 1479 nM. Ensifentrine was found to be relatively selective for PDE3 and PDE4 compared to other PDE enzyme family members. In human neutrophils, ensifentrine inhibited PDE3 and PDE4 leading to increased levels of cAMP. Based on the *in vitro* inhibition of both PDE3 and PDE4, the established pharmacological class was determined to be "phosphodiesterase 3 (PDE3) inhibitor and phosphodiesterase 4 (PDE4) inhibitor".

While there are no animal models that fully recapitulate all aspects of COPD, the most frequently employed animal models use cigarette smoke, lipopolysaccharide, and elastase to induce various features of COPD. The Applicant did not conduct *in vivo* pharmacology studies using these models; however, the Applicant conducted exploratory *in vivo* pharmacology studies for allergic rhinitis and asthma. Ensifentrine showed bronchoprotective, bronchodilator, and anti-inflammatory effects in these models. Given the differences in etiology between COPD and allergic conditions, the clinical relevance of these studies to the current application is not clear. Nevertheless, the totality of *in vitro* and *in vivo* pharmacology data submitted was considered sufficient to characterize the pharmacological activity of ensifentrine and inform labeling for the indication of COPD.

5.2. Clinical Pharmacology/Pharmacokinetics

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established Pharmacologic Class	Ensifentrine is a small molecule inhibitor of both PDE3 and PDE4.
Mechanism of action	Ensifentrine is a small molecule inhibitor of the PDE3 and PDE4 enzymes. In vitro studies showed that ensifentrine inhibited recombinant enzyme PDE3 activity with subnanomolar potency. The inhibitory potency at PDE4 ranged from approximately 50 to 1479 nM. PDE3 primarily hydrolyzes the second-messenger molecule cAMP but is also capable of hydrolyzing cGMP. PDE4 hydrolyzes cAMP only. Inhibition of PDE3 with ensifentrine results in smooth muscle relaxation which causes bronchodilation. Inhibition of PDE4 results in an anti-inflammatory effect.
Proposed dose and administration route	The proposed dose is 3 mg BID. The administration route is oral inhalation using a standard jet nebulizer with a mouthpiece.
Active moieties	Unchanged ensifentrine is the pharmacologically active moiety.
QT prolongation	Following a single dose of 9 mg ensifentrine (three times the proposed unit dose), no clinically relevant effects on prolongation of the QTc interval were observed in the thorough QT study.
	General Information
Bioanalysis	A method for the quantitation of ensifentrine in human plasma using LLE HPLC with MS/MS detection was developed and validated. A method for the quantitation of ensifentrine in human urine (b) (4) polypropylene collection containers) using UPLC/MS/MS was developed and validated. The bioanalytical assays were considered acceptable.
Healthy subjects vs. subjects with COPD	Population pharmacokinetic analysis indicated that relative bioavailability in subjects with COPD is approximately 35% lower when compared to healthy subjects.
Bioavailability across different formulations	The intended to-be-marketed formulation for ensifentrine is a sterile suspension of (b) (4) ensifentrine in pH-neutral phosphate buffered saline (b) (4) for inhaled delivery via a standard jet nebulizer (low phosphate suspension). The low phosphate ensifentrine suspension was used in phase 1 studies RPL554-PK-102, RPL554-PK-103, RPL554-CV-101, phase 2b study RPL554-CO-205, and the two pivotal phase 3 clinical studies RPL554-CO-301 and RPL554-CO-302. Earlier phase 1 and 2 clinical studies used the high phosphate suspension formulation (b) (4) higher for phosphate concentration) to characterize ensifentrine PK and PD upon single and multiple dosing. Although the population PK analyses did not identify formulation (high or low phosphate concentration) as a significant covariate for ensifentrine PK, the formulation impact on local airway efficacy is unclear.

Characteristic	Drug Information
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	Parameter / Mean±SD C_{max} 551 to 773 pg/mL (Studies RPL554-CV-101 and RPL554-PK-102) C_{24} 107 pg/mL (Study RPL554-CO-205)
Range of effective dose(s) or exposure	In a phase 2 dose-ranging study (Study RPL554-CO-205) using the to-be-marketed low-phosphate suspension, a dose-dependent (from 0.375, 0.75, 1.5, and 3 mg BID) improvement from baseline of peak FEV_1 at Week 4 (primary endpoint) by ensifentrine was demonstrated in subjects with COPD with stable background tiotropium treatment. All dosing groups demonstrated statistically significant improvement of peak FEV_1 over placebo group with placebo-adjusted mean values ranging from 0.078 to 0.124 L. Similarly, the dose-response relationship of ensifentrine was also generally established for secondary endpoints including improvement from baseline of FEV_1 AUC_{0-3h} and AUC_{0-12h} at Week 4. The numerical change from baseline FEV_1 values following the 3 mg dose showed a separation from lower doses at all time points over the 12-hour dosing interval at Week 4. In the pivotal phase 3 studies (Studies RPL554-CO-301 and RPL554-CO-302), ensifentrine 3 mg BID treatment demonstrated a statistically significant increase in mean FEV_1 AUC_{0-12h} at Week 12 (the primary endpoint) compared to placebo. The magnitude of mean FEV_1 AUC_{0-12h} improvement from baseline was consistent across the phase 2 dose-ranging study and phase 3 studies.
Maximally tolerated dose or exposure	For the to-be-marketed low-phosphate suspension, the highest studied single dose was 9 mg in the thorough QT Study RPL554-CV-101. The highest-studied multiple dose was 3 mg BID.
Dose proportionality	Thorough QT Study RPL554-CV-101 demonstrated that ensifentrine C_{max} and AUC_{0-last} increased greater than dose-proportionally (i.e., ~4.3-fold increase from 3 mg to 9 mg) following single dose inhalation of the to-be-marketed low-phosphate suspension. Following single-dose administration of a high-phosphate suspension developed during the early stage of the ensifentrine clinical program in healthy subjects, Study RPL554-007-2014 demonstrated that C_{max} and AUC_{0-inf} of ensifentrine increased in an approximately dose-proportional manner from 1.5 to 24 mg.
Time to and accumulation ratio at steady state	Population PK analysis predicted accumulation of ensifentrine of 1.3- and 1.4-fold for C_{max} and AUC, respectively, in healthy subjects and 1.4 and 1.5-fold for C_{max} and AUC, respectively, in subjects with COPD. Steady-state was attained by Day 3 following twice-daily dosing.
Clinical studies used to-be-marketed formulation	The to-be-marketed formulation (low phosphate ensifentrine suspension) was used in the phase 2b study RPL554-CO-205; phase 1 studies RPL554-PK-102, RPL554-PK-103, RPL554-CV-101; and the two pivotal phase 3 clinical trials RPL554-CO-301 and RPL554-CO-302.

Characteristic	Drug Information
	Absorption
Bioavailability	A dedicated PK study to evaluate the absolute bioavailability following oral inhalation of ensifentrine was not conducted. Charcoal block Study RPL554-PK-101 demonstrated that following single 6 mg dose oral inhalation of ensifentrine high-phosphate suspension in healthy subjects, co-administration of active charcoal suspension only reduced AUC value by 10.4%. The results indicate that gastrointestinal absorption of ensifentrine following high-phosphate suspension is low.
T_{max}	Following inhaled administration of ensifentrine in healthy subjects and subjects with COPD, ensifentrine C_{max} was attained around 0.6 to 1.5 hours after dosing (Study RPL554-PK-102, RPL554-PK-103, and RPL554-CV-101).
Food effect (fed/fasted) Geometric least square mean and 90% CI	Due to the route of administration (inhaled) of ensifentrine, the effect of food on ensifentrine bioavailability has not been studied as food is not expected to impact the delivery, performance, or PK of this inhaled drug product.
	Distribution
Volume of distribution	The apparent V/F of ensifentrine is 4520 L based on a two-compartment population PK model.
Plasma protein binding	In vitro plasma protein binding of ensifentrine is approximately 90%.
Drug as substrate of transporters	Based on in vitro studies, ensifentrine is not a substrate of the efflux transporter P-gp. Ensifentrine is a substrate of BCRP. Ensifentrine is not a substrate of the uptake transporters OATP1B1 or OATP1B3.
	Elimination
Mass balance results	A human mass balance/metabolism study was not performed.
CL/F	The CL/F of ensifentrine is 430 L/h based on a two-compartment population PK model.
Half-life	Following single-dose administration of 3 mg ensifentrine to-be-marketed low-phosphate suspension in healthy subjects, the terminal elimination half-life is about 8 to 10 hours (Study RPL554-CV-101, RPL554-PK-102, and RPL554-PK-103).
Metabolic pathway(s)	Studies YFK/001 and YFK/002 identified ensifentrine metabolites in plasma samples obtained from humans. Following administration of a single nebulized dose of 24 mg, unchanged ensifentrine was identified as the major drug-related component in human plasma, accounting for 96 and 99% of the drug-related material identified in T_{max} and time-normalized (0-24 h) plasma samples, respectively. The primary metabolic routes for ensifentrine are oxidative (hydroxylation, O-demethylation) followed by conjugation (e.g., glucuronidation). In vitro results indicate that, at therapeutic concentrations, ensifentrine was predominantly metabolized by CYP2C9 and to a lesser extent by CYP2D6. In a clinical DDI study, ensifentrine C_{max} and $AUC_{0\text{-}inf}$ were 1.4-fold and 1.6-fold higher; respectively, when a 3 mg single dose of ensifentrine was concomitantly administered with CYP2C9 inhibitor fluconazole (200 mg twice daily for 10 days).
Primary excretion pathways (% dose)	After a 3 mg nebulized dose, urinary elimination of unchanged ensifentrine was negligible (<0.3% of the dose) (Study RPL554-PK-102).

Characteristic	Drug Information
	Intrinsic Factors and Specific Populations
Body weight	No significant impact of body weight on the PK of ensifentrine was identified in the population PK analyses.
Age	No significant impact of age on the PK of ensifentrine was identified in the population PK analyses.
Renal impairment	The effect of renal impairment on the exposure to ensifentrine for up to 24 weeks was evaluated in a population PK analysis. The eGFR varied from 25.5 to 191 mL/min, representing a range of moderate to no renal impairment. While continuous covariates of renal function did not show a significant correlation with ensifentrine exposure, categorical characterization of renal function indicated a 25% reduction in the apparent clearance in subjects with moderate renal impairment. No dosage adjustment in patients with mild or moderate renal impairment is required. The PK of ensifentrine in severe renal impairment (creatine clearance <30 mL/min) or subjects with end-stage renal disease have not been evaluated.
Hepatic impairment	The PK of ensifentrine were evaluated in subjects with moderate (Child-Pugh Class B) (n=10) to severe (Child-Pugh Class C) (n=2) hepatic impairment. Ensifentrine C_{max} and AUC_{inf} were approximately 2.3-fold and 2.2-fold higher in subjects with moderate hepatic impairment compared with healthy controls. Ensifentrine C_{max} and AUC_{inf} were approximately 1.2-fold and 2.3-fold higher in subjects with severe hepatic impairment compared with healthy controls (n=7). Population PK analysis did not identify markers of liver function (ALT, AST, bilirubin, and ALP) as significant covariates for exposure of ensifentrine. Based on these results and the general low plasma concentration of ensifentrine following the 3 mg inhalation (free drug C_{max} <0.1 ng/mL or <0.2 nM), no dosage adjustment in patients with hepatic impairment is proposed and ensifentrine should be used with caution in patients with hepatic impairment.
	Drug Interaction Liability (Drug as Perpetrator)
Inhibition/induction of metabolism	At therapeutically relevant concentrations, ensifentrine does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4.
Inhibition/induction of transporter systems	At therapeutically relevant concentrations, ensifentrine is not an inhibitor of either BCRP or P-gp. In addition, ensifentrine does not inhibit the transporters, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 or MATE2-K, at therapeutically relevant concentrations.

Characteristic	Drug Information
	Immunogenicity (if Applicable)
Bioanalysis	NA
Incidence	NA
Clinical impact	NA

Source: Reviewer's summary

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transferase; AUC_{inf}, area under the concentration-time curve extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to the last measured time; BCRP breast cancer resistance protein; BID, twice daily; C_{24}, concentration at 24 hours; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CI, confidence interval; CL/F, apparent clearance; C_{max}, maximum concentration; COPD, chronic obstructive pulmonary disease; CYP, cytochrome P450; DDI, drug-drug interaction; eGFR, estimated glomerular filtration rate; FEV_1, forced expiratory volume in 1 second; HPLC, high-performance liquid chromatography; L, liters; LLE, liquid-liquid extraction; MATE, multidrug and toxin extrusion transporter; MS/MS, tandem mass spectrometry; NA, not applicable; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PD, pharmacodynamics; PDE, phosphodiesterase; P-gp, P-glycoprotein; PK, pharmacokinetics; SD, standard deviation; T_{max}, time to reach maximum concentration; UPLC/MS/MS, ultra-performance liquid tandem mass spectrometry; V/F, apparent volume of distribution; vs., versus

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

The proposed dose of ensifentrine inhalation suspension is one 3 mg unit-dose ampule administered twice daily, once in the morning and once in the evening, by oral inhalation using a standard jet nebulizer with a mouthpiece.

Phase 2 dose-ranging trial RPL554-CO-205 provided primary support for the selection of the 3 mg twice daily (BID) dosing used in the phase 3 trials.

Study RPL554-CO-205 was a randomized, double-blind, placebo-controlled, parallel group, dose-ranging trial to examine dose-dependent effects on efficacy and safety compared with placebo in subjects with COPD on stable background tiotropium therapy. The to-be-marketed ensifentrine low-phosphate formulation was used in Study RPL554-CO-205. The treatment groups were placebo, ensifentrine 0.375, 0.75, 1.5, and 3 mg following a BID regimen for 4 weeks. Each group enrolled about 82 to 84 subjects with COPD with total 415 subjects randomized. The primary endpoint was change from baseline FEV_1 in peak FEV_1 (maximum value during 3 hours following dose) at Week 4. The listed first two secondary endpoints were change from baseline FEV_1 to average the area under the concentration-time curve $(AUC)_{0\text{-}3h}$ and $AUC_{0\text{-}12h}$ FEV_1 on Day 1 and at Weeks 1 to 4 ($AUC_{0\text{-}3h}$ only).

A dose-dependent (from 0.375, 0.75, 1.5, and 3 mg BID) improvement from baseline to peak FEV_1 at Week 4 (primary endpoint) by ensifentrine was demonstrated (Table 6).

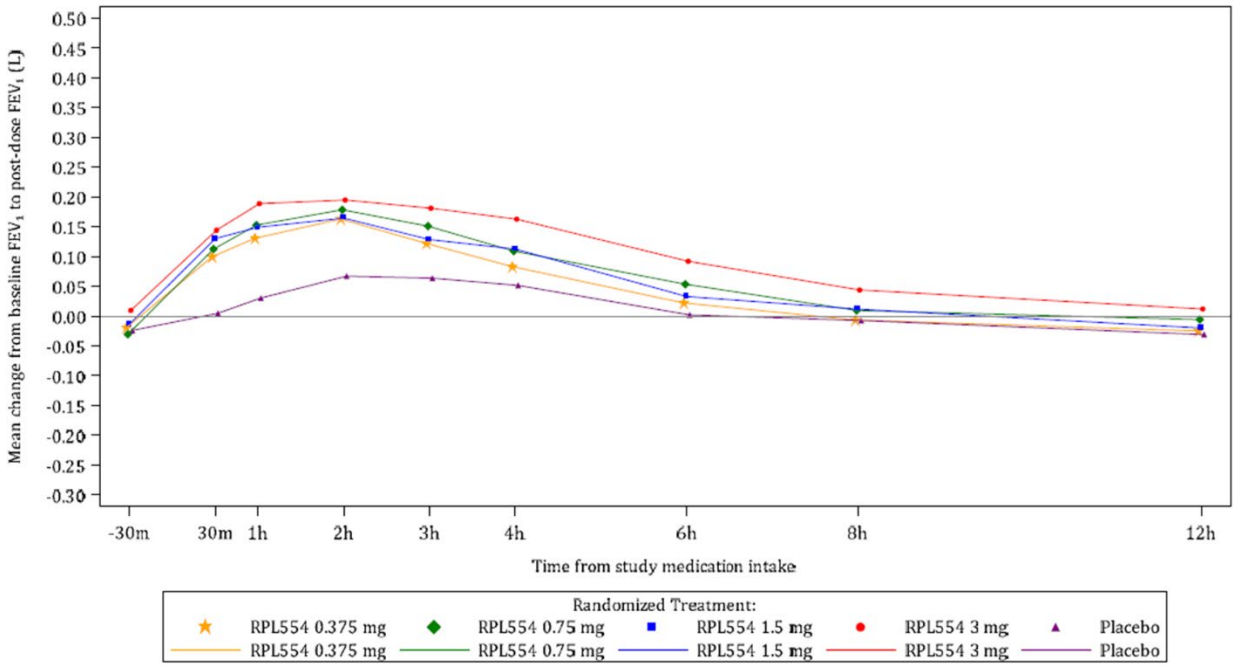
Table 6. Change From Baseline FEV_1 to Peak FEV_1 (Over 3 Hours) at Week 4 (Full Analysis Set)

	RPL554 0.375 mg (N=83)	RPL554 0.75 mg (N=83)	RPL554 1.5 mg (N=81)	RPL554 3 mg (N=82)	Placebo (N=84)
Baseline FEV_1 (L)					
n[a]	73	70	75	76	79
Mean (SD)	1.263 (0.431)	1.319 (0.432)	1.245 (0.364)	1.254 (0.400)	1.268 (0.397)
Peak FEV_1 at Week 4 (L)					
Mean (SD)	1.471 (0.456)	1.536 (0.476)	1.465 (0.446)	1.501 (0.457)	1.389 (0.480)
Change from baseline FEV_1 (L)					
Mean (SD)	0.209 (0.241)	0.217 (0.205)	0.220 (0.241)	0.247 (0.241)	0.121 (0.227)
Placebo-corrected treatment effect – MMRM[c] (L)					
LS mean difference vs placebo	0.078	0.091	0.107	0.124	
95% CI	(0.005, 0.150)	(0.018, 0.164)	(0.034, 0.180)	(0.052, 0.197)	
p-value	0.0368	0.0148	0.0040	0.0008	

Source: Study RPL554-CO-205 CSR, page 81, Table 16.
[a] Number of subjects with valid values at baseline and Week 4.
[c] Comparison was done using a closed testing procedure; testing began at the highest dose of RPL554 compared with placebo. If found statistically significant then the next highest dose was compared with placebo. This continued until a result was found to be non-significant or all RPL554 doses were compared with placebo.
Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume in 1 second; L, liter; LS, least squares; MMRM, mixed model for repeated measures; N, number of subjects in treatment group; n, number of subjects in subset; SD, standard deviation; vs, versus

In addition, a numerical dose separation trend of mean change from baseline FEV_1 0-12h time profile was noted for 3 mg dose when compared to all three lower doses (Figure 1).

Figure 1. Mean Change From Baseline FEV_1 Over Time at Week 4



Source: Study RPL554-CO-205 CSR, page 83, Figure 2
Abbreviations: FEV_1, forced expiratory volume in 1 second h, hours; L, liters

However, no clinically relevant change from baseline of morning trough FEV_1 following 4-week ensifentrine treatment was noticed when compared to placebo group. The placebo-adjusted least square mean change of trough FEV_1 from baseline was 1.7, -13, 7.7, and 27.2 mL for 0.375, 0.75, 1.5, and 3 mg ensifentrine dosing groups, respectively.

In addition, the Applicant conducted another phase 2 dose ranging study, Study RPL554-CO-203, in which a not-to-be-marketed ensifentrine product with high phosphate formulation was used. Study RPL554-CO-203 was a randomized, double-blind, placebo-controlled, parallel group dose-ranging trial to examine the effect of ensifentrine in subjects with moderate to severe COPD. The treatment groups were placebo, ensifentrine 0.75, 1.5, 3, and 6 mg following a BID regimen for 4 weeks. Each group enrolled about 80 to 82 subjects with COPD with a total 405 subjects randomized. The primary endpoint was change from baseline FEV_1 in peak FEV_1 (over 3 hours following dose) at Week 4.

The placebo-adjusted least square mean change from baseline FEV_1 to peak FEV_1 was 0.146, 0.153, 0.200, and 0.139 liters (L) for the 0.75, 1.5, 3, and 6 mg ensifentrine dosing groups, respectively. The results indicate no additional numerical bronchodilatory effect of the 6 mg dose of ensifentrine.

Therefore, it was reasonable for the Applicant to carry the 3 mg dose with a BID regimen to the pivotal phase 3 trials.

6.2. Clinical Studies/Trials Intended to Demonstrate Efficacy

6.2.1. Results of Pooled Analyses, Trials RPL554-CO-301 and RPL554-CO-302

The evaluation of efficacy was based on the analyses of the individual trials. Pooled efficacy analyses will not be presented.

6.2.2. Trial RPL554-CO-301 (Trial 301)

6.2.2.1. Design, Trial 301

Trial RPL554-CO-301 (Trial 301) was a phase 3, multicenter, randomized, double-blind, parallel-group, placebo-controlled trial to evaluate the efficacy and safety of ensifentrine 3 mg twice daily delivered by standard jet nebulizer (PARI LC Sprint®) compared to placebo in subjects 40 to 80 years of age with moderate to severe COPD. The trial consisted of two cohorts: one with a treatment period of 24 weeks (randomized 1:1 for study drug to placebo) and one with a treatment period of 48 weeks (randomized 3:1 for study drug to placebo) for additional safety analyses. The overall trial was randomized 5:3 for the study drug to placebo. Randomization was stratified by the following factors: treatment duration (cohort), stable background maintenance long-active muscarinic antagonists (LAMA) or long-acting beta$_2$-agonists (LABA) therapy use (yes or no), and cigarette smoking (current or former). The full review of the trial protocol design, subject population, objectives, and endpoints are reviewed in Section 15.

The primary objective of Trial 301 was to evaluate the efficacy of ensifentrine on lung function compared to placebo over a 12-hour dosing interval in subjects with moderate to severe COPD. The efficacy endpoints in the order of the statistical analysis hierarchy are listed below:

- Primary Endpoint:
  - FEV_1 $AUC_{0\text{-}12h}$ at Week 12
- Secondary Endpoints:
  - Peak FEV_1 at Week 12
  - Weekly mean Evaluating-Respiratory Symptoms total symptom score at Week 24
  - SGRQ total score at Week 24
  - Morning trough FEV_1 at Week 12
  - FEV_1 $AUC_{0\text{-}4h}$ at Week 12
  - SGRQ responders at Week 24 (defined as an improvement in at least 4 units from baseline in total SGRQ score)

In addition, moderate/severe COPD exacerbation rate over 24 and 48 weeks and the time to first moderate/severe COPD exacerbation over 24 and 48 weeks were evaluated as 'other endpoints.' These exacerbation analyses were outside the statistical hierarchy for multiplicity control.

Overall, the trial design and proposed endpoints are generally reasonable for the stated objectives. Traditionally, for bronchodilator medications used for the maintenance treatment of COPD such as the proposed indication for ensifentrine, the Division prefers the use of the morning trough FEV_1 given that endpoints such as the FEV_1 $AUC_{0\text{-}12h}$, can be impacted by early bronchodilatory responses. However, the use of FEV_1 $AUC_{0\text{-}12h}$ as a primary endpoint is not considered an unreasonable primary endpoint for demonstrating efficacy in COPD.

6.2.2.2. Eligibility Criteria, Trial 301

The eligibility criteria for Trial 301 were reasonable to ensure inclusion of patients with moderate to severe COPD and history of current or former smoking. Eligibility criteria did not specifically target for enrollment of patients with frequent COPD exacerbations.

The key inclusion criteria included:

- Established clinical history of COPD (Celli et al. 2004) with a score of ≥2 on the mMRC dyspnea scale
- Pre- and post-bronchodilator FEV_1/FVC ratio of <0.70
- Post-bronchodilator FEV_1 ≥30% and ≤70% predicted
- At least a 10 packyear smoking history and could be either current or former smokers (defined as those who stopped smoking at least 6 months prior)

The key exclusion criteria included:

- A history of 'life-threatening' COPD (including a history of an ICU admission or intubation)
- Hospitalization for COPD, pneumonia (or COVID-19) in the past 12 weeks
- Any COPD exacerbation requiring oral/parenteral steroids within 3 months
- A history of lung resection/lung reduction surgery within 12 months
- Long-term oxygen therapy (>12 hours/day) requirement
- A history of asthma or other respiratory condition, cardiovascular disease, or unstable liver disease

The trial aimed to recruit approximately 50% of subjects using stable background maintenance COPD medication defined as either a LAMA or LABA. LAMA and LABA combination therapy was not permitted. Up to 20% of subjects were permitted to also be on inhaled corticosteroids (ICS), provided this was in combination with either LABA or LAMA therapy. ICS monotherapy was not permitted.

Overall, the eligibility criteria were reasonable for a phase 3 trial in COPD. However, patients on LABA/LAMA combination therapy were excluded from enrollment. Current treatment guidelines recommend LABA/LAMA as first line therapy for COPD severity similar to those

enrolled in this trial (GOLD 2023), and given that this product would likely be used as add-on therapy, the exclusion of patients on LABA/LAMA may limit generalizability of the results.

6.2.2.3. Statistical Analysis Plan, Trial 301

The primary efficacy analysis compared ensifentrine to placebo using an analysis of covariance (ANCOVA) model adjusted for treatment, region, background medication strata, and smoking status as factors and baseline FEV_1 as covariate. The estimated treatment difference was presented with 95% confidence interval and associated (two-sided) p-value. Intercurrent events of premature treatment discontinuation and major protocol deviation were handled with a treatment policy strategy. Missing data was addressed using multiple imputation (MI) based on missing at random (MAR) assumption and its impact was assessed by tipping point analyses. The analysis was conducted on all randomized subjects who received at least one dose (or partial dose) of study medication, classified according to randomized treatment (modified intent-to-treat [mITT] population).

To address multiplicity in the analysis of the efficacy endpoints, a sequential testing procedure was conducted in a pre-specified hierarchical order for the primary endpoint and key secondary endpoints. The rate of and time to first moderate/severe COPD exacerbations were analyzed as "other efficacy analyses". See Section 15 for additional information.

Overall, the statistical analysis plan for Trial 301 appears acceptable to support valid interpretation and conclusions from the efficacy data.

6.2.2.4. Results of Analysis, Trial 301

Patient Enrollment and Disposition

A total of 1,280 patients were screened for Trial 301, with 763 subjects randomized and considered the intent-to-treat population. Of these, 760 received study treatment and were defined as the mITT population. The safety analysis set (SAS) included subjects who received at least one dose (or partial dose) of study medication. In this trial, the SAS was equal to the mITT population. The majority of subjects completed study treatment. The subject enrollment and disposition for Trial 301 are reviewed in Table 7.

Table 7. Trial 301, Subject Enrollment and Disposition

	ENS n (%)	PBO n (%)	Total Population n (%)
All randomized population (ITT; N)	479	284	763
Subjects treated (mITT population)	477 (99.6)	283 (99.6)	760 (99.6)
SAS	477 (99.6)	283 (99.6)	760 (99.6)
Subjects who completed the full study	400 (83.5)	245 (86.3)	645 (84.5)
Completed treatment period in study (up to Week 24)	422 (88.1)	252 (88.7)	674 (88.3)
Completed treatment period on study medication	378 (78.9)	232 (81.7)	610 (79.9)

	ENS n (%)	PBO n (%)	Total Population n (%)
Subjects who discontinued study medication	99 (20.7)	51(18.0)	150 (19.7)
Subjects who withdrew from the study	79 (16.5)	39 (13.7)	118 (15.5)

Source: Clinical reviewer calculated/confirmed, JMP15.0, ADSL dataset and the following parameter(s): RANDFL, EOTSTT, DCTREAS
Abbreviations: ENS, ensifentrine; ITT, intent-to-treat; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

A numerically higher proportion of subjects in the ensifentrine group discontinued study medication than in the placebo group (20.7% compared to 18.0%). The primary reasons listed for discontinuation included: withdrawal of consent, adverse events, COPD exacerbation criteria, and COVID-19. The overall number of subjects that discontinued the study medication was similar between treatment arms and is unlikely to significantly impact the analysis.

Similarly, a numerically higher proportion of subjects withdrew from the study in the treatment group than in the placebo group (16.5% compared to 13.7%). The reasons listed for study withdrawal were similar to study drug discontinuation and included: withdrawal of consent, adverse events, and the protocol-defined COPD exacerbation and COVID-19 withdrawal criteria. The slight numeric difference in study withdrawal is unlikely to significantly impact the interpretation of the safety or efficacy results.

Subject Demographics

The baseline demographics for Trial 301 are summarized in Table 8. Overall, the population had a mean age of 65.0 years and was 57.9% male. Subjects had mean post-bronchodilator FEV_1 of 48.4% predicted and for those with diagnosis information available, had a mean of 8.2 years since their COPD diagnosis. Overall, the baseline demographics and COPD disease characteristics were balanced between the treatment groups.

Table 8. Trial 301, Baseline Demographics and Disease Characteristics (ITT)

Characteristic	ENS (N=479)	PBO (N=284)	Total Population (N=763)
Age, years			
Mean (SD)	65.1 (7.1)	64.9 (7.7)	65.0 (7.3)
Median (min, max)	65 (41, 80)	65 (44, 79)	65 (41, 80)
Age group, n (%)			
<65 years	220 (45.9)	133 (46.8)	353 (46.3)
≥65 years	259 (54.1)	151 (53.2)	410 (53.7)
Sex, n (%)			
Male	275 (57.4)	167 (58.8)	442 (57.9)
Female	204 (42.6)	117 (41.2)	321 (42.1)

Characteristic	ENS (N=479)	PBO (N=284)	Total Population (N=763)
Ethnicity, n (%)			
Not Hispanic or Latino	464 (96.9)	278 (97.9)	742 (97.2)
Hispanic or Latino	15 (3.1)	6 (2.1)	21 (2.8)
Race, n (%)			
White	437 (91.2)	251 (88.4)	688 (90.2)
Black/African American	16 (3.3)	9 (3.2)	25 (3.3)
Asian	13 (2.7)	11 (3.9)	24 (3.1)
Other	0	1 (0.4)	1 (0.1)
Not reported	13 (2.7)	12 (4.2)	25 (3.3)
Country/region, n (%)			
United States	87 (18.2)	58 (20.4)	145 (19.0)
Outside the U.S.	392 (81.8)	226 (79.6)	618 (0.8)
Smoking status, n (%)			
Current	269 (56.2)	164 (57.7)	433 (56.7)
Former	210 (43.8)	120 (42.3)	330 (43.3)
Packyears, mean years (SD)	41.1 (20.7)	41.8 (20.6)	41.3 (20.7)
Height and weight, mean (SD)			
Weight, kg	80.6 (17.7)	79.5 (18.3)	80.2 (17.9)
Height, cm	169.5 (9.4)	169.9 (9.8)	169.7 (9.6)
BMI, kg/m^2	28.0 (5.3)	27.4 (5.4)	27.7 (5.3)
COPD severity, n (%)			
Mild	1 (0.2)[1]	0	1 (0.1)
Moderate	295 (61.6)	165 (58.1)	460 (60.3)
Severe	180 (37.6)	119 (42.0)	299 (39.2)
Very severe	3 (0.6)	0	3 (0.4)
FEV_1, mean (SD)			
Pre-bronchodilator, L	1.42 (0.46)	1.39 (0.46)	1.41 (0.46)
Post-bronchodilator, L	1.53 (0.46)	1.51 (0.47)	1.52 (0.46)
Reversibility[2] status, n (%)			
Reversible	122 (25.5)	67 (23.6)	189 (24.8)
Non-reversible	357 (74.5)	217 (76.4)	574 (75.2)

Source: Clinical reviewer calculated in JMP 15.0 using the ADSL dataset and the following parameter: RANDFL and ADVS data set with the following parameters: RANDFL(Y), AVISIT, PARAMCD, AVAL
[1] Protocol deviation
[2] Reversibility defined as ≥12% and ≥200 mL increase in FEV_1 following albuterol/salbutamol.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ITT, intent-to-treat; L, liters; max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SD, standard deviation; U.S., United States

The baseline demographics and disease characteristics were acceptable and consistent with a moderate to severe COPD population; however, the proportion of Black/African American subjects was limited to 3.3% (25/763 subjects) and only 2.8% (21/763) subjects were reported as Hispanic or Latino (where race and ethnicity were recorded). While these percentages are similar to that of the published U.S. epidemiologic data (American Lung Association 2023), COPD may be underreported in these minority groups within the U.S. population (Ejike et al. 2019) and the limitations in racial and ethnic diversity may impact generalizability of the results.

During the design of the clinical development program for ensifentrine, the Applicant specified that randomization would be stratified based on background COPD medication usage. As reviewed in Table 9, 68.5% of subjects in Trial 301 were on a background therapy which

included LAMA alone, LAMA/ICS combinations, LABA alone, and LABA/ICS combinations. In general, COPD background medication use was similar between treatment groups.

Table 9. Trial 301, Baseline Background Medication Usage (ITT)

Subgroup	ENS (N=479) n (%)	PBO (N=284) n (%)	Total Population (N=763) n (%)
No background medications	148 (30.9)	92 (32.4)	240 (31.5)
Background medications	331 (69.1)	192 (67.6)	523 (68.5)
LAMA	151 (31.5)	76 (26.8)	227 (29.8)
LAMA/ICS	4 (0.8)	5 (1.8)	9 (1.2)
LABA	89 (18.6)	45 (15.9)	134 (17.6)
LABA/ICS	87 (18.2)	66 (23.2)	153 (20.0)

Source: Clinical reviewer calculated/confirmed, JMP 15.0, ADCM dataset and the following parameter(s): RANDFL, BMSTRATA, BMSTRANM
Abbreviations: ENS, ensifentrine; ICS, inhaled corticosteroid; ITT, intent-to-treat; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

Those on LAMA/LABA combination therapy, a common COPD medication class, were excluded from participation in the trial. As noted in Section 6.2.2.2, based on the GOLD guidelines at the time the phase 3 trials were enrolling subjects, LAMA/LABA combination therapy was recommended for the enrolled population (GOLD Stage B, C, and D) given that the population had an mMRC score of greater than or equal to 2 (GOLD 2022). This would have included the enrolled population based on the spirometric and mMRC scores outlined in the inclusion criteria. Additionally, in the most recent updates to the GOLD clinical recommendations, LAMA/LABA combination therapy is now recommended as first line therapy for the trial population. The lack of LAMA/LABA combination therapy may limit the overall generalizability of this clinical development program, particularly as this product would likely be used as add-on therapy.

The most common comorbidities included hypertension (58.6%), type 2 diabetes (16.1%) and coronary artery disease (10.4%). This is consistent with the known comorbidities seen in patients with COPD and was evenly distributed between the treatment group and placebo. The most common concomitant medications included salbutamol (43.6%), tiotropium (21.8%), and acetylsalicylic acid (20.8%). Overall, these parameters were generally balanced between treatment groups and there are no review concerns regarding the study subjects' past medical history and other concurrent medications, which are both reasonable for a COPD population. The subjects' past medical history and other concomitant medication are reviewed in more detail in Section 16.1.

Analysis of Efficacy – Primary Endpoint

For the primary efficacy endpoint for Trial 301, change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12, ensifentrine treatment demonstrated a statistically significant increase of 0.087 L compared to placebo (95% CI: 0.055, 0.119, $p<0.0001$) in the mITT population, as shown in Table 10.

Table 10. Trial 301, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 (mITT)

Parameter	ENS (N=477)	PBO (N=283)
Baseline[1]		
n	477	282[2]
Mean (SD)	1.420 (0.487)	1.403 (0.468)
Week 12 (imputed[3])		
n	477	282
Mean (SD)	1.489 (0.500)	1.384 (0.495)
Change from baseline at Week 12 (imputed[3])		
n	477	282
Mean (SD)	0.069 (0.1978)	-0.018 (0.200)
LS mean change from baseline at Week 12[4]	0.061	-0.026
(95% CI)	(0.025, 0.097)	(-0.064, 0.013)
LS mean difference vs. placebo (95% CI)[4]		0.087 (0.055, 0.119)
p-value		<0.0001

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADRE, ADREMI and ADREMIAV datasets
[1] Baseline FEV1 is the mean of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes pre-dose on Day 1.
[2] One subject in placebo group with missing baseline FEV_1 data is not included in the analysis.
[3] Number of subjects with observed data is 433 and 261 for ENS and PBO, respectively. Any missing data postbaseline was imputed using multiple imputation. The multiple imputation model included treatment, region, background medication strata, smoking strata, baseline FEV_1 and FEV_1 AUC_{0-12h} at Week 12.
[4] ANCOVA models were run on 100 imputed datasets and their LS means, LS mean treatment differences, associated 95% CIs and p-values were combined using Rubin's rule.
Abbreviations: ANCOVA, analysis of covariance, AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group in the mITT Population; n, number of subjects in subset; PBO, placebo; SD, standard deviation, vs., versus

To examine the robustness of the primary analysis result to missing data, an average imputation analysis and a tipping point analysis were conducted. The least squares (LS) mean difference versus placebo remained statistically significant under the average imputation analysis (Section 16.4), indicating that the impact of the MAR assumption on overall missing data is not likely considerable. In the tipping point analyses on the primary endpoint, the LS mean difference versus placebo remained statistically significant ($p<0.05$) under most scenarios after multiple imputations for missing data and adding shift variables (-1 L to 1 L in the ensifentrine arm; -1 L to 1 L in the placebo arm), except under a few implausible extreme shifting scenarios (Section 16.4). This finding, along with the result from average imputation analysis, suggests that the primary analysis result is robust to underlying missing data assumptions (MAR). While the demonstrated change in the average FEV_1 AUC_{0-12h} is statistically significant and robust to missing data, the effect size is modest but supportive of the benefit of ensifentrine (reviewed in additional detail in Section 6.3.1).

Analysis of Efficacy – Secondary Endpoints

A summary of the key secondary endpoints in Trial 301 is shown in Table 11. Overall, the key secondary endpoints results were consistent with the primary endpoint findings with ensifentrine treatment demonstrating statistically significant improvements compared to placebo.

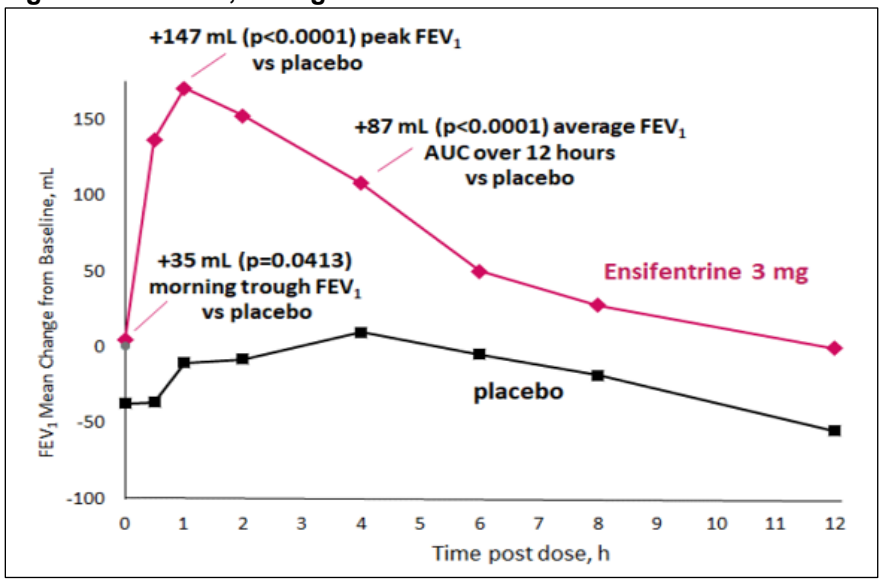
Table 11. Trial 301, Summary of Key Secondary Endpoints (mITT)

	ENS (N=477) vs. PBO (N=283)		
Endpoint	**LS Mean Diff (SE)**	**95% CI**	**p-Value**
CFB in Peak FEV_1 at Week 12, L	0.147 (0.018)	0.112, 0.183	<0.0001
CFB in ERS total symptom score at Week 24, units	-0.951 (0.375)	-1.685, -0.217	0.0111
CFB in SGRQ total score at Week 24, units	-2.298 (1.027)	-4.312, -0.285	0.0253
CFB in Morning trough FEV_1 at Week 12, L	0.035 (0.017)	0.001, 0.068	0.0413
CFB in FEV_1 AUC_{0-4h} at Week 12, L	0.139 (0.017)	0.106, 0.173	<0.0001
Odds ratio in proportion of SGRQ responders at Week 24[1]	1.49	1.07, 2.07	0.0194

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI, ADQSERSM and ADQSMI datasets All continuous variables used in change from baseline analysis including peak FEV_1, E-RSTM: COPD, SGRQ total score, morning trough FEV_1 and FEV_1 AUC_{0-4h} were analyzed using ANCOVA approach, as used for the primary efficacy analysis.
[1] SGRQ responder defined as an improvement in at least 4 units from baseline in total SGRQ score. A logistic regression model was used with fixed effects for treatment, region, actual background medication strata and actual smoking strata at baseline and baseline SGRQ total score as covariate, using MI based on MAR.
Abbreviations: AUC_{0-4h}, area under curve from 0 to 4 hours; CFB, change from baseline; CI, confidence interval; diff, difference; ENS, ensifentrine; ERS, evaluating-respiratory symptoms, FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; PBO, placebo; SE, standard error; SGRQ, St. George Respiratory Questionnaire, vs., versus

Based on the spirometric data, the effect of ensifentrine on bronchodilation appears to be largely driven by effects early in the treatment interval as based on the key secondary endpoints of peak FEV_1 and the FEV_1 AUC_{0-4h} and can been observed visually in Figure 2.

Figure 2. Trial 301, Change From Baseline in FEV_1 Over 12 Hours at Week 12 (mITT)



Source: Applicant provided (Integrated Summary of Effectiveness; Figure 4, page 73). Independently calculated/confirmed by the Statistical Reviewer
Abbreviations: AUC, area under the concentration-time curve; FEV_1, forced expiratory volume in 1 second; h, hours; mITT, modified intent-to-treat; vs, versus

Although the use of FEV_1 AUC_{0-12h} as the primary endpoint for this trial was not considered unreasonable, the morning trough FEV_1 is the Division's preferred endpoint for the evaluation of maintenance COPD therapies, particularly for bronchodilators, as this endpoint is not impacted by early bronchodilation that may not be sustained over the dosing interval. In Trial 301, the

morning trough FEV_1 with ensifentrine treatment increased by 0.035 L compared to placebo (95% CI: 0.004, 0.068; p=0.0413). While this improvement is statistically significant, the effect size is numerically modest and not as robust as the primary endpoint to the missing data. Nevertheless, the improvement in the morning trough FEV_1 demonstrated by ensifentrine treatment compared to placebo is supportive of the benefit and consistent with the primary endpoint.

The Applicant also evaluated patient-reported outcomes including the SGRQ to assess the effect of ensifentrine on various quality of life measures. There was a greater proportion of SGRQ responders (defined as an improvement of at least 4 units in the total SGRQ score) in the ensifentrine treatment group compared to placebo. This responder analysis was statistically significant and warrants inclusion in Section 14 of the label.

Additional review of the secondary endpoints is included in Section 16. Overall, the secondary endpoints are supportive of the benefit of ensifentrine in patients with moderate to severe COPD.

Efficacy Subgroup Analyses

The Applicant performed multiple subgroup analyses on the primary efficacy endpoint based on gender at birth, age, region, smoking status, FEV_1 reversibility, and a history of chronic bronchitis. Results for the evaluated subgroups were generally consistent with the overall population.

An additional subgroup analysis of the primary endpoint was also performed based on the background COPD medication strata as summarized in Table 12.

Table 12. Trial 301, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 by Background Medication Subgroup (mITT)

Subgroup	ENS (N=477) n (%)	Placebo (N=283) n (%)	LS Mean Difference[1]	95% CI[1]
No background medication	146 (30.6)	91 (32.2)	0.060	-0.003, 0.123
Any LAMA or LABA use	331 (69.4)	192 (67.8)	0.102	0.066, 0.137
LABA	89 (18.7)	45 (15.9)	0.145	0.083, 0.207
LABA/ICS	87 (18.2)	66 (23.3)	0.059	-0.009, 0.128
LAMA	151 (31.7)	76 (26.9)	0.110	0.053, 0.167
LAMA/ICS	4 (0.8)	5 (1.8)	0.181	N/A

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
[1] Based on the primary efficacy analysis by background medication subgroups.
Abbreviations: AUC_{0-12h}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; L, liters; LABA, long acting beta-agonist; LAMA, long acting muscarinic antagonist; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; N/A, not applicable

While the small sample size limits the ability to make definitive conclusions regarding the effect of ensifentrine based on background COPD medication subgroups, results for the subgroup analysis are generally consistent with the overall population.

However, as previously noted, patients on LABA/LAMA combination therapy were excluded from this trial. As such, inferences regarding the bronchodilatory effect of ensifentrine on patients taking LAMA/LABA combination therapy, a common class of COPD medications used in the studied population, cannot be made. See Section 6.3.1 for additional analysis.

Other Efficacy Endpoints – COPD Exacerbations

The Applicant included the overall frequency of moderate/severe COPD exacerbations and the time to first moderate/severe COPD exacerbation as other endpoints in their phase 3 trials. The definition of a COPD exacerbation was acceptable and consistent with other clinical development programs in this population. COPD exacerbations are a significant source of morbidity and mortality, and reductions in COPD exacerbation frequency and time to first COPD exacerbation in patients with frequent exacerbations have been considered clinically meaningful endpoints in other COPD medication development programs. Therefore, while exacerbation-related endpoints analyses were considered exploratory in part as they were not multiplicity controlled, they are reviewed below.

COPD Exacerbation Rate

This trial did not enrich for subjects with COPD who had frequent exacerbations and only approximately one quarter of subjects across treatment groups experienced an exacerbation within the 15 months leading up to the start of the trial [ensifentrine 26.4% (118/477) and placebo 26.1% (74/283)]. Compared to the average rate of COPD exacerbations within the general COPD population (Hurst et al. 2010), baseline exacerbations in the studied population appeared to be low.

For the overall population and the majority of subgroups, while point estimates favored ensifentrine treatment, 95% CIs for the annualized rate of exacerbations did not exclude null. The only subgroup of subjects analyzed where ensifentrine treatment demonstrated a reduction in the COPD exacerbation rate compared to placebo with a 95% CI for the rate ratio excluding the null was in subjects without a history of a COPD exacerbation in the past 15 months. The significance of this finding is unclear and ultimately may have been due to chance.

The ability to make definitive conclusions regarding exacerbations based on the overall population and subgroups is limited due to the exploratory nature of the exacerbation endpoint and small sample size within each subgroup. Table 13 reviews the moderate/severe COPD annualized exacerbation rate in Trial 301 over 24 and 48 weeks with select subgroup analyses.

Table 13. Trial 301, Moderate or Severe COPD Exacerbation Annualized Event Rate Over 24 and 48 Weeks (mITT)

	ENS (N=477)		PBO (N=283)		ENS vs. PBO	
	n/Event	AER, LS Mean	n/Event	AER, LS Mean	RR	95% CI
Week 24 (both 24w and 48w cohorts)	477/42	0.26	283/40	0.41	0.64	0.40, 1.00
Week 48 cohort only	280/44	0.25	89/23	0.44	0.56	0.32, 1.00
Week 24 subgroups						
Background YES	331/31	0.31	192/29	0.47	0.65	0.39, 1.10
Background NO	146/11	0.17	91/11	0.30	0.57	0.22, 1.47
Previous exacerbation in past 15 months	118/20	0.56	74/10	0.42	1.31	0.60, 2.84
No previous exacerbation in past 15 months	359/22	0.17	209/30	0.39	0.42	0.24, 0.75
Current smoker	268/25	0.31	163/20	0.41	0.76	0.41, 1.41
Former smoker	209/17	0.21	120/20	0.42	0.51	0.26, 1.00

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADEXAC dataset
Abbreviations: AER, annualized event rate; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; RR, rate ratio; vs., versus; w, week

Additionally, we note that the annualized event rate based on 24 weeks includes the assumption that the events in the first 6 months will remain the same for the next 6 months. An additional review of the annualized event rate in the 48-week cohort was consistent with the 24-week cohort in that point estimates favored ensifentrine treatment, but the 95% CI did not exclude null.

Time to First COPD Exacerbation

Given the assumptions regarding annualized event rates in 24-week trials, analyses were also conducted based on the time to first moderate/severe COPD exacerbation over 24 and 48 weeks with select subgroup analyses (Table 14). The hazard ratios (HRs) for subjects treated with ensifentrine compared to placebo favored ensifentrine with 95% CIs excluding null for both the 24- and 48-week cohorts. While the 95% CI for the HRs excluded null and may suggest an exacerbation effect, definitive conclusions cannot be made as the results are considered exploratory.

Table 14. Trial 301, Time to First Moderate or Severe COPD Exacerbation (mITT)

	ENS (n/Event)	PBO (n/Event)	HR	95% CI
Time to first COPD exacerbation (ENS vs. PBO)				
Week 24	477/38	283/36	0.62	0.39, 0.97
Week 48	280/35	89/21	0.48	0.28, 0.82
Subgroup analysis (Week 24 only)				
Background YES	331/28	192/27	0.60	0.35, 1.02
Background NO	146/10	91/9	0.66	0.27, 1.62
Previous exacerbation in past 15 months	118/18	74/9	1.35	0.60, 3.02
No previous exacerbation in past 15 months	359/20	209/27	0.41	0.23, 0.74
Current smoker	268/22	163/19	0.73	0.39, 1.36
Former smoker	209/16	120/17	0.51	0.26, 1.02

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADTTE dataset
Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; HR, hazard ratio; mITT, modified intent-to-treat; n, number of subjects in subset; PBO, placebo; vs., versus

Subgroup analyses were limited by small sample size and low event rates, but the results were generally consistent with the overall population, though most 95% CIs did not exclude null.

While there were some positive trends for exacerbation results from Trial 301, the majority of 95% CIs included null, and the exacerbation endpoints were not multiplicity controlled. Therefore, these data do not warrant inclusion in labeling. Additional review and analysis of the COPD exacerbation endpoints are included in Section 6.3.2.

6.2.3. Trial RPL554-CO-302 (Trial 302)

The overall design, endpoint selection, eligibility criteria, and statistical analysis plan for Trial 302 are the same as Trial 301 (reviewed in Section 6.2.2.1 and Section 15). The key difference between the trials was that Trial 302 had a single cohort with a 24-week treatment period with no 48-week safety cohort.

6.2.3.1. Results of Analyses, Trial 302

Subject Enrollment and Disposition

A total of 1,714 patients were screened for Trial 302, with 790 subjects randomized and considered the intent-to-treat population. Of these, 789 received study treatment and were defined as the mITT population. The SAS included subjects who received at least one dose (or partial dose) of study medication and was also equal to the mITT in Trial 302. The majority of subjects completed the study treatment. The subject enrollment and disposition are reviewed in Table 15.

Table 15. Trial 302, Subject Enrollment and Disposition

	ENS n (%)	PBO n (%)	Total n (%)
All randomized population (ITT; N)	499	291	790
Subjects treated (mITT population)	498 (99.8)	291 (100)	789 (99.9)
(SAS)	498	291	789
Subjects who completed the full study	393 (78.8)	218 (74.9)	611 (77.3)
Completed treatment period on study medication	377 (75.6)	196 (67.4)	573 (72.5)
Subjects who discontinued study medication	121 (24.2)	95 (32.6)	216 (27.3)
Subjects who withdrew from the study	106 (21.2)	73 (25.1)	178 (22.5)

Source: Clinical reviewer calculated/confirmed, JMP15.0, ADSL dataset and the following parameter(s): RANDFL, EOTSTT, DCTREAS
Abbreviations: ENS, ensifentrine; ITT, intent-to-treat; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

The percentage of subjects who discontinued the study medication was higher in the placebo group, 95 (32.6%) subjects, compared to the ensifentrine group, 121 (24.2%) subjects. The primary reasons for withdrawal in each group included adverse events and withdrawal of consent and, in the placebo group, 16 (5.5%) subjects had "other" listed as their reason for study drug discontinuation, compared to 7 (1.4%) in the ensifentrine group.

Similarly, the number of subjects who withdrew from the study was numerically higher in the placebo group, 73 (25.1%) subjects, compared to the ensifentrine group, 106 (21.2%) subjects. The reasons reported for withdrawal from the study included withdrawal of consent, adverse events, and COVID-19.

The proportion of subjects who discontinued the study drug was numerically higher in the placebo group, however this does not impact the overall interpretation of efficacy based on the missing data sensitivity analyses (Section 16.4.1).

Subject Demographics

The baseline demographics for Trial 302 are summarized in Table 16. Overall, the population had a mean age of 65.1 years and was 48.5% male, which is similar to the baseline demographics for Trial 301 and other similar COPD clinical trials. The subjects had a mean (SD) post-bronchodilator FEV_1 of 1.4281 L (0.44) or 50.60% predicted with a mean time since COPD diagnosis of 6.85 years (in those with diagnosis history information available). The baseline demographics were generally balanced between treatment groups.

Table 16. Trial 302, Baseline Demographics and Disease Characteristics (ITT)

Characteristic	ENS N=499	PBO N=291	Total N=790
Age, years			
Mean (SD)	65.0 (7.38)	65.3 (7.30)	65.1 (7.35)
Median (min, max)	65 (42, 80)	66 (40, 80)	65 (40, 80)
Age group, n (%)			
<65 years	224 (44.9)	124 (42.6)	348 (44.1)
≥65 years	275 (55.1)	167 (57.4)	442 (55.9)
Sex, n (%)			
Male	245 (49.1)	138 (47.4)	383 (48.5)
Female	254 (50.9)	153 (52.6)	407 (51.5)

Characteristic	ENS N=499	PBO N=291	Total N=790
Ethnicity, n (%)			
Not Hispanic or Latino	473 (94.8)	277 (95.2)	750 (94.9)
Hispanic or Latino	26 (5.2)	14 (4.8)	40 (5.1)
Race, n (%)			
White	472 (94.6)	276 (94.8)	748 (94.7)
Black/African American	24 (4.8)	11 (3.8)	35 (4.4)
Asian	1 (0.2)	1 (0.3)	2 (0.3)
Other	1 (0.2)	3 (1.0)	4 (0.5)
Not reported	0	0	0
Country/region, n (%)			
United States	281 (56.3)	174 (59.8)	455 (57.6)
Outside the U.S.	218 (43.7)	117 (40.2)	335 (42.4)
Smoking status, n (%)			
Current	277 (55.5)	160 (55.0)	437 (55.3)
Former	222 (44.5)	131 (45.0)	353 (44.7)
Packyears, mean years (SD)	42.7 (22.9)	41.9 (20.9)	42.4 (22.2)
Height and weight, mean (SD)			
Weight, kg	79.8 (19.2)	81.6 (21.6)	80.5 (20.1)
Height, cm	168.6 (9.5)	168.6 (10.5)	168.6 (9.9)
BMI, kg/m^2	28.0 (6.0)	28.7 (6.8)	28.2 (6.3)
COPD severity, n (%)			
Mild	1 (0.2)	0	1 (0.1)
Moderate	266 (53.3)	143 (49.1)	409 (51.8)
Severe	231 (46.3)	148 (50.9)	379 (48.0)
Very severe	1 (0.2)	0	1 (0.1)
FEV_1, mean (SD)			
Pre-bronchodilator, L	1.2868 (0.43)	1.2775 (0.43)	1.2834 (0.43)
Post- bronchodilator, L	1.4332 (0.44)	1.4194 (0.45)	1.4281 (0.44)
Reversibility[1] status, n (%)			
Reversible	168 (33.7)	82 (28.2)	540 (68.4)
Non-reversible	331 (66.3)	209 (71.8)	250 (31.6

Source: Clinical reviewer calculated/confirmed; JMP 15.0, ADSL dataset and the following parameter(s): RANDFL
[1] Reversibility defined as ≥12% and ≥200 mL increase in FEV_1 following albuterol/salbutamol.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ITT, intent-to-treat; L, liters; max, maximum; min, minimum; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SD, standard deviation; U.S., United States

Similar to Trial 301, the percentage of Black/African American (4.4%, 35/790) race and Hispanic or Latino (2.8%, 40/790) ethnicity, where reported, was similar to available epidemiologic COPD data (American Lung Association 2023), although the rates of COPD are likely underreported in these minority groups within the U.S. population (Ejike et al. 2019).

There was a numerically higher proportion of subjects in the ensifentrine group, 168/499 (33.7%) subjects, that had reversibility on screening pulmonary function tests (PFTs) (defined as ≥12% and ≥200 mL increase in FEV_1 following albuterol/salbutamol), compared to the placebo group, 82/291 (28.2%) subjects. This is unlikely to significantly impact the efficacy analysis as the difference in the proportion between the two groups is relatively small and the alternative mechanism of action the ensifentrine-mediated bronchodilation compared to albuterol.

Overall, the population and baseline demographics for Trial 302 are acceptable and generally consistent with a moderate to severe COPD population.

The Applicant specified that randomization would be stratified based on background COPD medications usage and outlined limitations on the proportion of subjects that could be using background medications. The baseline background medication use is outlined in Table 17. In general, the COPD background medication use was similar between treatment groups.

Table 17. Trial 302, Baseline Background Medication Usage (ITT)

Subgroup	ENS (N=499) n (%)	PBO (N=291) n (%)	Total Population (N=790) n (%)
No background medications	224 (44.9)	131 (45.0)	355 (44.9)
Background medications	275 (55.1)	160 (55.0)	435 (55.1)
LAMA	168 (33.7)	90 (30.9)	285 (32.7)
LAMA/ICS	1 (0.2)	0	1 (0.1)
LABA	34 (6.8)	23 (7.9)	57 (7.2)
LABA/ICS	72 (14.4)	47 (16.2)	119 (15.1)

Source: Clinical reviewer calculated/confirmed, JMP 15.0, ADCM dataset and the following parameter(s): RANDFL, BMSTRATA, BMSTRANM
Abbreviations: ENS, ensifentrine; ICS, inhaled corticosteroid; ITT, intent-to-treat; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

As with Trial 301, LAMA/LABA combination therapy were excluded from this trial. This may limit the generalizability of the results given that LAMA/LABA combination therapy is current standard of care. A higher overall percentage of Trial 302 subjects (44.9%, 355/790) were on no maintenance COPD medication compared to Trial 301 (31.5%, 240/763).

The most common comorbidities included hypertension (58.3%), gastroesophageal reflux disease (26.9%), menopausal symptoms (20.9%), and depression (22.4%). This is consistent with the known comorbidities in patients with COPD and was evenly distributed between the treatment group and placebo. The most common concomitant medications included salbutamol (67.7%), tiotropium (24.7%), and acetylsalicylic acid (19.6%). Overall, the parameters were balanced between treatment groups and there are no review concerns regarding the subjects' past medical history and other concomitant medications, which were both reasonable for a COPD population. The subjects' past medical history and other common concomitant medications are reviewed in more detail in Section 16.1.

Analysis of Efficacy – Primary Endpoint

For the primary efficacy endpoint for Trial 302, the change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12, ensifentrine demonstrated a statistically significant increase of 0.094 L compared to placebo (95% CI: 0.067, 0.124, p<0.0001), as shown in Table 18.

Table 18. Trial 302, Change From Baseline in FEV_1 $AUC_{0\text{-}12h}$ (L) at Week 12 (mITT)

Parameter	ENS (N=498)	PBO (N=291)
Baseline[1]		
n	498	291
Mean (SD)	1.285 (0.451)	1.279 (0.473)
Week 12 (imputed[2])		
n	498	291
Mean (SD)	1.332 (0.457)	1.232 (0.439)

Parameter	ENS (N=498)	PBO (N=291)
Change from baseline at Week 12 (imputed[2])		
n	498	291
Mean (SD)	0.047 (0.169)	-0.047 (0.179)
LS mean change from baseline at Week 12[3]	0.048	-0.046
95% CI	(0.030, 0.066)	(-0.070, -0.022)
LS mean difference vs. placebo (95% CI)[3]		0.094 (0.065, 0.124)
p-value		<0.0001

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADRE, ADREMI and ADREMIAV datasets
[1] Baseline FEV1 is the mean of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes pre-dose on Day 1.
[2] Number of subjects with observed data is 424 and 231 for ENS and PBO, respectively. Any missing data postbaseline was imputed using multiple imputation. The multiple imputation model included treatment, region, background medication strata, smoking strata, baseline FEV_1 and FEV_1 $AUC_{0\text{-}12h}$ at Week 12.
[3] ANCOA models were run on 100 imputed datasets and their LS means, LS mean treatment differences, associated 95% CIs and p-value were combined using Rubin's rule.
Abbreviations: ANCOVA, analysis of covariance, $AUC_{0\text{-}12h}$, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine, FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group in the mITT population; n, number of subjects in subset; PBO, placebo; SD, standard deviation; vs., versus

Similar to the results of Trial 301, the demonstrated change in FEV_1 $AUC_{0\text{-}12h}$ is statistically significant and robust against the deviation from the missing data assumption (MAR); the clinical effect size is modest but supportive of benefit (reviewed in additional detail in Section 6.3.1).

Analysis of Efficacy – Secondary Endpoints

A summary of the key secondary endpoints in Trial 301 is described in Table 19. Only the peak FEV_1 at Week 12 demonstrated a statistically significant difference between ensifentrine compared with placebo.

Table 19. Trial 302, Summary of Key Secondary Endpoints (mITT)

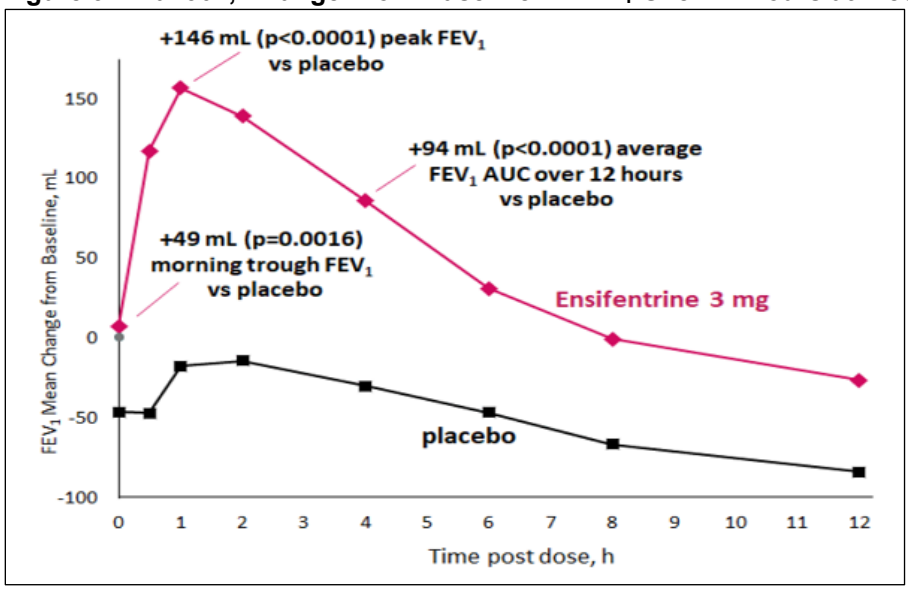
	ENS (N=498) vs. PBO (N=291)		
Endpoint	**LS Mean Diff (SE)**	**95% CI**	**p-Value**
CFB in Peak FEV_1 at Week 12, L	0.146 (0.017)	0.113, 0.179	<0.0001
CFB in ERS total symptom score at Week 24, units	-0.617 (0.412)	-1.424, 0.191	0.1334
CFB in SGRQ total score at Week 24, units	-0.478 (1.118)	-2.671, 1.715	0.6694
CFB in Morning trough FEV_1 at Week 12, L	0.049 (0.016)	0.019, 0.080	0.0016[1]
CFB in FEV_1 $AUC_{0\text{-}4h}$ at Week 12, L	0.136 (0.0154)	0.105, 0.166	<0.0001[1]
Odds ratio in proportion of SGRQ responders[2] at Week 24	0.92	0.661, 1.285	0.6284

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI, ADQSERSM and ADQSMI datasets
All continuous variables used in change from baseline analysis including peak FEV_1, E-RSTM: COPD, SGRQ total score, morning trough FEV_1 and FEV_1 $AUC_{0\text{-}4h}$ were analyzed using ANCOVA approach, as used for the primary efficacy analysis.
[1] 95% confidence interval excludes the null hypothesis but not statistically significant due to losses earlier in the statistical hierarchy.
[2] SGRQ responder defined as an improvement of at least 4 units from baseline in total SGRQ score. A logistic regression model was used with fixed effects for treatment, region, actual background medication strata and actual smoking strata at baseline and baseline SGRQ total score as covariate, using MI based on MAR.
Abbreviations: ANCOVA, analysis of covariance; $AUC_{0\text{-}4h}$, area under the concentration-time curve from 0 to 4 hours; CFB, change from baseline; CI, confidence interval; diff, difference; ENS, ensifentrine; ERS, evaluating-respiratory symptoms; FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; MAR, missing at random; MI, multiple imputation; mITT, modified intent-to-treat; N, number of subjects in treatment group; PBO, placebo; SE, standard error; SGRQ, St. George Respiratory Questionnaire; vs., versus

The results for morning trough FEV_1 and FEV_1 AUC_{0-4h} favored ensifentrine treatment and had a 95% CI that excluded the null; however, these results cannot be considered statistically significant due to earlier failure in the statistical analysis hierarchy for the Evaluating-Respiratory Symptoms total score. Results for SGRQ total score and SGRQ responder analyses did not exclude null; however, these results are typically included in labeling to provide additional clinical information to providers.

Similar to the results of Trial 301, the bronchodilatory effect of ensifentrine appears to be largely driven by effects early in the treatment interval as demonstrated in Figure 3.

Figure 3. Trial 302, Change From Baseline in FEV_1 Over 12 Hours at Week 12 (mITT)



Source: Applicant provided (Integrated Summary of Effectiveness; Figure 13, page 92). Independently calculated/confirmed by the Statistical Reviewer
Abbreviations: AUC, area under the concentration-time curve; FEV_1, forced expiratory volume in 1 second; h, hours; mITT, modified intent-to-treat; vs, versus

Overall, the secondary endpoints including the morning trough FEV_1, offer support for the primary endpoint and the overall benefit of ensifentrine compared to placebo, however this support is limited due to lack of statistical significance for the majority of the secondary endpoints. Additional review of the remaining secondary endpoints is included in Section 16.

Efficacy Subgroup Analyses

The Applicant performed multiple subgroup analyses on the primary endpoint based on gender at birth, age, region, smoking status, FEV_1 reversibility, and having a history of chronic bronchitis. Overall, the subgroup evaluations were limited by sample size but generally consistent with overall population.

An additional subgroup analysis of the primary endpoint was also performed based on background COPD medications as summarized in Table 20.

Table 20. Trial 302, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 by Background Medication Subgroup (mITT)

Subgroup	ENS (N=498) n (%)	PBO (N=291) N (%)	LS Mean Difference[1]	95% CI[1]
No background medication	223 (44.8)	131 (45.0)	0.115	0.069, 0.161
Any LAMA or LABA use	275 (55.2)	160 (55.0)	0.076	0.039, 0.114
LABA	34 (6.8)	23 (7.9)	0.040	-0.047, 0.126
LABA/ICS	72 (14.5)	47 (16.2)	0.093	0.029, 0.156
LAMA	168 (33.7)	90 (30.9)	0.079	0.027, 0.131
LAMA/ICS	1 (0.2)	0	N/A	N/A

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
[1] Based on the primary efficacy analysis by background medication subgroups.
Abbreviations: AUC_{0-12h}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; L, liters; LABA, long acting beta-agonist; LAMA, long acting muscarinic antagonist; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; N/A, not applicable; PBO, placebo

The ability to make definitive conclusions based on the COPD medication subgroup analysis is limited due to sample size, but the analyses were supportive of the primary endpoint analysis in the overall population. However, we note that use of LAMA/LABA combination therapy, a common COPD medication class, was not allowed in the trial and that this may limit generalizability of results to the target population.

Other Efficacy Endpoints – COPD Exacerbations

Similar to Trial 301, the Applicant included the overall frequency of the moderate/severe COPD exacerbations and the time to first moderate/severe COPD exacerbation as "other endpoints". These endpoints were outside of the statistical hierarchy for multiplicity control, and, as mentioned previously, are considered exploratory.

COPD Exacerbation Rate

Similar to Trial 301, Trial 302 also did not enrich for subjects with COPD who had frequent exacerbations and only approximately one fifth of subjects experienced an exacerbation within the 15 months leading up to the start of the trial – ensifentrine 20.5% (102/498 subjects), placebo 21.3% (62/291 subjects). Also, like Trial 301, the baseline exacerbations in the studied population appeared low compared to the general COPD population.

For the overall population, the rate ratio for the annualized moderate/severe COPD exacerbation rate favored ensifentrine and the 95% CI excluded null. These data and select subgroup analyses are shown in Table 21.

Table 21. Trial 302, Moderate or Severe COPD Exacerbation Annualized Event Rate Over 24 Weeks (mITT)

	ENS (N=498)		PBO (N=291)		ENS vs. PBO	
	n/Event	AER, LS Mean	n/Event	AER, LS Mean	RR	95% CI
Week 24	498/52	0.24	291/53	0.42	0.57	0.38, 0.87
Subgroups						
Background YES	275/28	0.24	160/30	0.44	0.55	0.32, 0.96
Background NO	223/24	0.28	131/23	0.46	0.60	0.32, 1.14
Previous exacerbation in past 15 months	102/12	0.30	62/19	0.80	0.38	0.18, 0.78
No previous exacerbation in past 15 months	396/40	0.21	229/34	0.30	0.71	0.42, 1.18
Current smoker	276/22	0.19	160/29	0.42	0.44	0.25, 0.80
Former smoker	222/30	0.30	131/24	0.40	0.75	0.41, 1.36

Source: Statistical Reviewer calculated/confirmed, SAS 9.4, ADEXAC dataset
Abbreviations: AER, annualized event rate, CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; RR, rate ratio; vs., versus

While rate ratios for the annualized rate of exacerbation favored ensifentrine with 95% CIs that excluded the null in the overall population, these results are exploratory as they were not multiplicity controlled. In addition, although the rate ratio point estimate suggests approximately a 40% relative reduction in exacerbation rates when comparing ensifentrine to placebo, the absolute difference in annual rate is modest and it is not clear if this is clinically relevant, particularly in a population that does not experience frequent exacerbations.

The Applicant conducted subgroup analyses on the COPD exacerbation rate and, overall, the point estimates favored ensifentrine treatment and supported the results in the overall population. However, the ability to draw conclusions based on these subgroup analyses is limited by the sample size, wide confidence intervals, and low event rate.

Additionally, we note that the annualized event rate based on 24 weeks includes the assumption that the events in the first 6 months will remain the same for the next 6 months.

Time to First COPD Exacerbation

Given the assumptions regarding annualized event rates in 24-week trials, analyses were conducted based on the time to first moderate/severe COPD exacerbation over 24 weeks with select subgroup analyses (Table 22). Treatment with ensifentrine led to an increase in the time to the first protocol-defined moderate/severe COPD exacerbation compared to subjects on placebo over 24 weeks with a hazard ratio of 0.58 and a 95% CI that excludes the null.

Table 22. Trial 302, Time-to-First Moderate or Severe COPD Exacerbation (mITT)

	ENS (n/Event)	PBO (n/Event)	HR	95% CI
Time-to-first COPD exacerbation (ENS vs. PBO)	498/46	291/45	0.58	0.38, 0.87
Subgroups				
Background YES	275/24	160/27	0.51	0.29, 0.89
Background NO	223/22	131/18	0.69	0.37, 1.29
Previous exacerbation in past 15 months	102/11	62/17	0.35	0.16, 0.75
No previous exacerbation in past 15 months	396/35	229/28	0.72	0.44, 1.18
Current smoker	267/21	160/24	0.48	0.27, 0.86
Former smoker	222/25	131/21	0.70	0.39, 1.25

Source: Statistical Reviewer calculated/confirmed, SAS 9.4, ADTTE dataset
Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; HR, hazard ratio; n, number of subjects; PBO, placebo; vs., versus

The small sample size and low event rates limit the ability to draw conclusions from the subgroup analyses. When evaluating the effect of ensifentrine based on background medication strata, there did not appear to be a difference in the time to first COPD exacerbation between treatment and placebo in those who were not on background COPD medications when compared to those who were on background COPD medications where a difference was suggested. The clinical significance of this is unclear in this setting.

As with Trial 301, Trial 302 was not designed as a formal exacerbation trial; however, there was a reduction in the annualized COPD exacerbation frequency and time to first COPD exacerbation, although these endpoints were not multiplicity controlled and are considered exploratory. Additionally, the overall clinical meaningfulness of this reduction is unclear given the modest absolute difference between the annualized event rates and the limited exacerbation history in the trial's population. Additional review and analysis of the COPD exacerbation endpoints are included in Section 6.3.2.

6.3. Key Efficacy Review Issues

6.3.1. Spirometric Effects

Issue

The Applicant has developed ensifentrine, an inhaled small molecule PDE3 and PDE4 inhibitor, to be used for the maintenance treatment of COPD. To support this, they have submitted the results of two phase 3 multicenter, randomized, double-blind, placebo-controlled trials that studied ensifentrine 3 mg twice daily delivered via a standard jet nebulizer. Both trials demonstrated statistically significant improvements in the primary endpoint, the change from baseline of the FEV_1 $AUC_{0\text{-}12h}$; however, the overall effect size appears to be driven by an early bronchodilatory response.

Background

The primary mechanism of action of ensifentrine, inhibition of PDE3 and PDE4, leads to airway smooth muscle relaxation with subsequent bronchodilation.

Ensifentrine has been developed for the maintenance treatment of COPD. To support this, the Applicant used the average FEV_1 $AUC_{0\text{-}12h}$ at Week 12 as the primary endpoint for their phase 3 program. While the use of FEV_1 $AUC_{0\text{-}12h}$ as the primary endpoint was not considered unreasonable, the Division had concerns regarding its use as a treatment effect may be driven by a bronchodilatory response shortly after dosing with minimal or no effect at the end of the dosing interval. Due to this concern, the Division recommended use of morning trough FEV_1 as the primary endpoint in the phase 3 program because this provides an assessment of the impact of the medication at the end of the dosing interval.

Assessment

To evaluate the impact of ensifentrine on lung function in subjects with moderate to severe COPD, we focused our review on the primary and key secondary endpoints measured by spirometry obtained at Week 12 in both phase 3 trials. This included: the primary endpoint of FEV_1 $AUC_{0\text{-}12h}$, which reflects changes throughout the dosing interval; the peak FEV_1 and FEV_1 $AUC_{0\text{-}4h}$, which reflect changes early in the dosing interval; and the morning trough FEV_1, which reflects changes at the end of the dosing interval.

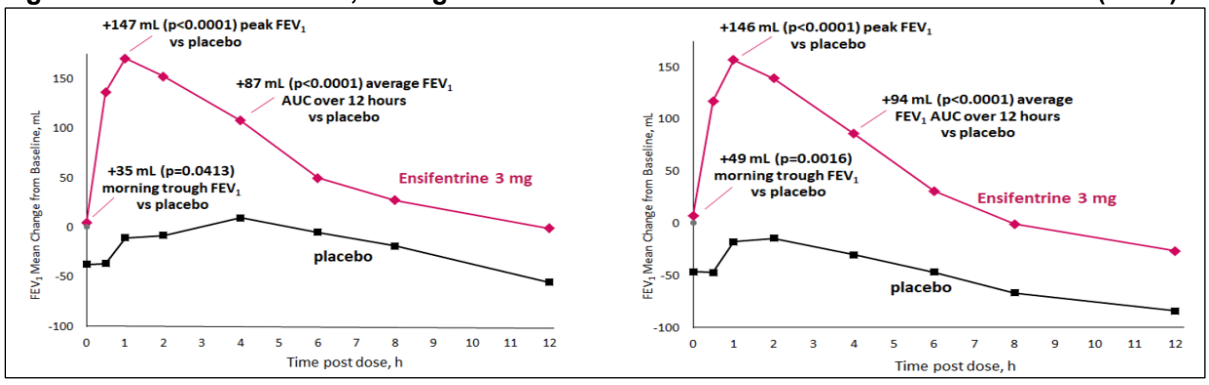
Figure 4 is a graphic representation of the FEV_1 over the 12-hour dosing interval.

Table 23. Trials 301 and 302, Summary of Spirometric Changes from Baseline at Week 12 (mITT)

	Trial 301			Trial 302		
Endpoint	ENS vs. PBO, L	95% CI	p-Value	ENS vs. PBO, L	95% CI	p-Value
FEV_1 $AUC_{0\text{-}12h}$	0.087	0.055, 0.118	<0.0001	0.094	0.064, 0.123	<0.0001
Morning trough FEV_1	0.035	0.001, 0.068	0.0413	0.049	0.018, 0.080	0.0016[1]
Peak FEV_1	0.147	0.111, 0.183	<0.0001	0.146	0.113, 0.179	<0.0001
FEV_1 $AUC_{0\text{-}4h}$	0.139	0.105, 0.172	<0.0001	0.136	0.105, 0.166	<0.0001[1]

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
[1] Not statistically significant despite 95% CI excluding null due to losses earlier in the statistical hierarchy.
Abbreviations: $AUC_{0\text{-}4h}$, area under the concentration-time curve from 0 to 4 hours; $AUC_{0\text{-}12h}$, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiration volume in 1 second; L, liters; mITT, modified intent-to-treat; PBO, placebo; vs., versus

Figure 4. Trials 301 and 302, Change From Baseline in FEV_1 Over 12 Hours at Week 12 (mITT)



Source: Applicant provided (Integrated Summary of Effectiveness; Figures 4 and 13, pages 73 and 92). Independently calculated/confirmed by the Statistical Reviewer
Abbreviations: AUC, area under the concentration-time curve; FEV_1, forced expiration volume in 1 second; h, hours; mITT, modified intent-to-treat; vs, versus

In both phase 3 trials, ensifentrine treatment increased the FEV_1 in subjects with moderate to severe COPD over the dosing interval compared to placebo, as measured by the FEV_1 $AUC_{0\text{-}12h}$. In addition, as shown in Figure 4 and observed in the peak FEV_1 and average FEV_1 $AUC_{0\text{-}4h}$ endpoints (summarized in Table 23), the positive effect of ensifentrine compared to placebo appears to be driven by bronchodilation early in the dosing interval. This is not unexpected with a medication such as ensifentrine given the bronchodilatory mechanism of action.

Improvements in lung function with ensifentrine compared to placebo were also demonstrated at the end of the dosing interval, as measured by the morning trough FEV_1; however, the effect size was clinically small and more modest than seen earlier in the dosing interval.

Evaluation of the potential impact of background medication on the effect of ensifentrine is limited due to sample size. A summary of primary endpoint analyses from Trials 301 and 302 by key background medication subgroup is shown in Table 24.

Table 24. Trials 301 and 302, Summary of Key Background Medication Subgroup Analyses of Primary Endpoint at Week 12 (mITT)

	Trial 301		Trial 302	
Subgroup	**ENS vs. PBO, L**	**95% CI**	**ENS vs. PBO, L**	**95% CI**
No background medication	0.060	-0.003, 0.123	0.115	0.069, 0.161
Any LAMA or LABA use	0.102	0.066, 0.138	0.076	0.040, 0.114
LAMA	0.079	0.053, 0.167	0.079	0.027, 0.131
LABA/ICS	0.059	-0.009, 0.128	0.093	0.029, 0.156

Source: Statistical Reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
Abbreviations: CI, confidence interval; ENS, ensifentrine; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; L, liters; LAMA, long-acting muscarinic antagonist; mITT, modified intent-to-treat; PBO, placebo; vs., versus

Overall, the impact of ensifentrine on the average FEV_1 $AUC_{0\text{-}12h}$ compared to placebo based on background COPD therapy subgroup appears consistent with the overall population. There did not appear to be a difference in the effect of ensifentrine compared to placebo in subjects on or off background COPD therapy. The exclusion of LABA/LAMA combination therapy, a common medication class used by patients with COPD, limits the generalizability of the bronchodilatory effect of ensifentrine.

Conclusion

Treatment with ensifentrine 3 mg twice daily demonstrated improvement compared to placebo in the FEV_1 as measured by spirometry in the moderate to severe COPD population studied in Trials 301 and 302. The impact appears to be largely driven by bronchodilation early in the treatment interval, as demonstrated by initial improvements in the FEV_1 $AUC_{0\text{-}12h}$, peak FEV_1, and average FEV_1 $AUC_{0\text{-}4h}$. The spirometric benefit of ensifentrine also persists to the end of the dosing interval, as measured by the morning trough FEV_1, although this effect is more modest. The overall evaluation of the impact of ensifentrine on lung function as measured by spirometry in subjects with moderate to severe COPD is favorable and sufficient to support the benefit of ensifentrine 3 mg twice daily compared to placebo.

6.3.2. COPD Exacerbations

Issue

COPD exacerbations are a significant cause of morbidity, mortality, and health care expenditures. Trials 301 and 302 were not designed as formal exacerbation trials; however, the Applicant included 'moderate and severe COPD exacerbation frequency' and the 'time to first moderate to severe COPD exacerbation' as other endpoints in their phase 3 program. These endpoints were outside of the statistical hierarchy for multiplicity control. We consider them to be exploratory; however, (b) (4)

Background

The reduction in COPD exacerbations has been established as an endpoint in multiple clinical development programs, although in instances where it has been included in the USPI, the endpoints are typically included in the statistical hierarchy and are often primary endpoints in dedicated exacerbation trials. These endpoints are considered clinically meaningful given the link between increased frequency of moderate/severe acute exacerbations of COPD and increased disease sequelae. This is particularly relevant in those patients who are experiencing frequent COPD exacerbations; however, it is less clear if this link can be applied to COPD patients with infrequent exacerbations, such as those evaluated in the ensifentrine phase 3 program.

In addition, as part of the pre-specified trial design, the trial excluded patients who were taking LAMA/LABA combination medications. This is a common COPD medication class included in the standard of care paradigm outlined by the GOLD currently and at the time the trials were enrolling. Many of these medications have also been previously shown to demonstrate a reduction in COPD exacerbations, and the lack of the ability to assess the effect of ensifentrine on COPD exacerbations in patients who are also receiving LAMA/LABA combinations may limit the generalizability of the results.

Assessment

A summary of the annualized COPD exacerbation rate and time to first COPD exacerbation in Trials 301 and 302 is presented below in Table 25.

Table 25. Trials 301 and 302, Summary of Moderate or Severe COPD Exacerbation Effect at Week 24 (mITT)

	Trial 301		Trial 302	
	ENS (N=477)	PBO (N=283)	ENS (N=498)	PBO (N=291)
Annualized event rate, LS mean	0.26	0.41	0.24	0.42
Subjects with event, n (%)	38 (8.0)	36 (12.7)	46 (9.2)	45 (15.5)
COPD exacerbation events	42	40	52	53
Moderate COPD exacerbation events	34	35	44	48
Severe COPD exacerbation events	8	5	8	5
	ENS vs. PBO		ENS vs. PBO	
	RR	95% CI	RR	95% CI
Annualized COPD exacerbation rate	0.64	0.40, 1.00	0.57	0.38, 0.87
H/o background therapy	0.65	0.39, 1.10	0.55	0.32, 0.96
No h/o background therapy	0.57	0.22, 1.47	0.60	0.32, 1.14
	ENS vs. PBO		ENS vs. PBO	
	HR	95% CI	HR	95% CI
Time to first COPD exacerbation	0.62	0.39, 0.97	0.58	0.38, 0.87
H/o background therapy	0.60	0.35, 1.02	0.51	0.29, 0.89
No h/o background therapy	0.66	0.27, 1.62	0.69	0.37, 1.29

Source: Statistical Reviewer calculated/confirmed, SAS 9.4, ADEXAC, ADTTE, and ADSL datasets
Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; h/o, history of; HR, hazard ratio; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; RR, rate ratio; vs., versus

Overall, COPD exacerbations were not common during the treatment periods, which is not surprising as neither trial enriched for frequent exacerbators and excluded patients on LAMA/LABA. In the 15 months leading up to the trial, only 26% and 21% of subjects had experienced a COPD exacerbation in Trials 301 and 302 respectively. For both annualized rate and time to first exacerbation, point estimates for rate and hazard ratios favored ensifentrine-treated subjects. However, these differences were driven by relatively small numbers of events between groups, and while 95% CIs for some comparisons excluded null, the exacerbation results are not statistically significant because the endpoints were analyzed outside of the statistical hierarchy for multiplicity control. In addition, the clinical relevance of the magnitude of the reduction in COPD exacerbation rate or time to first COPD exacerbation is not clear, particularly in a population that is infrequently exacerbating.

In clinical practice, we anticipate that ensifentrine will likely be used as an add-on therapy within the pre-existing COPD treatment paradigm. Although the analysis of the COPD exacerbation results based on subgroup is further limited by sample size, the effect of ensifentrine in subjects on COPD background therapy is generally consistent with the overall population. However, given the exclusion of LABA/LAMA combination therapy – a common medication class that includes multiple medications with demonstrated reductions in COPD exacerbations – it is unclear if the benefit of ensifentrine on exacerbations will persist in a real-world clinical setting. This adds further uncertainty to any potential beneficial effect on exacerbations.

As exacerbation results were not included in the statistical hierarchy, there were relatively few exacerbation events in a group of infrequent exacerbators, and patients on LAMA/LABA were excluded, the clinical relevance of the magnitude of the reduction in exacerbations is unclear. Therefore, definitive conclusions regarding exacerbation benefit cannot be made.

Conclusion

Available data do not support inclusion of exacerbation-related claims in the label.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

Coronary artery vasculopathy findings associated with hemodynamic changes (i.e., increased heart rate and decreased blood pressure) were observed in repeat-dose inhalation toxicology studies (from 6-week to 40-week) in dogs. The dog is considered to be very susceptible to inotropic/vasodilating agent-induced cardiovascular lesions that were observed with PDE3 inhibitors (NDA 20863 for PLETAL (cilostazol) (Cilostazol USPI 1999). The vasodilator-induced cardiovascular lesions in the dog are attributed to anatomical differences in coronary circulation between humans and dogs. Thus, these lesions are of questionable clinical relevance. Increased heart rate is monitorable in the clinical setting.

In a fertility study, male rats administered ensifentrine by inhalation had decreased sperm motility, increased abnormal sperm morphology, atrophy/degeneration in the testis, and intraluminal germ cell debris in the epididymis. These adverse effects resulted in impaired reproductive performance including decreased mating index and decreased fertility index, increased pre- and post-implantation loss, and decreased live embryos per litter in untreated females. The male reproductive tract-toxicity of ensifentrine is consistent with other approved PDE4 inhibitors (e.g., roflumilast (oral tablets), a PDE4 inhibitor). Rats are highly sensitive to PDE4 inhibitor-induced male reproductive tract toxicity. The clinical relevance of these findings is not clear.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

There are no inhaled small molecule PDE3 and PDE4 inhibitors that are currently approved; however, other PDE inhibitors are approved for a wide range of indications including COPD, psoriasis, acute decompensated heart failure, pulmonary arterial hypertension, and benign prostatic hyperplasia.

Potential risks noted in either PDE3 or PDE4 inhibitors are summarized below:

- Roflumilast (oral tablets), a PDE4 inhibitor, was approved on February 28, 2011 for the reduction in risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and history of exacerbations. Section 5 of the USPI (Warnings and Precautions) includes psychiatric events including suicidality and weight decrease. The adverse events (AEs) included in Section 6 of the USPI include multiple gastrointestinal (GI) symptoms such as diarrhea, nausea, and decreased appetite (Roflumilast USPI 2011).

- Apremilast (oral tablets), a PDE4 inhibitor, was approved on March 21, 2014 for the treatment of adult patients with active psoriatic arthritis, plaque psoriasis who are candidates for phototherapy or systemic therapy, and adult patients with oral ulcers associated with Behcet's Disease. Section 5 of the USPI (Warnings and Precautions) includes depression, weight decrease, and diarrhea, nausea, and vomiting (Apremilast USPI 2014).
- Cilostazol (oral tablets), a PDE3 inhibitor, was approved in 1999 for the reduction of symptoms of intermittent claudication. Section 5 of the USPI (Warnings and Precautions) includes hematologic adverse reactions including cases of thrombocytopenia or leukopenia progressing to agranulocytosis (reversible with discontinuation) (Cilostazol USPI 1999).

The potential risks of the related oral products are likely due to systemic exposure and may be less of a risk given the inhaled route of ensifentrine with low systemic exposure (see Section 8.1, free drug maximum plasma concentration [C_{max}] of ensifentrine < 0.1 ng/mL).

In addition to potential safety concerns based on drug class, administration of inhaled medications can result in paradoxical bronchospasm, including those medications that have a bronchodilatory mechanism of action. Therefore, given the route of administration, paradoxical bronchospasm is a potential safety concern.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

7.3.1. Adverse Events Identified in Postmarket Experiences

Ensifentrine is not currently approved in the United States or in any other foreign market; therefore, no postmarketing experience is available.

7.3.2. Expectations on Safety

Not applicable for this review.

7.3.3. Additional Safety Issues From Other Disciplines

Not applicable for this review.

7.4. FDA Approach to the Safety Review

The evaluation of safety for ensifentrine in COPD is primarily based on the clinical data from the phase 3 development program – Trials RPL554-CO-301 and RPL554-CO-302 (Trials 301 and 302). As both trials included 24-week placebo-controlled treatment periods and given the similarity in subject population and trial design, the 24-week safety data from these trials were pooled for safety analyses. This pooled dataset will be referred to as the Pooled 24-Week Safety

Database. Additional separate safety analyses were also performed on the 48-week treatment duration cohort from Trial 301. All safety analyses were conducted on the SAS, defined as all subjects that received at least one dose of study drug. All AEs presented are considered treatment emergent adverse events, defined as AEs that started or worsened in severity on or after the first dose of study medication.

7.5. Adequacy of the Clinical Safety Database

The duration of exposure for Trials 301 and 302 is presented separately in Table 26 and Table 27. Overall, the Pooled 24-Week Safety Database included 1,549 subjects (975 subjects receiving ensifentrine and 574 subjects receiving placebo).

Table 26. Trial 301, Duration of Exposure (SAS)

	24-Week Cohort		48-Week Cohort	
Parameter	**ENS (N=197)**	**PBO (N=194)**	**ENS (N=280)**	**PBO (N=89)**
Duration of treatment, days				
Mean (SD)	154.9 (38.9)	153.1 (42.1)	285.2 (98.7)	284.1 (101.8)
Subjects treated, by duration, n (%)				
<43 days	9 (4.6)	12 (6.1)	8 (2.9)	3 (3.4)
≥43 to <85 days	11 (5.6)	9 (4.6)	17 (6.1)	4 (4.5)
≥85 to <127 days	1 (0.5)	4 (2.1)	21 (4.3)	4 (4.5)
≥127 to <169 days	80 (40.6)	72 (37.1)	6 (2.1)	6 (6.7)
≥169 to <211 days	96 (48.7)	97 (50.0)	15 (5.4)	4 (4.5)
≥211 to <253 days	-	-	5 (1.8)	0
≥253 to <295 days	-	-	7 (2.5)	0
≥295 to <336 days	-	-	84 (30.0)	25 (28.1)
≥336 days	-	-	126 (45.0)	43 (48.3)

Source: Clinical Reviewer verified using JMP15.0. Data set: adda.xpt using the following parameters: SAFFL (Y), PARAMCD (TRTDUR), AVAL, AVALCAT4
Abbreviations: ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set; SD, standard deviation

Table 27. Trial 302, Duration of Exposure (SAS)

	Trial 302	
Parameter	**ENS (N=498)**	**PBO (N=291)**
Duration of treatment, days		
Mean (SD)	147 (47.9)	139.1 (52.4)
Subjects treated, by duration, n (%)		
<43 days	30 (6.0)	26 (8.9)
≥43 to <85 days	48 (9.6)	29 (10.0)
≥85 to <127 days	21 (4.2)	26 (8.9)
≥127 to <169 days	147 (29.5)	74 (25.4)
≥169 days	252 (50.6)	136 (46.7)

Source: Clinical reviewer verified using JMP15.0. Data set: adda.xpt using the following parameters: SAFFL (Y), PARAMCD (TRTDUR), AVAL
Abbreviations: ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set; SD, standard deviation

Overall, the safety database provided by the Applicant appears adequate to evaluate the safety of ensifentrine and is consistent with other COPD development programs.

7.6. Safety Results

7.6.1. Safety Results, Pooled Analyses, Trials 301 and 302

7.6.1.1. Overview of Adverse Events Summary, Pooled Analyses, Trials 301 and 302

An overview of AEs for the Pooled 24-Week Safety Database is provided in Table 28. Across AE categories, the proportion of subjects that experienced events were balanced between the treatment and placebo groups.

Table 28. Trials 301 and 302, Overview of Adverse Events, Pooled 24-Week Safety Database (SAS)

Event Category	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
SAE	60 (6.2)	36 (6.3)	-0.1 (-2.8, 2.3)
AE leading to permanent discontinuation of study drug	74 (7.6)	47 (8.2)	-0.6 (-3.5, 2.1)
AE leading to dose modification of study drug	26 (2.7)	12 (2.1)	0.6 (-1.2, 2.1)
Any AE	359 (36.8)	206 (35.9)	0.9 (-4.1, 5.8)
Severe	49 (5.0)	27 (4.7)	0.3 (-2.1, 2.5)
Moderate	159 (16.3)	78 (13.6)	2.7 (-1.0, 6.3)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Severity as assessed by the investigator.
Abbreviations: AE, adverse event; CI, confidence interval; ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAE, serious adverse event; SAS, safety analysis set

7.6.1.2. Deaths, Pooled Analyses, Trials 301 and 302

The deaths reported in the Pooled 24-Week Safety Database are summarized in Table 29. Overall, the deaths were infrequent and balanced between the treatment groups.

Table 29. Trials 301 and 302, Deaths, Pooled 24-Week Safety Database (SAS)

Preferred Term	ENS 3 mg N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Any AE leading to death	6 (0.6)	5 (0.9)	-0.3 (-1.5, 0.6)
Acute left ventricular failure	1 (0.1)	0	0.1 (-0.6, 0.6)
Adenocarcinoma metastatic	1 (0.1)	0	0.1 (-0.6, 0.6)
COVID-19[1]	2 (0.2)	0	0.1 (-0.6, 0.6)
Lung neoplasm malignant	1 (0.1)	0	0.1 (-0.6, 0.6)
Toxicity to various agents	1 (0.1)	0	0.1 (-0.6, 0.6)
Cholangiocarcinoma	0	1 (0.2)	-0.2 (-1.0, 0.2)

Preferred Term	ENS 3 mg N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Clostridium difficile infection	0	1 (0.2)	-0.2 (-1.0, 0.2)
Pneumonia bacterial	0	1 (0.2)	-0.2 (-1.0, 0.2)
Septic shock	0	1 (0.2)	-0.2 (-1.0, 0.2)
Shock hemorrhagic	0	1 (0.2)	-0.2 (-1.0, 0.2)
Small cell lung cancer metastatic	0	1 (0.2)	-0.2 (-1.0, 0.2)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
[1] COVID-19 includes combined preferred terms COVID-19 and COVID-19 pneumonia.
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; COVID-19, coronavirus disease 2019; ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

The death narratives provided by the Applicant were reviewed. The deaths were primarily due to infection (e.g., COVID-19, bacterial pneumonia, septic shock, and *Clostridium difficile* infection) or malignancy (e.g., metastatic adenocarcinoma of unknown primary, metastatic small cell lung cancer, cholangiocarcinoma). These are not unexpected conditions in a population with the average age, COPD diagnosis, and smoking history as was evaluated in Trials 301 and 302. There were no clear patterns in the cause of death across treatment groups.

Three additional deaths occurred in the 48-week cohort of Trial 301 after the first 24 weeks (data not shown). The listed cause of death for these subjects are COVID-19 pneumonia (ensifentrine), pancreatic carcinoma (placebo), and sepsis (placebo).

Overall, the ensifentrine phase 3 studies had a low incidence of death and the deaths occurring in the phase 3 program of ensifentrine did not raise any safety concerns.

7.6.1.3. Serious Adverse Events, Pooled Analyses, Trials 301 and 302

In the Pooled 24-Week Safety Database, 96 (6.2%) subjects experienced at least one serious adverse event (SAE). The most common SAEs reported were COPD exacerbations, pneumonia, and lung malignancies. In general, SAEs were balanced between ensifentrine and placebo groups. These data are summarized in Table 30.

Table 30. Trials 301 and 302, Subjects With Serious Adverse Events by System Organ Class and Preferred Term Occurring in >1 Subject, Pooled 24-Week Safety Database (SAS)

System Organ Class Preferred Term	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Any SAE	60 (6.2)	36 (6.3)	-0.1 (-2.8, 2.3)
Blood and lymphatic system disorders	0	1 (0.2)	-0.2 (-1.0, 0.2)
Cardiac disorders	3 (0.3)	3 (0.5)	-0.2 (-1.2, 0.5)
Ear and labyrinth disorders	1 (0.1)	1 (0.2)	-0.1 (-0.9, 0.4)
General disorders and administration site conditions	2 (0.2)	0	0.2 (-0.5, 0.7)
Hepatobiliary disorders	2 (0.2)	0	0.2 (-0.5, 0.7)
Infections and infestations	17 (1.7)	11 (1.9)	-0.2 (-1.8, 1.2)
COVID-19 pneumonia	5 (0.5)	3 (0.5)	-0.0 (-1.1, 0.8)
COVID-19	2 (0.2)	2 (0.3)	-0.1 (-1.1, 0.4)
Pneumonia	5 (0.5)	4 (0.7)	-0.2 (-1.3, 0.6)

System Organ Class Preferred Term	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Injury, poisoning and procedural complications	4 (0.4)	4 (0.7)	-0.3 (-1.4, 0.5)
Musculoskeletal and connective tissue disorders	2 (0.2)	0	0.2 (-0.5, 0.7)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	10 (1.0)	5 (0.9)	0.2 (-1.1, 1.2)
Lung adenocarcinoma	2 (0.2)	0	0.2 (-0.5, 0.7)
Lung neoplasm malignant	2 (0.2)	0	0.2 (-0.5, 0.7)
Nervous system disorders	3 (0.3)	1 (0.2)	0.1 (-0.7, 0.8)
Psychiatric disorders	1 (0.1)	0	0.1 (-0.6, 0.6)
Renal and urinary disorders	1 (0.1)	0	0.1 (-0.6, 0.6)
Reproductive system and breast disorders	1 (0.1)	0	0.1 (-0.6, 0.6)
Respiratory, thoracic and mediastinal disorders	21 (2.2)	13 (2.3)	-0.1 (-1.8, 1.4)
Acute respiratory failure	3 (0.3)	0	0.3 (-0.4, 0.9)
Chronic obstructive pulmonary disease	17 (1.7)	11 (1.9)	-0.2 (-1.8, 1.2)
Vascular disorders	4 (0.4)	2 (0.3)	0.1 (-0.9, 0.8)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified; adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; COVID-19, coronavirus disease 2019; ENS, ensifentrine; incl, including; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAE, serious adverse event; SAS, safety analysis set; SAS, safety analysis set

For all SAEs occurring in more than one subject, the 95% CIs for the risk difference between ensifentrine and placebo included the null. SAE narratives were reviewed and did reveal any patterns in events. Review of the SAE data from the 48-week cohort was consistent with the Pooled 24-week Safety Database. Overall, the analysis of the SAEs did not raise any safety concerns.

7.6.1.4. Adverse Events Leading to Treatment Discontinuation, Pooled Analyses, Trials 301 and 302

Adverse events leading to treatment discontinuation were noted in 7.6% (74/975) in the ensifentrine treatment group compared to 8.2% (47/574) of placebo. Overall, the AEs leading to treatment discontinuation appeared balanced between the ensifentrine and placebo group as summarized in Table 31.

Table 31. Trials 301 and 302, Subjects With Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term Occurring in >1 Subject, Pooled 24-Week Safety Database (SAS)

System Organ Class Preferred Term	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Any AE leading to discontinuation	74 (7.6)	47 (8.2)	-0.6 (-3.5, 2.1)
Cardiac disorders	3 (0.3)	5 (0.9)	-0.6 (-1.7, 0.2)
Ear and labyrinth disorders	0	1 (0.2)	-0.2 (-1.0, 0.2)
Eye disorders	0	1 (0.2)	-0.2 (-1.0, 0.2)
Gastrointestinal disorders	3 (0.3)	1 (0.2)	0.1 (-0.7, 0.8)
Vomiting	2 (0.2)	0	0.2 (-0.5, 0.7)
General disorders and administration site conditions	5 (0.5)	2 (0.3)	0.2 (-0.8, 0.9)
Chest discomfort	1 (0.1)	2 (0.3)	-0.2 (-1.2, 0.3)

System Organ Class Preferred Term	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Infections and infestations	30 (3.1)	16 (2.8)	0.3 (-1.6, 2.0)
COVID-19	21 (2.2)	9 (1.6)	0.6 (-1.0, 1.9)
Sepsis	2 (0.2)	0	0.2 (-0.5, 0.7)
COVID-19 pneumonia	2 (0.2)	2 (0.3)	-0.1 (-1.1, 0.4)
Pneumonia	1 (0.1)	3 (0.5)	-0.4 (-1.4, 0.1)
Injury, poisoning and procedural complications	1 (0.1)	1 (0.2)	-0.1 (-0.9, 0.4)
Investigations	0	2 (0.3)	-0.3 (-1.3, 0.0)
Metabolism and nutrition disorders	3 (0.3)	0	0.3 (-0.4, 0.9)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	6 (0.6)	2 (0.3)	0.3 (-0.7, 1.0)
Nervous system disorders	4 (0.4)	5 (0.9)	-0.5 (-1.6, 0.3)
Headache	2 (0.2)	1 (0.2)	0.0 (-0.8, 0.6)
Dizziness	1 (0.1)	2 (0.3)	-0.2 (-1.2, 0.3)
Psychiatric disorders	3 (0.3)	0	0.3 (-0.4, 0.9)
Renal and urinary disorders	1 (0.1)	0	0.1 (-0.6, 0.6)
Respiratory, thoracic and mediastinal disorders	25 (2.6)	16 (2.8)	-0.2 (-2.1, 1.4)
Acute respiratory failure	3 (0.3)	0	0.3 (-0.4, 0.9)
Cough	4 (0.4)	1 (0.2)	0.2 (-0.6, 0.9)
Chronic obstructive pulmonary disease	12 (1.2)	7 (1.2)	0.0 (-1.4, 1.1)
Dyspnea	5 (0.5)	7 (1.2)	-0.7 (-2.0, 0.2)
Skin and subcutaneous tissue disorders	0	1 (0.2)	-0.2 (-1.0, 0.2)
Vascular disorders	3 (0.3)	0	0.3 (-0.4, 0.9)
Hypertension	2 (0.2)	0	0.2 (-0.5, 0.7)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified; adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; COVID-19, coronavirus disease 2019; ENS, ensifentrine; incl, including; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

There were no identified risk differences in AEs leading to discontinuation of the study medication where the 95% CIs excluded the null or other significant safety trends identified.

The two most common AEs leading to discontinuation included COPD and COVID-19, which were both listed in the protocol as criteria for study drug withdrawal. Other etiologies included malignancies and cough, although the overall proportion of subjects experiencing these AEs was low. Additional review of the two trials individually and the 48-week cohort in Trial 301 (data not shown) was consistent with the pooled analysis and did not identify any additional safety concerns.

Overall, AEs leading to treatment discontinuation in the phase 3 program is reassuring and appears balanced between the treatment and placebo groups.

7.6.1.5. Adverse Events, Pooled Analyses, Trials 301 and 302

A total of 565 (36.5%) subjects experienced at least one AE during the 24-week treatment period and the rates of AEs were generally balanced between treatment groups. The most common AEs occurring in the Pooled 24-Week Safety Database are presented in Table 32 (limited to AEs occurring in ≥1% of subjects).

Table 32. Trials 301 and 302, Subjects With Common Adverse Events Occurring at ≥1.0% Frequency, Pooled 24-Week Safety Database (SAS)

Preferred Term	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Any AE	359 (36.8)	206 (35.9)	0.9 (-4.1, 5.8)
COVID-19	34 (3.5)	21 (3.7)	-0.2 (-2.3, 1.7)
Headache	26 (2.7)	19 (3.3)	-0.6 (-2.6, 1.1)
Nasopharyngitis	22 (2.3)	19 (3.3)	-1.1 (-3.0, 0.6)
Chronic obstructive pulmonary disease	18 (1.8)	11 (1.9)	-0.1 (-1.7, 1.3)
Back pain	18 (1.8)	6 (1.0)	0.8 (-0.6, 2.0)
Hypertension	17 (1.7)	5 (0.9)	0.9 (-0.4, 2.0)
Urinary tract infection	13 (1.3)	6 (1.0)	0.3 (-1.0, 1.4)
Upper respiratory tract infection	10 (1.0)	7 (1.2)	-0.2 (-1.6, 0.9)
Diarrhea	10 (1.0)	4 (0.7)	0.3 (-0.8, 1.3)
Pneumonia	8 (0.8)	6 (1.0)	-0.2 (-1.5, 0.7)
Dyspnea	6 (0.6)	8 (1.4)	-0.8 (-2.2, 0.2)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; COVID-19, coronavirus disease 2019; ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

The most common AEs reported included COVID-19, headache, and nasopharyngitis, and all were balanced between ensifentrine treatment and placebo. Although the confidence intervals did not exclude the null, the preferred terms (PTs) of back pain, hypertension, UTI, and diarrhea occurred at a higher rate with ensifentrine treatment compared to placebo.

Overall, review of common AEs is reassuring and did not demonstrate significant safety concerns.

Submission Specific Safety Concerns

There were no prespecified AEs of special interest in the clinical program; however, during the discussions between the Applicant and Division, the following safety topics of interest were proposed based on known clinical effects of other PDE inhibitors, the non-clinical program, and the COVID-19 pandemic:

- Gastrointestinal disorders
- Cardiac and vascular disorders
- COVID-19
- Psychiatric disorders.

During the Division's review of the safety data, syncope and arthritis were noted as potential safety concerns and are reviewed below.

Gastrointestinal Disorders

Roflumilast and other oral PDE inhibitors list GI disorders including diarrhea in Section 5 of the USPI. As such, GI AEs were specifically analyzed. The additional targeted safety analysis performed based on a combination of gastrointestinal AEs is reviewed in Table 33.

Table 33. Trials 301 and 302, Adverse Events Related to Gastrointestinal Disorders, Pooled 24-Week Safety Database (SAS)

Gastrointestinal Disorders	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
AE grouping related to GI disorders	31 (3.2)	13 (2.3)	0.9 (-0.9, 2.5)
Diarrhea	10 (1.0)	4 (0.7)	0.3 (-0.8, 1.3)
Nausea	6 (0.6)	3 (0.5)	0.1 (-1.0, 0.9)
Vomiting	5 (0.5)	1 (0.2)	0.3 (-0.5, 1.0)
Abdominal pain	3 (0.3)	1 (0.2)	0.1 (-0.7, 0.8)
Dry mouth	3 (0.3)	1 (0.2)	0.1 (-0.7, 0.8)
Abdominal pain upper	2 (0.2)	0	0.2 (-0.5, 0.7)
Pancreatitis chronic	1 (0.1)	0	0.1 (-0.6, 0.6)
Dry throat	2 (0.2)	1 (0.2)	0.0 (-0.8, 0.6)
Dyspepsia	2 (0.2)	1 (0.2)	0.0 (-0.8, 0.6)
Pancreatitis chronic	1 (0.1)	0	0.1 (-0.6, 0.6)
Abdominal discomfort	0	1 (0.2)	-0.2 (-1.0, 0.2)
Constipation	0	1 (0.2)	-0.2 (-1.0, 0.2)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; ENS, ensifentrine; GI, gastrointestinal; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SAS, safety analysis set

Overall, there may be a trend towards increased GI adverse events with ensifentrine treatment, although the 95% confidence interval did not exclude the null for the AE grouping or any individual AE. Diarrhea was one of the more commonly reported GI AEs in the Pooled P3 Safety Database, occurring in 10/975 (1.0%) subjects in the treatment group and 4/574 (0.7%) subjects in the placebo group. The events of diarrhea primarily occurred after 84 days (12 weeks) of treatment and the Applicant reports that all events of diarrhea were reported as mild to moderate in intensity. It is unclear if this can be entirely attributed to ensifentrine treatment. The additional common GI AEs included nausea and vomiting, although these events appeared generally balanced between the treatment groups. There were no SAEs reported related to GI disorders. Review of the GI adverse events from the individual trials as well as the 48-week cohort in Trial 301 were similar to the pooled analysis.

During discussions with the Division, the Applicant was also asked to evaluate for changes in body weight. Only one (0.1%) subject in the ensifentrine group was reported to have an AE of ‘abnormal loss of weight’ and none in the placebo group; however, body weight was only measured at screening.

Overall, the review of AEs related to gastrointestinal disorders did not reveal an overt safety concern.

Cardiac and Vascular Disorders

In the nonclinical development program, ensifentrine treatment in dogs resulted in coronary artery vasculopathy associated with an increased heart rate and decreased blood pressure (see Section 7.1). As such, cardiac and vascular events were evaluated as a safety topic of interest. Table 34 summarizes AEs occurring in the system organ class of cardiac and vascular disorders and listed by narrow FDA medical query.

Table 34. Trials 301 and 302, Subjects With Cardiac and Vascular Adverse Events by SOC and FDA Medical Query (Narrow), Pooled 24-Week Safety Database (SAS)

System Organ Class FMQ (Narrow)	ENS 3 mg N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Cardiac disorders			
Systemic hypertension	21 (2.2)	6 (1.0)	1.1 (-0.3, 2.4)
Arrhythmia	15 (1.5)	6 (1.0)	0.5 (-0.9, 1.6)
Tachycardia	5 (0.5)	2 (0.3)	0.2 (-0.8, 0.9)
Cardiac conduction disturbance	1 (0.1)	0	0.1 (-0.6, 0.6)
Palpitations	1 (0.1)	1 (0.2)	-0.1 (-0.9, 0.4)
Heart failure	4 (0.4)	3 (0.5)	-0.1 (-1.1, 0.6)
Myocardial Ischemia	2 (0.2)	5 (0.9)	-0.7 (-1.8, 0.0)
Vascular disorders			
Hypotension	5 (0.5)	0	0.5 (-0.2, 1.2)
Thrombosis venous	2 (0.2)	1 (0.2)	0.0 (-0.8, 0.6)
Thrombosis arterial	1 (0.1)	2 (0.3)	-0.2 (-1.2, 0.3)
Thrombosis	3 (0.3)	4 (0.7)	-0.4 (-1.5, 0.3)
Hemorrhage	9 (0.9)	8 (1.4)	-0.5 (-1.9, 0.6)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.
Abbreviations: CI, confidence interval; ENS, ensifentrine; FMQ, FDA, U.S. Food and Drug Administration; FDA medical query; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; PT, preferred term; SAS, safety analysis set; SOC, system organ class

The systemic hypertension FDA medical query (FMQ), which includes the PTs hypertension, blood pressure increased, and hypertensive crisis, suggested a trend towards an increase with ensifentrine treatment with a risk difference of 1.1%, although the confidence interval did not exclude the null. On review of the blood pressure measurements obtained during the study visits, the postbaseline elevated readings of systolic blood pressure were similar between the treatment and placebo groups. Additionally, 0.2% (2/975) subjects in the ensifentrine treatment group listed hypertension as the reason for study drug discontinuation.

Hypotension, noted in the nonclinical studies, was reported as an AE in 0.5% (5/975) of subjects treated with ensifentrine but not reported in any subjects in the placebo group, leading to a risk difference of 0.5 with a 95% CI that did not exclude the null. Tachycardia, also noted in the nonclinical studies, was also only reported in five (0.5%) subjects in the ensifentrine group and two (0.3%) subjects in the placebo group. However, AEs included in the arrhythmias FMQ (include PTs: atrial fibrillation, atrial flutter, bradycardia, sinus tachycardia supraventricular extrasystoles, tachycardia, tachycardia paroxysmal, ventricular extrasystoles), appear to have occurred at a numerically higher rate in the ensifentrine group, 1.5% (15/975) subjects, compared to the placebo group, 1.0% (6/574) subjects. Review of blood pressure and heart rate measurements obtained during study visits did not reveal any apparent imbalanced in HR or blood pressure readings in the hypotensive range between treatment groups.

One subject with reported significant preexisting heart disease died during the treatment period (Trial 302: ensifentrine group; completed 33 days of treatment) with a listed cause of death of acute ventricular failure. Cardiac and vascular SAEs were balanced between treatment groups.

Overall, there were numerical increases in cardiac and vascular events in ensifentrine-treated subjects compared to placebo. The 95% Cis for these numerical differences did not exclude null and the clinical significance of this is unclear. The review of the cardiac and vascular events in the individual trials as well as the 48-week safety cohort was similar to the pooled analysis. In general, review of cardiac and vascular disorders is reassuring. See Section 7.7.1 for additional review.

COVID-19

Trials 301 and 302 both took place entirely during the COVID-19 pandemic. The Applicant designed the protocol such that patients with a positive COVID-19 test result indicting an active infection (at screening or randomization) or a COVID-19 related hospitalization within the 12 weeks prior to screening were excluded. Additionally, subjects with a positive COVID-19 test result indicating an active infection were required to discontinue the study medication and withdraw from the study.

Given that patients with COPD are at increased risk for respiratory viral infections such as COVID-19 and the potential variability in reporting preferred terms, an additional analysis of COVID-19 as a special safety signal of interest was performed and included the following PTs: COVID-19, asymptomatic COVID-19, COVID-19 pneumonia, and SARS-CoV-2 test positive.

Overall, the rates of reported COVID-19 AEs in the pooled studies (ensifentrine 4.4%, placebo 4.4%) were balanced between the treatment groups. Review of the COVID-19 safety data for the individual trials as well as the 48-week cohort was similar to the pooled analysis. There does not appear to be a safety concern related to COVID-19 based on this review.

Psychiatric Disorders

Psychiatric disorders are included in the Warnings and Precaution sections of roflumilast and other PDE inhibitors. The most commonly reported psychiatric AEs include insomnia, anxiety, and depression, but increased suicidal ideation and behavior, was also noted in the clinical trials for roflumilast (Roflumilast USPI 2011). As such, psychiatric disorders were included as a safety topic of interest for ensifentrine. The AEs related to Psychiatric Disorders for the Pooled Phase 3 Safety Database are summarized in Table 35.

Table 35. Trials 301 and 302, Adverse Events Related to Psychiatric Disorders, Pooled 24-Week Safety Database (SAS)

Psychiatric Disorders	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
AE grouping related to psychiatric disorders	12 (1.2)	2 (0.3)	0.9 (-0.1, 1.8)
Insomnia	6 (0.6)	2 (0.3)	0.3 (-0.7, 1.0)
Adjustment disorder with depressed mood	2 (0.2)	0	0.2 (-0.5, 0.7)
Depression	1 (0.1)	0	0.1 (-0.6, 0.6)
Major depression	1 (0.1)	0	0.1 (-0.6, 0.6)
Suicide attempt	1 (0.1)	0	0.1 (-0.6, 0.6)
Anxiety	2 (0.2)	1 (0.2)	0.0 (-0.8, 0.6)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: AE, adverse event; CI, confidence interval; ENS, ensifentrine; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo, SAS, safety analysis set

There were no psychiatric safety signals identified where the 95% CI excluded the null; however, there does appear to be a numerical increase in the number of events in the ensifentrine group [1.2% (12/975) subjects] compared to placebo [0.3% (2/597) subjects]. Review of the psychiatric events in the individual trials as well as the 48-week safety cohort were similar to the pooled analysis. We also note that there was one (0.1%) suicide attempt in the ensifentrine group in the phase 3 studies and one suicide in the phase 2 program (Trial RPL554-CO-203).

This potential trend towards an increase in psychiatric events, along with the two reported suicidal behaviors in the clinical development program, raises concern that, despite the inhaled route of inhalation, ensifentrine may increase psychiatric events compared to placebo. See Section 7.7 for additional review of this topic.

Syncope

While not specified as a safety signal of interest, syncope was reported in seven (0.7%) subjects in the treatment group but no subjects in the placebo group, leading to a risk difference (95% CI) that excluded the null: -0.7 (0.1, 1.5). While there were limited reported events of syncope, given the imbalance between the groups, these events may have been related to ensifentrine treatment however, as it occurred at a frequency < 1%, this was not included in in Section 6 of the USPI.

Other

Another potential imbalance identified in the pooled review of the reported AEs was arthritis. The FMQ analyzed for this included osteoarthritis, arthritis, rheumatoid arthritis, and spinal osteoarthritis. The risk difference between ensifentrine and placebo was 0.8, and while the confidence interval excluded the null (0.2, 1.6), the clinical relevance is not clear.

Analysis of the trials individually, as well as the review of the 48 Week cohort in Trial 301, did not reveal additional safety concerns (data not shown).

7.6.1.6. Laboratory Findings, Pooled Analyses, Trials 301 and 302

The Applicant conducted analyses of clinical laboratory results including chemistry and hematologic parameters. Analyses included the changes in mean values over time, changes in individual subjects over time, and potentially clinically significant values. There were no apparent clinically meaningful trends or mean changes from baseline observed in parameters of hematology and clinical chemistry. Postbaseline newly occurring or worsening potentially clinically significant values for hematology and clinical chemistry were infrequent and similar across groups.

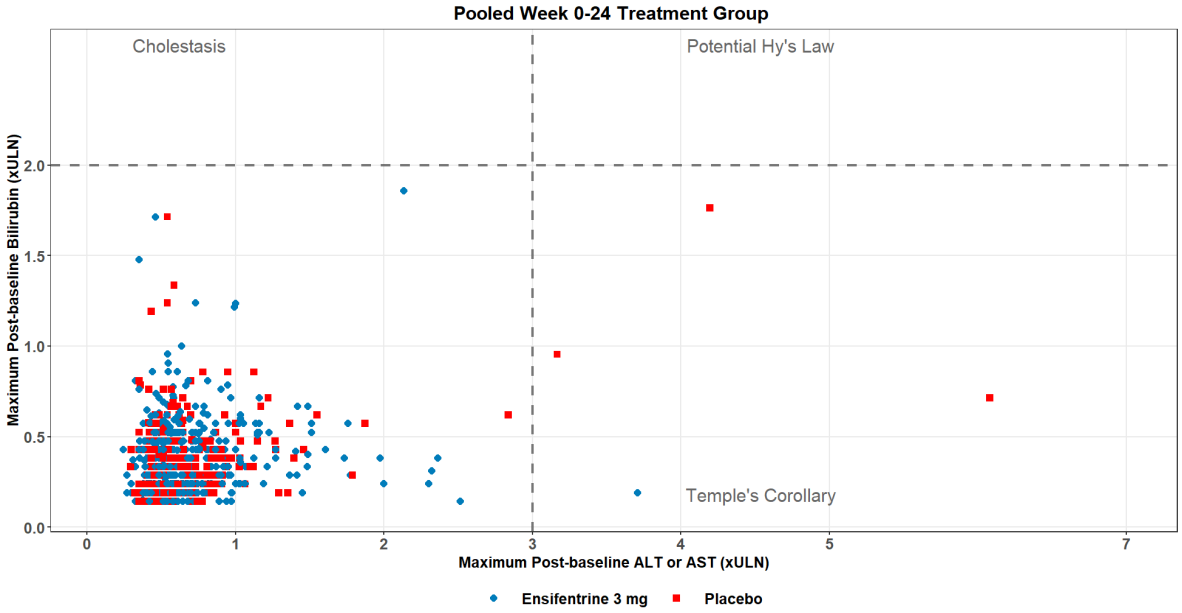
On review of kidney function, there was a higher rate of elevation in creatinine (≥1.5 times baseline) in the ensifentrine-treated group compared to placebo (risk difference [95% CI]: 1.6 [0.6, 2.8]). There were no differences noted between ensifentrine and placebo in creatinine increases ≥2x baseline or ≥3x baseline or in any increase in estimated glomerular filtration rate. Given the inconsistency in these results, these finding are unlikely to be of clinical significance.

Overall, no safety concerns were identified in the analysis of laboratory values.

7.6.1.7. Assessment of Drug-Induced Liver Injury, Pooled Analyses, Trials 301 and 302

There were no clinically meaningful trends or mean changes from baseline in the liver biochemistry values in the Pooled 24 Week Safety Database. Additional analysis of the shift from baseline was unrevealing. Hy's law screening analysis was reassuring and without overt concerning signal for potential cases of serious drug-induced liver injury as shown in Figure 5.

Figure 5. Trials 301 and 302, Hepatocellular Drug-Induced Liver Injury Screening Plot, Pooled 24-Week Safety Database (SAS)



Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adlb.xpt; Software: R
Each data point represents a subject plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.
A potential Hy's Law case was defined as having any postbaseline total bilirubin equal to or exceeding 2X ULN within 30 days after a postbaseline ALT or AST equal to or exceeding 3X ULN, and ALP less than 2X ULN (note ALP values are not circled). All subjects with at least one postbaseline ALT or AST and bilirubin are plotted. The within-30-days analysis window rule does not apply to cholestatic DILI and Temple's Corollary cases.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; SAS, safety analysis set; ULN, upper limit of normal

Overall, there was not an identified concern for drug-induced liver injury with ensifentrine treatment.

7.6.1.8. Vital Signs, Pooled Analyses, Trials 301 and 302

No clinically significant changes in mean values over time and shift analysis of vital signs (systolic and diastolic blood pressure, heart rate, and respiratory rate) were identified in the Pooled 24 Week Safety Database or 48-week safety cohort. Overall, no safety concerns were identified in the analysis of vital signs.

7.6.1.9. Subgroups, Pooled Analyses, Trials 301 and 302

A safety analysis by key demographic subgroup including age grouping, gender at birth, race, ethnicity, and region (U.S. and ex-U.S. sites) was performed. There were no significant differences noted between subgroups. A nominal trend was noted for an increase in AEs in the placebo group compared to ensifentrine with increased age, but the 95% confidence interval for the risk difference between the groups did not exclude the null.

7.6.2. Safety Results, Trial 301

The safety analysis based on Trial 301 was performed and consistent with the findings of the pooled phase 3 data.

7.6.3. Safety Results, Trial 302

The safety analysis based on Trial 302 was performed and consistent with the findings of the pooled phase 3 data.

7.7. Key Safety Review Issues

7.7.1. Cardiac and Vascular Disorders

Issue

Cardiac and vascular disorders were evaluated as safety topics of interest based on findings during the nonclinical development program for ensifentrine.

Background

In the nonclinical development program, ensifentrine treatment in dogs resulted in coronary artery vasculopathy associated with an increased heart rate and decreased blood pressure. A 6-week mechanistic inhalation toxicology study in dogs established a direct link between hemodynamic changes (i.e., increased heart rate and decreased blood pressure) and coronary artery vasculopathy at high dose. A no observed adverse effect level was identified at an inhaled dose of 0.3 mg/kg/day in dogs, at which there was no increase of heart rate, no decrease of blood pressure, and no coronary artery vasculopathy. While the no observed adverse effect level in dogs provided adequate nonclinical safety margins, cardiac and vascular disorders were evaluated as safety topics of interest.

Many patients with COPD will have concurrent cardiac conditions because COPD shares similar risk factors with these conditions. This was noted within the populations of the phase 3 trials, where hypertension was the most common condition reported in the Past Medical History, occurring in over 55% of subjects. A history of coronary artery disease was also commonly reported, albeit less frequently. The overlap of risk factors and occurrence of these conditions with COPD may complicate the analysis of the potential effects of a medication.

Assessment

A summary of selected cardiac and vascular safety analyses is shown in Table 36. See Section 7.6.1.5 for additional analysis.

Table 36. Trials 301 and 302, Selected Cardiac and Vascular Adverse Events by SOC and FDA Medical Query (Narrow), Pooled 24-Week Safety Database (SAS)

System Organ Class FMQ (Narrow)	ENS N=975 n (%)	PBO N=574 n (%)	Risk Difference (%) (95% CI)
Cardiac disorders			
Systemic hypertension	21 (2.2)	6 (1.0)	1.1 (-0.3, 2.4)
Arrhythmia	15 (1.5)	6 (1.0)	0.5 (-0.9, 1.6)
Tachycardia	5 (0.5)	2 (0.3)	0.2 (-0.8, 0.9)
Vascular disorders			
Hypotension	5 (0.5)	0	0.5 (-0.2, 1.2)

Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and placebo.
Abbreviations: CI, confidence interval; ENS, ensifentrine; FDA, U.S. Food and Drug Administration; FMQ, FDA medical query; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; PT, preferred term; SAS, safety analysis set

The safety analysis showed that the systemic hypertension FMQ was numerically more frequent in the ensifentrine-treated groups compared to placebo with a risk difference of 1.1, although the 95% CI includes the null. When evaluating tachycardia and hypotension, there also appears to be a numeric increase in the rates of tachycardia, tachyarrhythmias, and hypotension; however, the number of events was small, and the 95% CI of the risk difference included the null.

Trials 301 and 302 did not include formal major adverse cardiovascular event (MACE) analyses. However, there did not appear to be an increased risk for other cardiac or vascular disorders including myocardial ischemia/infarctions or cerebrovascular infarctions with ensifentrine treatment, although this may be limited by the time course of evaluation in the trials. The majority of the safety data available is limited to 24 weeks, but the review of the 48-week safety data from Trial 301 is consistent with the pooled analysis and remains reassuring.

Conclusion

Overall, the targeted safety review of cardiac and vascular events with ensifentrine treatment was reassuring. The rates of hypertension, hypotension, and tachycardia were numerically increased with ensifentrine treatment compared to placebo; however, the number of events were small, and the 95% CI of the risk differences did not exclude the null. The safety data does not support inclusion of tachycardia and hypotension in the USPI at this time; however, hypertension will be included in Section 6 because it occurred more commonly in the ensifentrine group with an incidence of ≥1%.

There was not an overt signal for increased risk of myocardial ischemic events or other events associated with coronary artery disease, although the analysis may be limited based on the time course and other confounding baseline risk factors between COPD and coronary artery disease. Overall, the targeted review of the cardiac and vascular disorders was reassuring.

7.7.2. Psychiatric Disorders

Issue

Psychiatric disorders including depression and – in some cases – suicidality are included in the Warnings and Precautions section of the US Prescribing Information on other PDE inhibitors, prompting psychiatric disorders to be included as a safety topic of interest. There were two episodes of suicidal behavior (one successful suicide in phase 2 and one suicide attempt in phase 3) within the development program of ensifentrine.

Background

Unlike other currently available PDE inhibitors such as roflumilast, ensifentrine is an inhaled medicine and there appears to be low systemic absorption of ensifentrine based on clinical pharmacokinetic (PK) studies conducted by the Applicant. In addition, the Applicant reports that there were no drug-related clinical signs or histological findings in animals administered ensifentrine by inhalation that would indicate a direct effect on the central nervous system. Due to this, it is unclear if the increase in psychiatric disorders seen with oral PDE inhibitors would be expected in patients treated with inhaled ensifentrine.

Depression, anxiety, and other psychiatric conditions are common in the general population and in patients with chronic conditions including COPD. In the phase 3 programs, the baseline rates of depression and anxiety were 9.2% and 5.0% respectively in Trial 301 and 22.4% and 17.9% respectively in Trial 302.

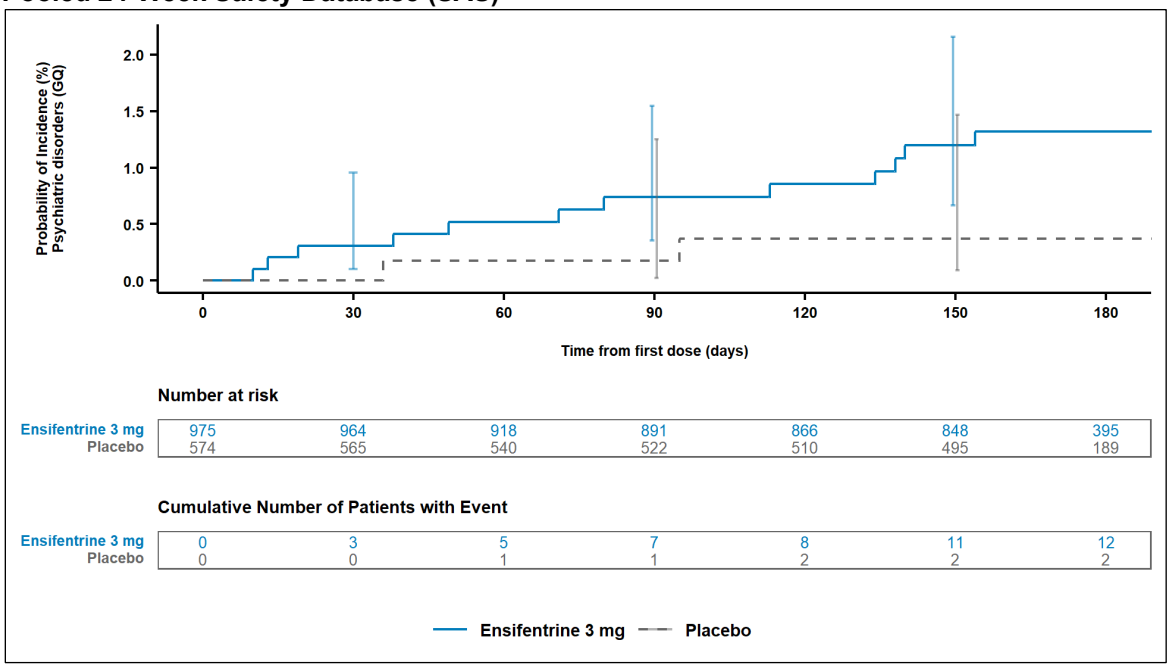
Assessment

On review of AEs related to psychiatric disorders, there is a numerical increase in the overall rate of psychiatric events with ensifentrine treatment compared to placebo, although the confidence interval does not exclude the null. Notably, the majority of events were limited to non-serious AEs, with the exception of one SAE of suicide attempt in phase 3.

The most common AE in this grouping was insomnia, noted in six (0.6%) subjects in the ensifentrine group and two (0.3%) subjects in the treatment group. The other conditions included a suicide, depression, adjustment disorder with depressed mood, and major depression – occurring in 0.4% (4/975) ensifentrine treated subjects and no subjects in the placebo group. Anxiety and a suicide attempt were also included in this analysis. See Section 7.6.1.5 for more details.

Figure 6 demonstrates the time to onset of AEs related to psychiatric disorders.

Figure 6. Trials 301 and 302, Time to Onset of Adverse Events Related to Psychiatric Disorders, Pooled 24-Week Safety Database (SAS)



Source: Clinical Data Scientist and Clinical Reviewer calculated and verified. Adae.xpt; Software: R
This figure depicts Kaplan-Meier estimates of the cumulative percentage of subjects that experience an incident (i.e., first) event by a given time point.
Abbreviations: GQ, grouped query; SAS, safety analysis set

As shown in Figure 6, with increased time, there appears to be a progressive separation between the ensifentrine group and the placebo group, although the low rate of events limits formal analysis in this setting. Analysis of the 48-week cohort did not reveal a significant number of events and the events that did occur appeared balanced between groups, which is reassuring.

Conclusion

In the review of the clinical development program for ensifentrine, there was a numerical increase in the number of psychiatric adverse events in the treatment group compared to placebo that included two serious events involving suicidality (one suicide in phase 2, one suicide attempt in phase 3). These data, in combination with the known psychiatric safety concerns included in the warnings and precautions section of other approved oral PDE inhibitors, support the inclusion of psychiatric adverse events in the warnings and precaution section of the USPI for ensifentrine.

8. Therapeutic Individualization

8.1. Intrinsic Factors

Age

The Applicant proposed no dose adjustment for geriatric patients (aged 65 years and older), and the review team agree that this approach is acceptable. In population PK analysis, age was not associated with significant difference in ensifentrine exposure. Furthermore, no overall differences in safety or effectiveness of ensifentrine were observed between subjects 65 years of age and older and younger adults.

Hepatic Impairment

The Applicant proposed no dose adjustment for subjects with hepatic impairment and is proposing to use ensifentrine with caution in subjects with hepatic impairment in the label. The review team agree that this approach is acceptable.

The Applicant conducted a dedicated hepatic impairment (HI) study (Study RPL554-PK-103) to evaluate ensifentrine PK in moderate and severe HI subjects compared to matched healthy subject controls. Results of this study showed that the systemic exposure to ensifentrine was higher (approximately 2.3-fold for C_{max} and $AUC_{0\text{-}inf}$) in moderate or severe HI subjects compared to matched control subjects with normal hepatic function; however, the free drug C_{max} of ensifentrine (<0.1 ng/mL or <0.2 nM) is lower than the in vitro half maximal inhibitory concentration values for PDE inhibition. Therefore, apparent PDE-related clinically relevant systemic pharmacological effect is expected to be less likely. In addition, population PK analysis did not identify markers of liver function (alanine aminotransferase, aspartate aminotransferase, bilirubin, and alkaline phosphatase) as a significant covariate for exposure of ensifentrine. Therefore, the observed increase in systemic exposure of ensifentrine in moderate or severe HI subjects observed in RPL554-PK-103 is not considered clinically relevant and does not warrant a dose adjustment.

Renal Impairment

The Applicant proposed no dose adjustment for subjects with mild or moderate renal impairment. Subjects with severe renal impairment have not been studied. The review team agree that this approach is acceptable.

After a 3 mg nebulized dose, urinary elimination of unchanged ensifentrine was negligible (<0.3% of the dose). A dedicated study with ensifentrine evaluating the effect of renal impairment on the PK of ensifentrine was not conducted. The effect of renal impairment on the exposure to ensifentrine for up to 24 weeks was evaluated in a population PK analysis. Estimated glomerular filtration rate varied from 25.5 to 191 mL/min, representing a range of moderate to no renal impairment. While continuous covariates of renal function did not show a significant correlation with ensifentrine exposure, categorical characterization of renal function indicated 25% reduction in the apparent clearance in subjects with moderate renal impairment. The pharmacokinetics of ensifentrine in severe renal impairment (creatine clearance <30 mL/min) or subjects with end-stage renal disease have not been evaluated.

8.2. Extrinsic Factors

Food Effect

Due to the route of administration (inhalation) of ensifentrine, the effect of food on ensifentrine bioavailability has not been studied because food is not expected to impact the delivery, performance, or PK of this inhaled drug product.

Drug Interactions

Effect of Ensifentrine on Other Drugs (Ensifentrine as Perpetrator)

In vitro studies show that ensifentrine does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4, breast cancer resistance protein or P-glycoprotein (P-gp), OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 or MATE2-K at therapeutically relevant concentrations. Based on these in vitro data, the potential of ensifentrine for drug-drug interactions (DDIs) as a perpetrator in the context of the expected clinical exposure (free drug C_{max} of 0.075 ng/mL; 0.15 nM) following the proposed dosing regimen of 3 mg BID regimen, suggests no clinically significant DDIs are expected. Maximal systemic exposures in humans (approximately 0.15 nM), are ≤0.1% of the half maximal inhibitory concentration values for inhibition of CYPs or transporters. Therefore, DDI potential for ensifentrine as a perpetrator is not expected at clinically relevant concentrations.

Effect of Other Drugs on Ensifentrine (Ensifentrine as Victim)

Based on in vitro studies, ensifentrine metabolism is primarily mediated by CYP2C9, with metabolism also via CYP2D6 to a lesser extent. Thus, a clinical DDI study was conducted with fluconazole, a moderate CYP2C9 inhibitor. The results of this DDI study with ensifentrine (using to-be-marketed formulation) dosed with steady-state CYP2C9 inhibitor, fluconazole, showed an increase in systemic exposure of ensifentrine (1.4-fold increase in C_{max} and 1.6-fold increase in $AUC_{0\text{-}inf}$). Based on population PK analysis, there were no significant effects of inhibitors of CYP2D6 on the PK of ensifentrine. Since the increase in ensifentrine exposure was less than 2-fold with concomitant administration of fluconazole (CYP2C9 inhibitor), no dose adjustment is needed when co-administering ensifentrine with CYP2C9 inhibitors.

8.3. Plans for Pediatric Drug Development

The Applicant has requested a full waiver of the Pediatric Research Equity Act requirements given that COPD almost exclusively involves an adult population (FDA DPMH 2024). This was agreed upon by the Division on 9/29/2020 and remains acceptable.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

Animal Data

The following nonclinical information was used in support of the drug's labeling. Additional details are available in Section 13.

Table 37. Nonclinical Data Supporting Labeling on Fertility, Pregnancy, and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	• In pregnant rats administered ensifentrine during organogenesis, ensifentrine did not cause adverse effects to the fetus at exposures up to 79 times the MRHDID. • In pregnant rabbits administered ensifentrine during organogenesis, ensifentrine did not cause adverse effects to the fetus at maternal exposures up to 9 times the MRHDID.
8.3 Females and Males of Reproductive Potential	• In a male rat fertility study, ensifentrine exposure caused atrophy/degeneration in the testis and intraluminal germ cell debris in the epididymis at exposures 13 times the MRHDID and greater. At ensifentrine exposures 30 times the MRHDID, male rats had decreased sperm motility and increased abnormal sperm morphology as well as decrease reproductive performance including decreased mating index and decreased fertility index, increased pre- and post-implantation loss, and decreased live embryos per litter in untreated females. The sperm counts, sperm motility, and sperm morphology as well as atrophy/degeneration in the testis and intraluminal germ cell debris in the epididymis were not present at the end of a 4-week treatment-free period. • In a female rat fertility study, ensifentrine had no effect on female fertility and reproductive performance indices up to 31 times the exposure at the MRHDID.
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	• In a pre- and postnatal development study in pregnant rats, no adverse effects were observed in the offspring exposed daily to ensifentrine from birth (in utero) through lactation at maternal pulmonary deposited doses up to approximately 79 times the MRHDID.

Source: Reviewer generated table
Abbreviation: MRHDID, maximum recommended human daily inhalation dose

Table 38. Reproductive Toxicity Safety Margins

Study	NOAEL (mg/kg)	AUC (ng·h/mL)	Exposure Margins[1] (Multiples)
FEED rat, male	5.75	136	12.7
FEED rat, female	18.1	334	31.2
EFD rat maternal and fetal	15.4	849	79.2
EFD rabbit maternal and fetal	12.4	92.8	8.7
PPND rat maternal and developmental	18.6	849	79.2

Source: Reviewer generated table
[1] Clinical exposure of ensifentrine in healthy subjects at a single inhalation dose of 3 mg low phosphate formulation dose (Study RPL554-PK-102. The geometric mean $AUC_{0\text{-}inf}$ following a single dose of 3 mg (page 59) is 5.361 (CV=54%) ng·h/mL. Multiply 5.361 by 2=10.722 ng*h/mL; that will be the daily AUC value following 3 mg BID at steady state).
Abbreviations: AUC, area under the concentration-time curve; $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; BID, twice daily; CV, coefficient of variation; EFD, embryo-fetal development; FEED, fertility and early embryonic development; h, hours; NOAEL, no observed adverse effect level; PPND, pre- and postnatal development

Clinical Experience

There were no clinical studies assessing pregnancy, lactation, or reproduction included in this application.

9. Product Quality

The Office of Pharmaceutical Quality (OPQ) review team has assessed NDA 217389 with respect to chemistry, manufacturing, and controls and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such OPQ recommends approval of this NDA from a quality perspective.

9.1. Device or Combination Product Considerations

Ensifentrine is a single medication administered through a standard jet nebulizer. There are no device or combination product considerations.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

Good Clinical Practice Compliance

The Applicant stated that the study was conducted in accordance with good clinical practices as described in Integrated Addendum to International Council for Harmonisation Guidelines EG (R2), Good Clinical Practice (November 2016). The study protocol and amendments, informed consent, and other necessary documents were reviewed and approved by an Independent Ethics Committee or an Institutional Review Board as appropriate. Written Informed Consent was obtained prior to study participation.

Data Quality Assurance

The Applicant conducted reviews of clinical site data/metrics to identify clinical sites with aberrant or implausible patterns using the following criteria:

- Higher than expected enrollment defined as ≥3 times that of the night highest enrolling sites in the study
- Screen failure rate of <10% based on Cenduit report for number screened, number randomized, and number screen failed
- Sites with an AE rate <0.2 based on randomized subjects
- Report from unblinded reviewer of PK data that a site has questionable results across multiple subjects

Sites that met 2 of 3 of the first three criteria or criteria #4 (note Trial 301 did not include criteria #4) were reviewed to determine if a request would be issued to review data at that site. As part of these reviews, the Applicant noted the following issues:

- Site 1721 (Trial 301) – Met criteria #2 and 3
- Site 1768 (Trial 301) – Met criteria #1, 2, and 3
- Site 1741 (Trial 302) – Met criteria #4

All PFTs and electrocardiograms were reviewed by independent experts at the sites which indicated significant findings suggesting widespread issues with data reliability and significant noncompliance with good clinical practice at these sites. Serious breach assessments were conducted, and it was determined that the good clinical practice noncompliance at the sites did not impact the scientific integrity of the study or pose risks to subject safety. Sites 1768 and 1741 were closed at the completion of the investigation and any remaining subjects were discontinued from the study; however, all subjects at Site 1721 had completed the study prior to the conclusion of the study. The Applicant discussed the removal of subjects with the Division prior to the finalization of the statistical analysis plan and database lock. This was considered a reasonable approach.

The Applicant also provided audit certificates from the sites that underwent audits (18 sites in Trial 301 and 22 sites in Trial 302).

Financial Disclosure

The Applicant adequately disclosed potential financial interests and arrangements by clinical investigators as recommended in the guidance for industry Financial Disclosure by Clinical Investigators (See Section 25 for more information). In addition, the Applicant stated that no Investigators/Sub-investigators participating in either trial were part-time or full-time employees.

11. Advisory Committee Summary

An advisory committee meeting or other external consultations were not held as part of this application.

III. Additional Analyses and Information

12. Summary of Regulatory History

The Applicant requested a Pre-IND meeting with the Agency in November 2016 under Pre-IND 133146 to discuss the development plans for a novel small molecule inhibitor of phosphodiesterase (PDE) 3 and PDE4 enzymes delivered via nebulization for the treatment of obstructive and inflammatory diseases of the respiratory tract. On January 12, 2017, the Agency shared Pre-IND feedback with the Applicant regarding concerns for the use of Peak forced expiratory volume in 1 second (FEV_1) as primary endpoint in their study and the FDA recommended using trough FEV_1 instead. The Applicant submitted their opening IND 133146 (RPL554/ensifentrine suspension) on March 9, 2017. The study was considered safe to proceed on April 11, 2017.

On March 20, 2018, a Written Response Only (WRO) document was issued in response to a Type C meeting request to discuss the Agency's advice relating to Chemistry, Manufacturing, and Controls (CMC) and nonclinical and clinical development of the Applicant's ensifentrine nebulizer formulation. FDA informed the Applicant of the following:

- Microscopic findings in the lungs and bronchi in vehicle-control animals in the 6-week inhalation toxicity studies conducted in rats and dogs raised potential safety concerns with respect to local toxicity in the lungs and respiratory tract. An assessment of the toxicity attributable to vehicle components was not possible in these studies based on the lack of an air-only comparator arm.
- Inhaled excipients in the proposed ensifentrine drug product formulations must be appropriately qualified for safety. In particular, any novel excipient for the inhalation route or an excipient that is delivered at a dose that exceeds levels in currently FDA-approved inhalation products will need to be qualified for safety.

On September 20, 2018, a Type C Guidance meeting was held to discuss the Applicant's study design plans for ensifentrine for qualification of the vehicle formulation(s) and to seek advice from the Agency relating to the clinical development while planning for phase 3 studies. The Division noted that in the 40-week dog study (Study #PL15XK), a no observed adverse effect level (NOAEL) was not established with respect to coronary artery vasculopathy. Further, NOAELs with respect to increased heart rate were also not established in either the 6- or 40-week dog studies. The Division stated that prior to clinical trials exceeding a duration of 6 weeks, the Applicant should conduct a mechanistic study with dogs that receive ensifentrine by inhalation to establish that increased heart rate and decreased blood pressure are responsible for the findings of coronary artery vasculopathy.

On May 9, 2019, the Agency issued a response to a Carcinogenicity Special Protocol Assessment. The Committee recommended doses of 0 (b) (4), 0.7, 2.2, and 7.0 mg/kg/day for both male and female rats. The high dose (for both males and females) was based on achieving an AUC ratio greater than 25-fold of the clinical exposure at the projected dose of 3 mg BID. The mid- and low- doses were selected based on approximately one third AUC spacing. The

Committee noted that the Applicant submitted a draft report for the 13-week dose range-finding study. The recommendations provided by the Committee were contingent on the results of the final report not being notably different from those in the draft report.

On November 27, 2019, written responses were provided to the Applicant in response to a Type C Guidance meeting request where the Applicant was seeking guidance on their proposal for maintaining the blind in phase 3 studies. FDA had concerns with the proposal to use a clear, colorless vehicle as placebo due to the potential for unblinding. FDA recommended the following: 1) the use a dose of the product that is clinically ineffective but visually similar to the dose planned for use in phase 3 trials as placebo or 2) the development of a placebo formulation that is similar in appearance to the drug product.

On May 11, 2020, the End of Phase 2 meeting minutes were provided discussing the development of ensifentrine as a nebulized suspension for the maintenance treatment of COPD. The nonclinical safety package, toxicology studies, and clinical pharmacology plan appeared adequate to support submission of an NDA but noted that the dose-ranging program with to-be-marketed formulation was limited. FDA continued to recommended trough FEV_1 as primary endpoint but reviewed that the FEV_1 $AUC_{0\text{-}12h}$ as a primary endpoint was not unreasonable. It was also communicated that if morning trough FEV_1 was not used as the primary endpoint, it would be factored into the efficacy assessments and it should be a key secondary endpoint in the statistical hierarchy. In addition, (b) (4) The Division also continued to note concerns regarding the lack of visually matching placebo and recommended the inclusion of assessments of subject perception of their treatment assignment in the phase 3 trails.

On June 8, 2020, the Applicant submitted their initial Pediatric Study Plan (iPSP) planning to request a waiver for pediatric studies. On September 29, 2020, an initial agreement for the iPSP was issued.

On January 6, 2023, written feedback was issued in response to a Type C meeting request. FDA reviewed safety topics of interest and found them to be acceptable. The Agency also requested additional analyses related to gastrointestinal, psychiatric, cardiovascular, and COVID associated adverse events. The FDA reviewed concerns (b) (4)

The Pre-NDA meeting was held on April 24, 2023, to present the phase 3 clinical safety and efficacy results as well as to obtain guidance from the Agency on the content and format for the future NDA. The Agency requested the Applicant submit all safety database data regardless of indication. The FDA agreed that the Applicant's completed program supported an NDA submission. Due the previously reviewed lack of visually matched placebo, it was reviewed that the Applicant should provide additional efficacy subgroup analyses on efficacy endpoints based on the subject perception assessments with analyses based on the concordant and discordant responses to treatment assignment. At this meeting, FDA also reviewed plans for exclusion of

subjects at sites with data reliability and Good Clinical Practice (GCP) noncompliance concerns that were considered acceptable. The Division's concerns regarding the ability of the data from the development program related to COPD exacerbations were also reiterated.

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

The Applicant has conducted a comprehensive program of pharmacology, pharmacokinetic (PK), and toxicology studies to support clinical development and the NDA submission of ensifentrine (referred to during development as RPL554). All pivotal toxicity studies have been submitted and reviewed previously under IND 133146. The impurities and extractables and leachables studies have been submitted and reviewed under NDA 217389. The key findings from the nonclinical studies are cited and summarized in this review to support the approval of NDA 217389.

Pharmacological studies demonstrated that ensifentrine inhibited both PDE3 and PDE4. In cell-free isolated enzyme assays, ensifentrine was relatively selective for PDE3 and PDE4 versus members of the other PDE enzyme families (PDE1, PDE2, PDE5, PDE7, PDE8, PDE9, PDE10, and PDE11). In cell-based assays, ensifentrine inhibited PDE3 and PDE4, leading to increased levels of cAMP.

The Applicant has conducted subchronic and chronic toxicology studies with ensifentrine in both rats and dogs. Target organs of toxicity were identified as mandibular and parotid salivary glands (acinar cell hypertrophy), testis (tubular degeneration/atrophy), and epididymis (intraluminal germ cell debris) in rats, and heart (coronary artery vasculopathy) in dogs. The nonclinical findings were not considered clinically dose-limiting except for the reproductive organ toxicities. The nonclinical general toxicology studies provided sufficient support for the clinical development program.

In animal reproduction studies, no adverse developmental effects were observed with inhalation administration of ensifentrine to pregnant rats and rabbits during organogenesis at maternal exposures up to 79 and 9 times, respectively, the exposure at the maximum recommended human daily inhalation dose (MRHDID). No adverse pre- or postnatal developmental effects were observed after inhaled administration of ensifentrine to pregnant rats from the period of organogenesis through lactation doses up to 79 times the MRHDID on an area under the concentration-time curve (AUC) basis. The male reproductive tract toxicity observed in the rat repeat dose toxicity studies resulted in impaired reproductive performance including decreased mating index and decreased fertility index, increased pre- and post-implantation loss, and decreased live embryos per litter in untreated females at 30 times the MRDHID.

Ensifentrine was not mutagenic or clastogenic in in vitro and in vivo assays. A two-year inhalation study in Han Wistar rats and a 6-month oral study in Tg.rasH2 transgenic mice were conducted to assess the carcinogenic potential of ensifentrine. No evidence of tumorigenicity was observed in male and female rats at an exposure approximately 40 times the MRHDID. No

evidence of tumorigenicity was observed in male and female Tg.rasH2 mice at oral doses up to 80 mg/kg/day, the highest dose tested.

To conclude, NDA 217389 is recommended for approval from the nonclinical perspective.

Pharmacology

Ensifentrine is a small molecule inhibitor of both PDE3 and PDE4. Ensifentrine inhibited recombinant enzyme PDE3 activity with subnanomolar potency. Inhibitory potency at PDE4 ranged from approximately 50 to 1479 nM. Ensifentrine at 1 µM was found to be relatively selective for PDE3 and PDE4 versus members of the other PDE enzyme families (PDE1, PDE2, PDE5, PDE7, PDE8, PDE9, PDE10, and PDE11). A series of in vitro and in vivo primary pharmacology studies were conducted to characterize the mechanism of action of ensifentrine. However, the Applicant did not conduct any in vivo pharmacology studies using COPD animal models. The in vivo pharmacology studies were primarily for allergic conditions as well as asthma. Selected in vivo primary pharmacology studies are reviewed in Section 13.2.

Safety Pharmacology

A complete battery of safety pharmacology studies has been conducted with ensifentrine. The ensifentrine half maximal inhibitory concentration (IC_{50}) at hERG channels expressed in HEK293 cells was calculated to be 5.62 µM. The effects of ensifentrine on central nervous system function were examined in a modified Irwin test included in a 28-day inhalation toxicity study in Wistar rats. There were no effects observed at up to 1.65 mg/kg/day. This dose resulted in a systemic exposure that was approximately 3-fold higher than the clinical exposure at 24 mg/day. There were no effects on respiration rate, tidal volume, or minute volume in a single dose IV study in dogs at up to 0.3 mg/kg. It is also noted that there were no effects on respiratory parameters in dogs in the 6-week inhalation toxicology study at up to 5.5 mg/kg/day. Decreased blood pressure and increased heart rate – expected pharmacodynamic (PD) effects of PDE3 inhibition – were observed in both rats and dogs. The magnitude of the effect sizes was dose dependent. There was no observed effect on QTc interval (QTcF or QTcB) in dogs.

Absorption, Distribution, Metabolism, Excretion/PK

A single inhalation dose of ensifentrine suspension formulation in dogs and rats resulted in lower systemic exposure compared to oral administration. The half-life ($t_{1/2}$) was generally less than 1 hour in both rodents and non-rodents. The bioavailability of ensifentrine was generally low, 5% in rats and 20% in dogs, following oral administration. In a QWBA study in rats, intravenous [^{14}C]-ensifentrine distributed primarily to the gastrointestinal tract. Radioactivity was not detectable in the brain or spinal cord in the rats. An in vitro plasma protein binding study (equilibrium dialysis) in which ensifentrine was tested at 0.1 and 1 µM showed comparable plasma protein binding in human, rat, and dog plasma (85 to 92%). Unchanged ensifentrine was the primary component in plasma in rats, dogs, and healthy human volunteers (96 to 99%).

General Toxicology

As listed in Table 39, general toxicity studies with ensifentrine were conducted in rats and dogs with dosing durations up to 6 months and 9 months, respectively. All the pivotal studies have been reviewed previously under IND 133146 *(DARRTS Reference ID: 3162851, 3624707,*

3666991, 3952011, 4173292, and 5056867). Key findings discussed in the previous reviews are summarized below.

Table 39. List of General Toxicology Studies Reviewed

General Toxicology Study	GLP	Report Number	Review Reference ID
Rats			
• RPL554: Toxicity study by inhalation administration to rats for 6 weeks followed by a 2-week recovery period • Doses/groups: 0, 2.0, 6.97, and 15.5 mg/kg/day; 10/sex/group	Yes	SKY0007	4081145
• RPL554: Toxicity study by inhalation administration with a (b) (4) formulation to Han Wistar rats for 13 weeks • Doses/groups: 0, 0.688, 2.15, and 6.53 mg/kg/day; 10/sex/group	Yes	TM17HG	4431580
• RPL554: Toxicity study by inhalation administration to Han Wistar rats for 26 weeks with male fertility followed by a 4-week recovery period • Doses/groups: 0, 2.08, 5.75, and 15.5 mg/kg/day; 2/sex/group	Yes	GV97FS	4431580
Formulation-bridging study: • RPL554: Toxicity study by inhalation administration with a (b) (4) and suspension formulation to Han Wistar rats for 2 weeks • Doses/groups: 0, 2.77 (b) (4) 7.91 (suspension), and 7.21 mg/kg/day; 10/sex/group	Yes	LF13VH	4457701
Dogs			
• RPL554: Toxicity study by inhalation administration to beagle dogs for 6 weeks followed by a 2-week recovery period • Doses/groups: 0, 0.63, 2.04, and 5.52 mg/kg/day; 3/sex/group	Yes	SKY0008	4081145
• RPL554: Toxicity study by inhalation administration to Beagle dogs for 40 weeks followed by a 4-week recovery period • Doses/groups: 0, 0.972, 1.66, and 3.60 mg/kg/day; 3/sex/group	Yes	PL15XK	5324803
MOA study: • RPL554: Mechanistic study to evaluate the relationship between incidence of decreased blood pressure, increased heart rate and vasculopathy following inhalation administration to Beagle dogs for 6 weeks • Doses/groups: 0, 0.3, and 5.9 mg/kg/day; 3/sex/group • Study protocol was FDA-reviewed (DARRTS Ref: 4391812)	Yes	BT61JW	5324803

Source: Reviewer generated table
Abbreviations: DARRTS, Document Archiving, Reporting, and Regulatory Tracking System; FDA, U.S. Food and Drug Administration; GLP, good laboratory practices; ID, identification; MOA, mechanism of action

Repeat Dose Toxicology Studies in Rats

6-Week Rat Study (SKY0007)

In a good laboratory practice (GLP) study, Wistar Han rats (10 animals/sex/group) were administered ensifentrine (suspension) once daily at 0 (vehicle), 2.0, 6.97, and 15.5 mg/kg/day

(achieved inhalation dose) for 6 weeks. An additional 5 animals/sex/group in the control and 15.5 mg/kg/day groups were included in the 2-week recovery study. Target organs of toxicity were testis (tubular degeneration/atrophy) and epididymis (reduced sperm and luminal cell debris). However, there was no clear dose-relationship of these findings. Multiple lesions in the larynx, lungs & bronchi, and tracheal bifurcation were observed with comparable frequency between vehicle controls and animals administered 15.5 mg/kg/day. Due to the lack of an air-only control group, it is unclear if these findings are attributable to the vehicle formulation or represent background lesions. The NOAEL was identified as 15.5 mg/kg/day, associated with an AUC of 1,965 ng·hr/mL.

13-Week Rat Study (CC36VY)

In a GLP study, Wistar Han rats (10 animals/sex/group) were administered ensifentrine (b) (4) once daily at 0 (vehicle), 0.688, 2.15, and 6.53 mg/kg/day (achieved inhalation dose) for 13 weeks. Target organs of toxicity were the mandibular and parotid salivary glands (acinar cell hypertrophy) observed at all doses. These effects are not considered dose-limiting. Therefore, the high dose of 6.53 mg/kg/day, associated with an AUC of 338 ng·hr/mL, was used for the safety assessment.

26-Week Rat Study (GV97FS)

In a GLP study, Wistar Han rats (12 animals/sex/group) were administered ensifentrine (suspension) by inhalation once daily at 0 (vehicle), 2.08, 5.75, and 15.5 mg/kg/day (achieved doses) for 26 weeks with additional animals included in the control and 15.5 mg/kg/day groups for a 4-week recovery period. Target organs of toxicity were the mandibular and parotid salivary glands (acinar cell hypertrophy at all doses in males and females), testis (tubular degeneration/atrophy at 5.75 and 15.5 mg/kg/day), and epididymis (intraluminal germ cell debris at the 5.75 and 15.5 mg/kg/day). The study also incorporated a male fertility assessment following 10 weeks of dosing. The male reproductive tract toxicity in the high dose group resulted in impaired reproductive performance including decreased mating index and decreased fertility index, increased pre- and post-implantation loss, and decreased live embryos per litter in untreated females. The NOAEL was determined to be 5.75 mg/kg, associated with an AUC of 585 ng·hr/mL. The male reproductive tract toxicity of ensifentrine is consistent with other approved PDE4 inhibitors (e.g., roflumilast (oral tablets)). Rats are highly sensitive to PDE4 inhibitor-induced male reproductive tract toxicity. The clinical relevance of these findings is not clear.

(b) (4) **Bridging Study (LF13VH)**

In this 2-week GLP inhalation toxicology study, rats received ensifentrine at achieved doses of: 0 (b) (4) 2.77 (b) (4) 7.91 (suspension), and 7.21 (b) (4) mg/kg/day. Target organs of toxicity were the mandibular and parotid salivary glands (acinar cell hypertrophy) observed at all doses. Minimal to severe tubular degeneration/atrophy in the testes associated with the presence of epididymal cell debris was observed in males from control and all treatment groups. Vehicle-related findings in the larynx were observed in males and females from the suspension formulation group. The larynx findings were considered rat specific (i.e., nonspecific irritant effects associated with (b) (4)

with little or no relevance to humans. Based on the similarity of findings between the prior suspension and [redacted] formulation, the Applicant has established a scientific bridge between the two formulations that [redacted]

Repeat Dose Toxicology Studies in Beagle Dogs

6-Week Dog Study (SKY0008)

In a GLP study, beagle dogs (three animals/sex/group) were administered ensifentrine (suspension) once daily at 0 (vehicle), 0.63, 2.04, and 5.52 mg/kg/day (achieved inhalation dose) for 6 weeks. An additional two animals/sex/group in the control and 5.52 mg/kg/day groups were included in the 2-week recovery study. Mean body weight gain was decreased in the 5.52 mg/kg/day group. Dose-dependent increase of heart rate was observed in all treatment groups. Coronary artery vasculopathy was observed in the 2.04 and 5.52 mg/kg/day groups. These findings were considered to be clinically dose-limiting. There were no effects of treatment on respiratory parameters (respiratory rate, tidal volume, minute volume). The NOAEL was defined as 0.63 mg/kg/day, associated with an AUC of 34.45 ng·hr/mL.

6-Week Mechanistic Inhalation Toxicology Study (BT61JW)

In a GLP study, beagle dogs (three animals/sex/group) were administered ensifentrine once daily at 0 (vehicle), 0.3, and 5.9 mg/kg/day (achieved inhalation dose) for 6 weeks. The 6-week mechanistic inhalation toxicology study in dogs established a direct link between hemodynamic changes (i.e., increased heart rate and decreased blood pressure) and coronary artery vasculopathy at the high dose of 5.9 mg/kg/day. A NOAEL was identified at the dose of 0.3 mg/kg/day in dogs where there was no increase of heart rate, no decrease of blood pressure, and no coronary artery vasculopathy. The vasodilator-induced cardiovascular lesions in dogs attributed to anatomical differences in the coronary circulation are of questionable clinical relevance (Mesfin et al. 1989).

40-Week Dog Study (PL15XK)

In a GLP study, beagle dogs (four animals/sex/group) were administered ensifentrine (suspension) once daily at 0 (vehicle), 0.972, 1.66, and 3.60 mg/kg/day (achieved inhalation dose) for 40 weeks. An additional two animals/sex/group in the control and 3.60 mg/kg/day groups were included in the 4-week recovery study. The target organ of toxicity was the heart. In the heart, coronary artery vasculopathy was observed in both males and females in all treated groups. This vasculopathy was mainly characterized by medial hypertrophy but also included areas of vacuolation and mucinous degeneration in some vessels. The intramural vessels of the left ventricle including the papillary muscle were predominantly affected. The coronary artery vasculopathy findings were associated with hemodynamic changes (i.e., increased heart rate and decreased blood pressure). The high dose (3.60 mg/kg/day, AUC: 278.5 ng·h/mL) was used for safety margin calculations because the findings were not considered dose-limiting based on the weight of evidence (see summary of Study BT61JW, above).

Ensifentrine Toxicokinetics and Exposure Margins

Adequate exposure margins between the nonclinical general toxicology studies and proposed human doses of ensifentrine were obtained throughout the clinical development program.

Table 40. Summary of Ensifentrine Exposures in Repeat-Dose Toxicity Studies

Study	NOAEL (mg/kg)	AUC (ng·h/mL)	Systemic Exposure Margin[b]	Local (Lung) Margin[c] PDD (mg/g LW)	Local (Lung) Margin[c] Safety Margin[d]
26 weeks, rat, inhalation	5.75	209.5	20	0.096	16
40 weeks, dog, inhalation	3.6[a]	278.5	26	0.11	18
6 weeks, dog, MOA, inhalation	0.3	22.93	2.1	NA[e]	NA

Source: FDA Pharmacology Toxicology Reviewer table
[a] Increased heart rate and coronary artery (CA) vasculopathy at ensifentrine doses of 0.972 mg/kg/day and higher
[b] Clinical exposure of ensifentrine at a dose of 6 mg (3 mg BID) at the steady-state (DDI study RPL554-PK-102, 6 mg: $AUC_{0\text{-}24h}$=10,722 pg·h/mL, the geometric mean $AUC_{0\text{-}inf}$ following single dose 3 mg (page 59) is 5361 (CV=54%) pg·h/mL)
[c] 10% deposition factor for rats, 25% deposition factor for dogs. Lung weights for a 250 g rat, 10 kg dog, and 60 kg human are approximately 1.5, 110, and 1000 grams, respectively (Tepper et al. 2016).
[d] Human PDD=0.006 mg/g LW/d
[e] This study was designed to assess a systemic toxicity issue. Therefore, lung safety margins were not calculated.
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}24h}$, area under the concentration-time curve from time 0 to 24 hours; BID, twice daily; CA, coronary artery; CV, coefficient of variation; DDI, drug-drug interaction; FDA, U.S. Food and Drug Administration; h, hours; LW, lung weight; MOA, mechanism of action; NA, not applicable; NOAEL, no observed adverse effect level; PDD, pulmonary deposited dose

Genetic Toxicology

Ensifentrine was negative for genotoxicity in the following assays: in vitro Ames test for bacterial gene mutation, in vivo comet test (lung and liver) with rats, and in vivo micronucleus assay with mice.

Carcinogenicity

In the 2-year GLP carcinogenicity study, Wistar Han rats were administered 0 (b) (4) 0.74, 2.3, and 7.0 mg/kg/day ensifentrine by inhalation. The Applicant did have concurrence from the Executive Carcinogenicity Assessment Committee for dose selection. There were no statistically significant treatment-related tumor findings in male or female rats. Non-neoplastic target organs of toxicity were the mandibular and parotid salivary glands (diffuse hypertrophy of the acinar cells). Ensifentrine was not tumorigenic in male and female rats. There is a 40-fold exposure margin between the AUC at the NOAEL (no tumors, 7 mg/kg/day) in the 2-year rat inhalation carcinogenicity study and the clinical dose of 6 mg/day (3 mg twice daily [BID]).

Table 41. Summary of Exposure Margins for the Two-Year Rat Inhalation Carcinogenicity Study

				Local (Lung) Margin	
Species	Dose (mg/kg/day)	$AUC_{0\text{-}24\ h}$ (ng·h/mL)	Systemic Exposure Margin[b]	PDD (mg/g LW)	Safety Margin[c]
Rat[a]	0.74	BLD	NA	0.12	2.0
	2.3	234	21.8	0.038	6.3
	7.0 (NOAEL)	426	39.7	0.117	19.5

Source: FDA Pharmacology Toxicology Reviewer table
[a] Toxicokinetic sampling occurred at Week 26
[b] Clinical exposure of ensifentrine in COPD subjects at dose of 6 mg (3 mg BID) at the steady state (DDI study RPL554-PK-102, 6 mg: $AUC_{0\text{-}24h}$=10722 pg·h/mL, the geometric mean $AUC_{0\text{-}inf}$ following a single dose of 3 mg (page 59) is 5361 (CV=54%) pg·h/mL)
[c] Human PDD=0.006 mg/g LW/d, assuming 100% pulmonary deposition of an achieved inhaled dose of 3 mg BID
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}24h}$, area under the concentration-time curve from time 0 to 24 hours; BID, twice daily; BLD, below the limit of detection; COPD, chronic obstructive pulmonary disease; CV, coefficient of variation; DDI, drug-drug interaction; FDA, U.S. Food and Drug Administration; h, hours; LW, lung weight; NA, not applicable; NOAEL, no observed adverse effect level; PDD, pulmonary deposited dose

In the 6-month GLP carcinogenicity study, transgenic rasH2 mice were administered 0 (b) (4) aqueous sodium chloride containing (b) (4) polysorbate (b) (4) 7, 28, and 80 mg/kg/day ensifentrine by oral gavage. The Applicant did have concurrence from the Executive Carcinogenicity Assessment Committee for dose selection. There were no statistically significant treatment-related tumor findings in male or female mice. All animals in the positive control group (b) (4) were observed with neoplastic findings. Non-neoplastic target organs of toxicity were the kidney (hyaline casts), mammary (ducts dilatation), stomach (mucus cell hypertrophy and mucus/submucosal inflammatory cell infiltrate in glandular regions), and uterine cervix (inflammatory cell infiltrate). Ensifentrine was not tumorigenic in male and female transgenic rasH2 mice.

Table 42. Summary of Exposure Margins for the Tg.rasH2 Transgenic Mouse Oral Carcinogenicity Study

Species	Dose (mg/kg/day)	$AUC_{0\text{-}24\ h}$ (ng·h/mL)	Exposure Margin[b]
Mouse[a]	7	NR	NA
	28	2350	220
	80 (NOAEL)	6340	591

Source: FDA Pharmacology Toxicology Reviewer table
[a] Toxicokinetic sampling occurred at Week 4
[b] Clinical exposure of ensifentrine in COPD subjects at dose of 6 mg (3 mg BID) at the steady state (DDI study RPL554-PK-102, 6 mg: $AUC_{0\text{-}24h}$=10722 pg·h/mL, the geometric mean $AUC_{0\text{-}inf}$ following a single dose of 3 mg (page 59) is 5,361 (CV=54%) pg·h/mL)
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}24h}$, area under the concentration-time curve from time 0 to 24 hours; BID, twice daily; COPD, chronic obstructive pulmonary disease; CV, coefficient of variation; DDI, drug-drug interaction; FDA, U.S. Food and Drug Administration; h, hours; NA, not applicable; NOAEL, no observed adverse effect level; NR, not reported

Reproductive Toxicology

The following GLP studies were submitted and reviewed previously.

Table 43. List of Reproductive and Developmental Toxicology Studies Reviewed

Reproductive and Developmental Toxicology Studies	Report Number	Review Reference ID
RPL554: Study for effects on fertility and early embryonic development in the female Han Wistar rat by inhalation administration Doses/groups: 0, 2.07, 6.46, and 18.1 mg/kg/day; 22 females/group	DW07LD	5343302
RPL554: Toxicity study by inhalation administration to Han Wistar rats for 26 weeks with male fertility followed by a 4-week recovery period Doses/groups: 0, 2.08, 5.75, or 15.5 mg/kg/day; 12 males/group	GV97FS	5343302
RPL554: Study of effects on embryo-fetal development in the Han Wistar rat by inhalation administration Doses/groups: 0, 2.05, 5.64, and 15.4 mg/kg/day; 22 females/group	XC09SK	5343302
RPL554: Study of effects on embryo-fetal development in the New Zealand white rabbit by inhalation administration Doses/groups: 0, 2.07, 5.16, and 12.4 mg/kg/day; 22 females/group	PL12GF	5343302
RPL554: Study for effects on pre- and postnatal development in the Han Wistar rat by inhalation administration Doses/groups: 0, 2.04, 5.94, and 18.6 mg/kg/day; 22 females/group	8438109	5343302

Source: FDA Pharmacology Toxicology Reviewer Table
Abbreviations: FDA, U.S. Food and Drug Administration; ID, identifier

Fertility Studies

In a male fertility study integrated into the 26-week GLP repeat dose inhalation toxicity study (GV97FS), ensifentrine was administrated to male rats at inhalation doses of 0 (vehicle), 2, 6, and 16 mg/kg/day (4, 13, and 30 times the exposure at the MRHDID) for 10 weeks prior to mating with untreated females. Male rats had decreased sperm motility and increased abnormal sperm morphology at an inhalation dose of 16 mg/kg/day (approximately 30 times the MRHDID). Decreased sperm counts in the testis were observed at all doses. Atrophy/degeneration in the testis and intraluminal germ cell debris in the epididymis were observed at doses of 6 and 16 mg/kg/day (4 and 13 times the exposure at the MRHDID, respectively). Additional adverse effects at 16 mg/kg/day on reproductive performance included decreased mating index and decreased fertility index, increased pre- and post-implantation loss, and decreased live embryos per litter in untreated females. No developmental toxicity was observed in rats at 6 mg/kg/day (13 times the exposure at the MRHDID). The sperm counts, sperm motility, and sperm morphology were reversible at the end of a 4-week treatment-free period. Atrophy/degeneration in the testis and intraluminal germ cell debris in the epididymis were not present at the end of a 4-week treatment-free period. In conclusion, the NOAEL for

fertility in this study was determined to be 6 mg/kg/day in males ($AUC_{0\text{-}24h}$, 136 ng·h/mL). It is noted that ensifentrine induced reproductive tract toxicity (i.e., tubular degeneration/atrophy in the testes and intraluminal germ cell debris in the epididymis) in both 6-week and 6-month inhalation toxicity studies in rats. Given that (1) these findings at all doses did not appear to progress from 6-week to 6-month, (2) they were reversible at the end of 14-day or 4-week recovery period, and (3) the low dose and mid dose in the fertility study did not induce any adverse functional toxicities of mating and fertility performances, the mid dose of ensifentrine at 6 mg/kg/day in the fertility study was used for safety margin calculations.

Rats are highly sensitive to PDE4 inhibitor-induced male reproductive tract toxicity. However, the relevance of these findings to humans remains unclear. Roflumilast is an oral PDE4 inhibitor that was approved in 2011 as a treatment for COPD. In a 4-month study in rats, testicular atrophy was observed in all study groups including controls. The incidence of this finding increased with dose. Such findings in the testis were not reported in a 6-month rat study. A 3-month study in juvenile hamsters identified testicular tubular atrophy with 80 to 100% incidence in controls and all dose groups with no evidence of a dose-response effect. Tubular degeneration of the testis was also observed at the highest doses tested in dogs in studies of 1- and 6-month duration.

In a GLP female fertility study (DW07LD), ensifentrine was administrated to female rats at achieved inhalation doses of 2.07, 6.46, and 18.1 mg/kg/day from 2 weeks prior to mating to 7 days after mating. Ensifentrine had no effect on female fertility and reproductive performance indices up to 18.1 mg/kg/day (31 times the exposure at the MRHDID). In conclusion, the NOAEL for fertility in this study was determined to be 18.1 mg/kg/day in females ($AUC_{0\text{-}24h}$, 334 ng·h/mL).

Embryofetal Development Studies

In a GLP embryo-fetal development study (XC09SK), pregnant rats were administered ensifentrine at achieved inhalation doses of 2.05, 5.64, and 15.4 mg/kg/day (8, 33, and 79 times the exposure at the MRHDID) during the period of organogenesis from gestation days 6 to 17. Ensifentrine did not cause adverse effects to the fetus at exposures up to 79 times the MRHDID (on an AUC basis at a maternal inhalation dose of 15.4 mg/kg/day). In conclusion, the NOAEL for maternal and embryo/fetal toxicities was determined to be 15 mg/kg/day ($AUC_{0\text{-}24h}$, 849 ng·hr/mL) in rats.

In a GLP embryo-fetal development study (PL12GF), pregnant rabbits were administered ensifentrine at achieved inhalation doses of 2.07, 5.16, and 12.4 mg/kg/day (2, 2, and 9 times the exposure at the MRHDID) during the period of organogenesis from gestation days 6 to 19. Ensifentrine did not cause adverse effects to the fetus at maternal exposures up to 9 times the MRHDID. In conclusion, the NOAEL for maternal and embryo/fetal toxicities was determined to be 12 mg/kg/day ($AUC_{0\text{-}24h}$, 92.8 ng·hr/mL) in rabbits.

Pre- and Postnatal Development Study

In a GLP pre- and postnatal development study (8438109), pregnant rats were administered ensifentrine at achieved inhalation doses of 2.04, 5.94, and 18.6 mg/kg/day (approximately 8, 33, and 79 times the exposure at the MRHDID) from gestation day 6 to lactation/post-partum day 24. No adverse effects were observed in the offspring at maternal pulmonary deposited doses

up to approximately 79 times the MRHDID. In conclusion, the NOAEL for maternal and developmental toxicities was determined to be 19 mg/kg/day (849 ng·hr/mL, $AUC_{0\text{-}24h}$ from embryo-fetal development study XC09SK) in rats. Toxicokinetic analysis was not conducted.

Table 44. Ensifentrine Exposure Margins in Reproductive and Development Toxicity Studies

Study	Species	Dose (mg/kg)	$AUC_{0\text{-}24h}$ (ng·h/mL)	Exposure Margin[3]
FEED[1]	Male rat	2.08	41.2	3.8
		5.75 (NOAEL)	136	12.7
		15.5	324	30.2
	Female rat	2.07	52.2	4.9
		6.46	226	21.1
		18.1 (NOAEL)	334	31.2
EFD	Rat	2.05	84.1	7.8
		5.64	355	33.1
		15.4 (NOAEL)	849	79.2
	Rabbit	2.07	16	1.5
		5.16	17.5	1.6
		12.4 (NOAEL)	92.8	8.7
PPND[2]	Rat	2.04	84.1	7.8
		5.94	355	33.1
		18.6 (NOAEL)	849	79.2

Source: FDA Pharmacology Toxicology Reviewer Table
[1] AUCs of ensifentrine in the rat FEED study are assessed at Week 6 in the 26-week inhalation toxicology study.
[2] AUCs of ensifentrine in the rat PPND study are estimated from the EFD study in rats. Human proposed dose=0.1 mg/kg (6 mg/60 kg).
[3] Clinical exposure of ensifentrine in healthy subjects at a single inhalation dose of 3 mg low phosphate formulation dose (Study RPL554-PK-102. The geometric mean $AUC_{0\text{-}inf}$ following a single dose of 3 mg (page 59) is 5.361 (CV=54%) ng·h/mL. Multiply 5.361 by 2=10.722 ng·h/mL; that will be the daily AUC value following 3 mg BID at steady state).
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; BID, twice daily dosing; CV, coefficient of variation; EFD, embryofetal development; FDA, U.S. Food and Drug Administration; FEED, fertility and early embryonic development; h, hours; NOAEL, no observed adverse effect level; PPND, pre- and postnatal development

Referenced NDAs, BLAs, DMFs

None.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

Table 45. Nonclinical Studies Reviewed in the NDA Submission

Study	Study Report ID(s)/Section ID(s)/Location
In Vivo Pharmacology	
Bronchoprotective effects: The pharmacology of RPL554-final report, Effects of the mixed phosphodiesterase 3/4 inhibitors VMX554 and VMX565 on histamine induced bronchoconstriction in anaesthetised guinea pig; The pharmacology of RPL554: In vivo guinea pig data with aerosolized RPL554; Effect of RPL554 on allergic and methacholine induced bronchoconstriction in primates	VRP080118, VRP010301, VRP080121, VRP080125
Bronchodilator effects: RPL554, a dual phosphodiesterase (PDE) 3/4 inhibitor acts synergistically with muscarinic receptor antagonists and beta-adrenoceptor agonists in vivo.	(Keir and Page 2014)
Anti-inflammatory effects: The Pharmacology of RPL554 The anti-inflammatory effect of RPL554 in sensitised guinea pigs	VRP080118, VRP080429, VRP080120
Secondary Pharmacology	
In vitro pharmacology and ADME-tox, Study of RPL554	VRP071210
Impurities	3.2.S.3.2 Impurities
Extractables and leachables	3.2.P.2.4 Container Closure System; 1.11.2 Nonclinical Information Amendment to IR response Reference ID: 5250391

Source: Reviewer generated table
Abbreviations: ADME-tox, absorption, distribution, metabolism, and excretion-toxicology; ID, identifier; PDE, phosphodiesterase

In Vivo Pharmacology

The Applicant conducted exploratory in vivo pharmacology studies for allergic rhinitis and asthma. Ensifentrine showed bronchoprotective, bronchodilatory, and anti-inflammatory effects in these models.

In a histamine-induced bronchoconstriction model in guinea pigs (VRP080118 and VRP010301), inhalation administration of ensifentrine showed dose-dependent bronchoprotection against histamine-induced bronchoconstriction up to 5 hours. In an acetylcholine-induced airway obstruction model in guinea pigs (VRP080121), inhalation administration of ensifentrine showed a dose-dependent bronchoprotection against airway obstruction induced by acetylcholine. In a methacholine- and allergen-induced bronchoconstriction model in monkeys (VRP080125), ensifentrine reduced the effects of threshold doses of methacholine and allergen on peak inspiratory flow, tidal volume and breathing rate, dynamic compliance and resistance, maximum esophageal pressure, and oxygen saturation.

In a bombesin-induced airway obstruction model in guinea pigs, intravenous administration of ensifentrine showed dose-dependent relaxation of guinea pig airways as measured by lung resistance (Keir and Page 2014).

In ovalbumin-challenged guinea pigs (VRP080118, VRP080429, VRP080120), inhalation administration of ensifentrine inhibited the recruitment of eosinophils to the lungs.

Secondary Pharmacology

In a CEREP panel by broad ligand profiling in various in vitro receptor binding, functional, and enzyme assays, ensifentrine (10,000 nM) inhibited binding to the benzodiazepine ([central]), muscarinic 3 receptor (M3, human), κ-opioid receptor, and Ca^{2+} channel (L, diltiazem site, benzothiazepines) in the range of 53 to 63%. No significant binding was observed in >100 other receptors, channels, and enzymes evaluated. Ensifentrine (10,000 nM) had no significant agonist or antagonist effects on human α2, β1, or β2 adrenergic receptors.

Impurities/Degradants

The Applicant followed the recommendations of the International Council for Harmonisation (ICH) Guidance for Industry: M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk (March 2018) and evaluated the mutagenic potential of impurities based on literature, internal databases, and in silico assessment including Leadscope Genetox Statistical Models and Derek Nexus. Overall, the proposed impurity specifications are acceptable and there are no outstanding nonclinical safety concerns.

Six potential mutagenic impurities of concern were identified in the drug substance Table 46. They were controlled appropriately per ICH M7.

Table 46. Potential Mutagenic Impurities, Mutagenic Impurity Control, and Batch History

No.	pMGI/MGI	ICH M7 Class	Stage Measured	Detection Limit (ppm)	Measured Concentration in any batch to date[a]
1	(b) (4)	1	B, C, D, E	(b) (4)	ND
2		2	C, D		ND
3		3	Not observed[d]		Not observed[d]
4		3	Not observed[d]		Not observed[d]
5		3	Not observed[d]		Not observed[d]
6		3	Not observed[d]		Not observed[d]

ND=not detected; pMGI=potential mutagenic impurity.
a. registration and clinical batches.
b. (b) (4)
c.
d.

Source: Excerpted from Applicant's submission, NDA-217389-SD1- M3.2.S.3.2.-pg22
Abbreviations: ICH, International Conference on Harmonisation; MGI, mutagenic impurity; ND, not detected; No., number; pMGI, potential mutagenic impurity

The ensifentrine manufacturing process [redacted] (b) (4)

Other identified impurities are appropriately controlled per ICH Q3A, ICH Q3B, or United States Pharmacopeia Guidelines.

Extractables and Leachables

The Applicant conducted a comprehensive evaluation of extractables and leachables based on literature/databases and in silico assessment including Derek Nexus and Leadscope. Overall, the proposed extractables and leachables specifications are acceptable and there are no outstanding nonclinical safety concerns.

Three leachables [redacted] (b) (4) are present at higher than [redacted] (b) (4) but less than [redacted] (b) (4) They are not genotoxic. There are adequate safety margins to the proposed maximum human dose.

Table 47. PDE and Safety Margins for [redacted] (b) (4)

Leachable	Inhalation PDE (µg/day)	Maximum Potential Daily Exposure [a] (µg/day)	Margin of Safety [b]
[redacted] (b) (4)			

Source: Excerpted from Applicant's submission, NDA-217389-SD1-IR response-1.11.2 Nonclinical Information Amendment-p 3
[a] Maximum potential daily exposure for ensifentrine inhalation suspension considering a 5 mL daily dose.
[b] Margin of safety=PDE (µg/day)/maximum daily exposure (µg/day)
Abbreviations: p, page; PDE, permitted daily exposure

14. Clinical Pharmacology

Overall, ensifentrine is intended to be administered as a suspension via a nebulizer allowing for extended retention in the lung – the pharmacological site of action – thereby minimizing systemic exposure (maximum plasma concentration [C_{max}]). Therefore, plasma levels of ensifentrine do not predict the therapeutic effect. After administration of a single 6 mg oral inhalation of ensifentrine in high phosphate suspension, 10.4% of the observed systemic exposure (AUC) was attributed to absorption in the gastrointestinal tract.

The clinical data combined with the in vitro drug-drug interaction (DDI) data for ensifentrine indicates a lack of clinically relevant DDIs with concomitant medications administered in the COPD population.

The dose selection of 3 mg BID for pivotal phase 3 studies is supported by the results from the dose ranging study (Study RPL554-CO-205, see Section 6.1), in which ensifentrine demonstrated a dose-dependent improvement of forced expiratory volume in 1 second (FEV_1) change from baseline in subjects with moderate to severe COPD when compared to placebo.

A dedicated hepatic impairment study demonstrated that ensifentrine C_{max} and $AUC_{0\text{-}inf}$ were approximately 2.3-fold higher in subjects with moderate or severe hepatic impairment (HI) when compared to matched controls. Ensifentrine exposure appeared to be similar between moderate and severe HI subjects. Given that the free drug C_{max} (<0.1 ng/mL or <0.2 nM) of ensifentrine following 3 mg inhalation is lower than the IC_{50} values for PDE inhibition and that the population PK analysis did not identify liver function test results (alanine aminotransferase [ALT], aspartate aminotransferase [AST], bilirubin, and alkaline phosphatase [ALP]) as a significant covariate for exposure of ensifentrine, the Applicant proposed that no dosage adjustment in patients with hepatic impairment is needed. The clinical pharmacology review team agrees with this approach. The Applicant proposed no dose adjustment for subjects with hepatic impairment and is proposing to use ensifentrine with caution in subjects with hepatic impairment in the label. The review team agrees with this approach.

A dedicated study with ensifentrine evaluating the effect of renal impairment on the PK of ensifentrine was not conducted. The effect of renal impairment on the exposure to ensifentrine for up to 24 weeks was evaluated in a population PK analysis. While continuous covariates of renal function did not show a significant correlation with ensifentrine exposure, categorical characterization of renal function indicated 25% reduction in the apparent clearance in subjects with moderate renal impairment. The pharmacokinetics of ensifentrine in severe renal impairment (creatine clearance <30 mL/min) or subjects with end-stage renal disease have not been evaluated. The Applicant proposed no dose adjustment for subjects with mild or moderate renal impairment. The review team agrees with this approach.

14.1. In Vitro Studies

The Applicant has conducted several in vitro studies with human biomaterials as part of the nonclinical PK package in this submission. Key results are summarized in this section.

In Caco-2 cells, ensifentrine was found to have the potential for human intestinal absorption following oral dosing when administered via the oral route, though this was of limited clinical importance as ensifentrine is administered via the inhalation route. Based on multiple studies of the binding of ensifentrine to human plasma, the human plasma protein binding of ensifentrine was estimated to be approximately 90%. In vitro metabolism studies conducted in human liver microsomes (HLM) showed that the main routes of metabolism of ensifentrine HLM were oxidative, including O-demethylation, hydroxylation, and oxidation to yield a carboxylic acid. Overall, the metabolic pathways observed for ensifentrine in HLM had a high degree of concurrence with metabolic pathways in rat liver microsomes and dog liver microsomes and confirmed the absence of any unique human metabolites. In vitro studies were performed to characterize the contribution of cytochrome P450 (CYP) isoforms to ensifentrine metabolism. At therapeutic concentrations, ensifentrine was predominantly metabolized by CYP2C9. In

substrate phenotyping assays, ensifentrine was identified as a likely substrate of breast cancer resistance protein, but not a substrate of P-glycoprotein, OATP1B1, and OAT1B3.

An in vitro study was conducted to identify the ensifentrine-related components in human plasma collected from subjects in clinical study RPL554-007-2014 and compared these components to metabolites detected from incubation of ensifentrine with HLM (Study YFK/001 and YFK/002). This study identified ensifentrine as the major drug-related component in human plasma, accounting for 96% and 99% of the drug related material identified in peak ensifentrine plasma samples and time-normalized plasma samples, respectively, and demonstrated that the metabolites identified following incubation of ensifentrine with liver microsomes were consistent with the metabolites profile in human plasma.

An analysis of ensifentrine in pooled urine samples collected in study RPL554-PK-102 demonstrated negligible urinary excretion of unchanged ensifentrine (less than 0.3% of the dose).

In vitro DDI studies have been performed to evaluate the clinical DDI potential of ensifentrine to modulate activity of metabolizing enzymes and uptake/efflux transporters. Clinical DDI potential has been evaluated based on these in vitro studies and in consideration of ensifentrine free drug C_{max} value (i.e., <0.2 nM) following the proposed dosing regimen of 3 mg BID regimen. Ensifentrine showed weak inhibition of CYP2C8, CYP2C9, and CYP3A4, with IC_{50} values of 37, 31, and 25 µM, respectively. Ensifentrine showed inhibitory interactions with multiple efflux and uptake transporters but at IC_{50} values ranging from 1.4 µM (organic cation transporter 2) to 40.9 µM (OATP1B3) that far exceed the C_{max} following a 3 mg BID dose. Similarly, IC_{50} values of ensifentrine for inhibition of major CYP enzymes are also far greater than the C_{max} value. Therefore, DDI potential for ensifentrine as a perpetrator is not expected at clinically relevant concentrations. The Applicant has not conducted any CYP or transporter induction studies because these effects from ensifentrine as a perpetrator are unlikely due to the very low ensifentrine systemic concentrations following inhalation route of administrations and the high protein binding of ensifentrine. A summary of the clinical DDI potential of ensifentrine based on in vitro evaluations is presented in Table 48.

Table 48. Summary of the Clinical Drug-Drug Interaction Potential of Ensifentrine as a Perpetrator Based on In Vitro Evaluations

CYP or Transporter	IC_{50} (µM)	R Values and Ratios Based on Clinical Dosing Regimen of 3 mg Twice Daily[a]	Interpretation
CYP2C8	37	$R_1 = 1 + (I_{max,u}/K_i^b) = 1.00$	Clinical DDI unlikely[c]
CYP2C9	31	$R_1 = 1 + (I_{max,u}/K_i^b) = 1.00$	Clinical DDI unlikely[c]
CYP3A4	25	$R_1 = 1 + (I_{max,u}/K_i^b) = 1.00$	Clinical DDI unlikely[c]
P-gp	8.5	$I_1/IC_{50} < 0.1$	Clinical DDI unlikely[d]
BCRP	4.4	$I_1/IC_{50} < 0.1$	Clinical DDI unlikely[d]
OATP1B1	26.1	R<1.1	Clinical DDI unlikely[e]
OATP1B3	40.9	R<1.1	Clinical DDI unlikely[e]
OAT1	9.6	$I_{max,u}/IC_{50} < 0.1$	Clinical DDI unlikely[f]
OAT3	10.0	$I_{max,u}/IC_{50} < 0.1$	Clinical DDI unlikely[f]
OCT2	1.4	$I_{max,u}/IC_{50} < 0.1$	Clinical DDI unlikely[f]
MATE1	5.3	$I_{max,u}/IC_{50} < 0.1$	Clinical DDI unlikely[f]
MATE2-K	9.7	$I_{max,u}/IC_{50} < 0.1$	Clinical DDI unlikely[f]

Source: Summary of Clinical Pharmacology Studies, Table 3.
Abbreviations: AUC, area under the concentration-time curve; CYP, cytochrome p450; DDI, drug-drug interaction; IC_{50}, half maximal inhibitory concentration; I_{max}, maximum drug inhibition; K_i, equilibrium dissociation constant representing equilibrium binding affinity for a ligand that reduces activity of its binding partner; MATE, multidrug and toxin extrusion transporter; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; R, ratio of victim AUC in the presence and absence of perpetrators (inhibitors or inducers)

14.2. In Vivo Studies

Formulation Development

The intended to-be-marketed formulation for ensifentrine is a sterile suspension of ensifentrine in pH neutral phosphate buffered saline containing (b) (4) for inhaled delivery via a standard jet nebulizer. Ensifentrine inhalation suspension is administered by the orally inhaled route via a standard jet nebulizer connected to an air compressor. The PARI LC Sprint® nebulizer with a PARI Vios® PRO compressor or equivalent was used for administration of ensifentrine inhalation suspension in the phase 2b and phase 3 clinical program.

Early nonclinical and clinical studies were conducted with an ensifentrine inhalation solution (b) (4) The high phosphate suspension formulation was used in multiple phase 1 and phase 2 studies to characterize ensifentrine PK and PD and explore the efficacious dose range in subjects with COPD. One PD study following inhaled ensifentrine solution dosing was conducted to inform on the anti-inflammatory effects of ensifentrine delivered to the lungs.

The high phosphate suspension formulation was subsequently optimized to a low phosphate formulation with (b) (4) The low phosphate ensifentrine suspension is the to-be-marketed formulation and was used in phase 1 studies RPL554-PK-102, RPL554-PK-103,

RPL554-CV-101, phase 2b study RPL554-CO-205, and the two pivotal phase 3 clinical studies RPL554-CO-301 and RPL554-CO-302. Although the population PK analyses did not identify formulation (high or low phosphate concentration) as a significant covariate for ensifentrine PK, the difference of local airway deposition of ensifentrine by two different formulations is unclear.

Clinical Pharmacology-Related Studies

Ensifentrine PK was assessed in several single- and multiple-dose clinical studies as a nebulized suspension formulation. As the nebulized suspension formulation, ensifentrine was dosed in healthy volunteers at doses ranging from 1.5 mg to 24 mg and in subjects with COPD at doses ranging from 0.375 to 12 mg. Studies were conducted using both high and low phosphate (to-be-marketed) suspension formulations. Dedicated clinical pharmacology studies to investigate the fraction of ensifentrine systemic exposure attributable to swallowed drug product (PK in the presence and absence of a charcoal block) following an inhaled dose, DDIs with a CYP2C9 inhibitor, PK in special populations (specifically hepatically impaired subjects), and a thorough QT study to assess the effect of ensifentrine on electrocardiogram (ECG) parameters including the QT interval have been completed. Out of the major clinical pharmacology studies conducted, only four studies used the to-be-marketed formulation (low phosphate suspension formulation) and this review will focus on the results from these studies: Study RPL554-PK-102, DDI study evaluating fluconazole effect on ensifentrine; Study RPL554-PK-103, hepatic impairment study; Study RPL554-CV-101, thorough QT study; and Study RPL554-CO-205, phase 2 b dose-ranging study.

In addition, the PK of ensifentrine following single and multiple ascending doses administered by nebulizer in healthy subjects and subjects with stable moderate COPD was evaluated in Study RPL554-007-2014, which used the high phosphate suspension formulation.

The Applicant conducted population PK analyses to investigate the effects of intrinsic and extrinsic factors on ensifentrine PK incorporating data from phase 3 study RPL554-CO-302 and other relevant phase 1 and phase 2 studies in healthy subjects and subjects with COPD, and the results indicated that formulation (high or low phosphate) was not a covariate for ensifentrine exposure (See Section 14.5 for details). In addition, multiple studies have been conducted to investigate the local PD effects (e.g., sputum cell counts and onset of bronchodilation) of inhaled ensifentrine in subjects with COPD. However, these studies did not use the to-be-marketed formulation, thus the inclusion of local PD data from these studies in the label (b) (4) has limited clinical relevance.

Study RPL554-007-2014 (Formulation: High Phosphate Suspension)

This was a phase 1, randomized, double-blind, three-part, placebo-controlled, dose escalation study to evaluate the PK, safety, and tolerability of single and multiple ascending doses of ensifentrine (high phosphate suspension formulation), administered by nebulizer in healthy subjects and subjects with stable moderate COPD. The study was conducted in the following three parts: Part A: single ascending doses of ensifentrine in 50 healthy male subjects (non-smokers or ex-smokers) (1.5 to 24 mg); Part B: multiple ascending doses of ensifentrine in 30 healthy male subjects (non-smokers or ex-smokers) (6 to 24 mg BID); Part C: multiple ascending doses of ensifentrine in 32 subjects (males or female subjects of non-childbearing

potential) with stable moderate COPD (post-bronchodilator 50 to 80% predicted normal FEV_1) (1.5 to 12 mg BID).

PK Results

Part A: The median time to maximum concentration (T_{max}) was 1.5 hours with all doses. Geometric mean C_{max} and AUC values generally increased proportionally with dose. Variability was high for both C_{max} and AUC with % coefficient of variation (CV) 28% or higher. The mean terminal $t_{1/2}$ values were between 8.16 and 10.4 hours. Key PK parameters are summarized in Table 49. Mean PK profiles are depicted in Figure 7.

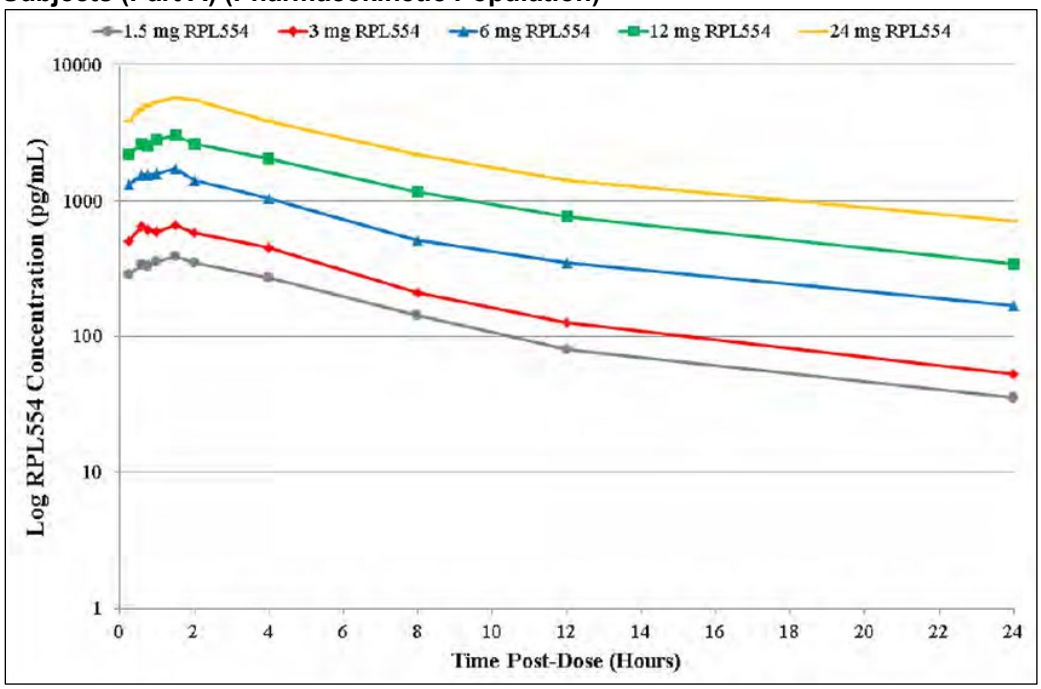
Table 49. Pharmacokinetic Parameters Following Single Dosing to Healthy Subjects (Part A) (Study RPL554-007-2014)

Ensifentrine Dose	C_{max} (pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	AUC_{0-12} (h*pg/mL) Geometric Mean (%CV)	AUC_{0-t} (h*pg /mL) Geometric Mean (%CV)	AUC_{0-inf} (h*pg /mL) Geometric Mean (%CV)	$t_{½}$ (h) Geometric Mean (%CV)	CL/F (L/h) Geometric Mean (%CV)
1.5 mg (n=7)	375 (31.5)	1.5 (0.58, 1.5)	2400 (38.1)	3020 (41.1)	3410 (43.6)	8.16 (18.9)	362 (39.4)
3 mg (n=7)	621 (77.0)	1.5 (0.22, 1.5)	3440 (65)	4310 (65.2)	4840 (65.9)	8.46 (7.12)	462 (45.8)
6 mg (n=7)	1640 (44.5)	1.5 (0.2, 1.53)	9570 (38.6)	12600 (36.5)	15100 (35.1)	10.4 (8.68)	361 (35.4)
12 mg (n=7)	2910 (33.1)	1.5 (0.2, 2)	19200 (28.4)	25600 (28.5)	30100 (28.7)	9.37 (13.8)	340 (26.2)
24 mg (n=7)	5910 (29.3)	1.5 (0.77, 2)	36800 (27.5)	49100 (26.9)	59200 (28.3)	10.1 (15.5)	335 (40.5)

Dose Normalized Exposure [a]				
Ensifentrine Dose	**C_{max}/Dose (pg/mL/mg) Geometric Mean**	**AUC_{0-t}/Dose (h*pg /mL/mg) Geometric Mean**	**AUC_{0-12}/Dose (h*pg /mL/mg) Geometric Mean**	**AUC_{0-inf}/Dose (p h*pg /mL/mg) Geometric Mean**
1.5 mg (n=7)	250	2010	1600	2270
3 mg (n=7)	207	1440	1150	1610
6 mg (n=7)	273	2100	1600	2520
12 mg (n=7)	243	2130	1600	2510
24 mg (n=7)	246	2050	1530	2470

Source: RPL554-007-2014 CSR, Table 11
Abbreviations: AUC_{0-inf}, area under the concentration-time curve from time 0 extrapolated to infinity; AUC_{0-t}, area under the concentration-time curve from time 0 to time t; AUC_{0-12}, area under the concentration-time curve from time 0 to 12 hours; CL/F, apparent clearance; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; L, liters; max, maximum; min, minimum; n, number of subjects in treatment group; T_{max}, time to peak concentration; $t_{1/2}$, half-life

Figure 7. Mean Ensifentrine Plasma Concentration-Time Curve Following Single Dosing to Healthy Subjects (Part A) (Pharmacokinetic Population)



Source: RPL554-007-2014 CSR, page 84, Figure 5

A metabolic profiling analysis was conducted using samples from seven Part A subjects dosed with 24 mg ensifentrine to identify the ensifentrine-related components present in human plasma. Ensifentrine was the major drug-related component in the T_{max} sample and the time-normalized pools (0 to 24 hours) for all subjects. The only metabolites detected were identified as 4'-hydroxy and 9-O-desmethyl ensifentrine. Both metabolites were present with an abundance of less than 1% of unchanged ensifentrine in a human plasma 0 to 24 hours' time-normalized pool.

Part B: C_{max} and AUC_{0-12} on Day 1 in Part B were similar to those following single dosing in Part A. There was no apparent increase in trough concentrations of ensifentrine between Days 2 and 6 and no statistically significant difference on adjacent dosing days with the 6 mg and 12 mg doses of ensifentrine. The trough levels on Day 7 were similar to the trough levels on Day 2 (12 hours after the second dose of ensifentrine), indicating that steady-state appeared to be achieved by Day 2 for high phosphate formulation (Figure 8).

Subjects in the 24 mg BID cohort had dosing stopped after approximately 2 days of dosing in Part B following one incidence of suspected QTc prolongation in a subject in the 12 mg and three subjects in the 24 mg dose group (one case was later confirmed by two independent cardiologists to be an error with the ECG machine automatic measurement) and one incident of T-wave inversion in one subject in the 24 mg dose group. Subjects in the 6 mg (10 of 10 subjects) and 12 mg (9 of 10 subjects) cohorts completed 6 days BID dosing.

BID dosing resulted in limited accumulation of ensifentrine with respect to C_{max} and AUC_{0-12} between Day 1 and Day 6. Key PK parameters are shown in Table 50. Ensifentrine concentrations in plasma following multiple dosing in healthy subjects, Part B, are shown in

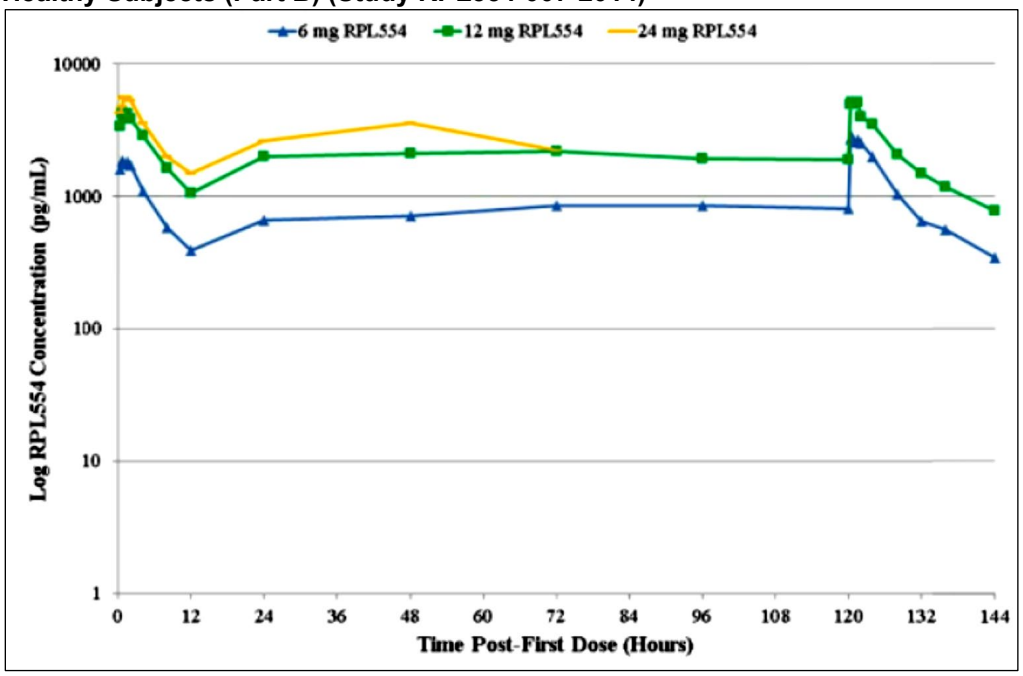
Figure 8. There was a dose dependent increase in ensifentrine plasma concentrations on both Day 1 and Day 6.

Table 50. Pharmacokinetic Parameters on Day 1 and Day 6 Following Multiple Dosing to Healthy Subjects (Part B) (Study RPL554-007-2014)

Ensifentrine Dose	Day 1		Day 6				Geometric Mean AR (Day 6/Day 1)	
	C_{max} (pg/mL) Geometric Mean (%CV)	AUC_{0-12} (h*pg/mL) Geometric Mean (%CV)	C_{max} (pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	AUC_{0-12} (h*pg/mL) Geometric Mean (%CV)	$t_{½}$ (h) Geometric Mean (%CV)	$AR_{(Cmax)}$	$AR_{(AUC0-12)}$
6 mg (N=7)	1840 (37.3)	10800 (33.9)	2770 (32.1)	0.58 (0.22, 1.5)	17600 (39.1)	12.3 (46.2)	1.5 (96)	1.63 (85.7)
12 mg (N=7)	4480 (35.4)	27700 (25.1)	5600 (40.3)	1.5 (0.23, 1.5)	34800 (40.6)	12.6 (17.6)	1.19 (36.2)	1.21 (29.3)
24 mg (N=7)	5760 (46.1)	34100 (42.9)	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]
Dose Normalized Exposure[b]								
Ensifentrine Dose	**C_{max}/Dose (pg/mL/mg) Geometric Mean**	**AUC_{0-12}/Dose (h*pg/mL/mg) Geometric Mean**	**C_{max}/Dose (pg/mL/mg) Geometric Mean**		**AUC_{0-12}/Dose (h*pg/mL/mg) Geometric Mean**		NA	
6 mg (N=7)	307	1800	4612		2933			
12 mg (N=7)	373	2308	467		2900			
24 mg (N=7)	240	1420	-[a]		-[a]			

Source: RPL554-007-2014 CSR, Table 12, Table 14.2.3.1, and Table 14.2.3.2
Abbreviations: AR, arithmetic ratio; AUC_{0-12}, area under the concentration-time curve from time 0 to 12 hours; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; max, maximum; min, minimum; N, number of subjects in treatment group; NA, not applicable; T_{max}, time to peak concentration; $t_{1/2,}$ half-life

Figure 8. Mean Ensifentrine Plasma Concentration-Time Curve Following Multiple Dosing to Healthy Subjects (Part B) (Study RPL554-007-2014)



Source: RPL554-007-2014 CSR, Figure 7
Note: trough concentrations were only measured on Day 2 to Day 5

Part C: Mean values for C_{max} and AUC_{0-t} in subjects with COPD increased in a less than dose-proportional manner on Day 1 and Day 6 (Table 51 and Figure 9). Wide inter-subject variability in exposure was noted across all doses of ensifentrine in COPD subjects and this was similar to that observed in healthy subjects. The exposure was lower in COPD subjects compared to

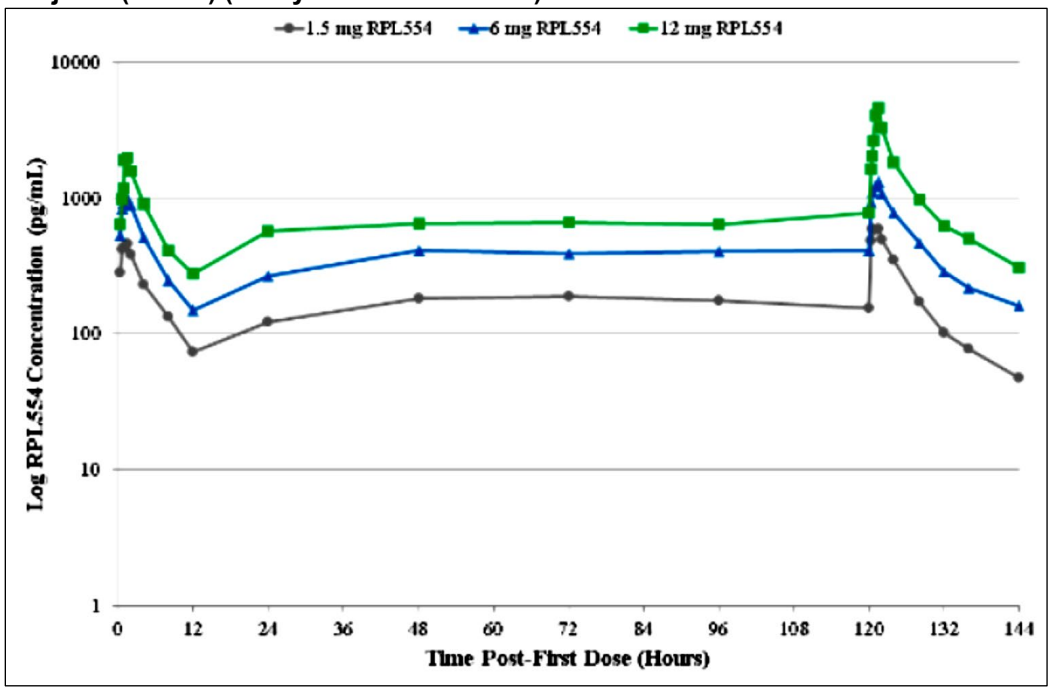
healthy subjects. A small degree of accumulation was observed from Day 1 to Day 6 at doses of 1.5 and 6 mg, similar to that observed in healthy volunteers. Steady state was reached by Day 2 (Figure 9). Key PK parameters for Part C are summarized in Table 51.

Table 51. Pharmacokinetic Parameters on Day 1 and Day 6 Following Multiple Dosing in Subjects With COPD (Part C) (Study RPL554-007-2014)

Ensifentrine Dose	Day 1		Day 6				Geometric Mean AR (Day 6/Day 1)	
	C_{max} (pg/mL) Geometric Mean (%CV)	AUC_{0-12} (h*pg/mL) Geometric Mean (%CV)	C_{max} (pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	AUC_{0-12} (h*pg/mL) Geometric Mean (%CV)	$t_{½}$ (h) Geometric Mean (%CV)	AR_{Cmax}	$AR_{AUC0-12}$
1.5 mg (N=7)	475 (16.2)	2470 (22.9)	609 (33.6)	1.27 (0.58, 1.53)	3340 (33.4)	10.6 (31.5)	1.23	1.27
6 mg (N=7)	985 (52.7)	4390 (71.4)	1160 (66.9)	1.5 (0.62, 2.0)	5880 (89.0)	11.9 (39.7)	1.3	1.44
12 mg (N=7)	1570 (76)	8010 (53.9)	3930 (67.3)	1.5 (1.02, 1.52)	18000 (50.6)	12.2 (20)	2.51	2.25
Dose Normalized Exposure [a]								
Ensifentrine Dose	C_{max}/Dose (pg/mL/mg) Geometric Mean	AUC_{0-12}/Dose (h*pg/mL/mg) Geometric Mean	C_{max}/Dose (pg/mL/mg) Geometric Mean		AUC_{0-12}/Dose (h*pg/mL/mg) Geometric Mean		NA	
1.5 mg (N=7)	317	1647	406		2753			
6 mg (N=7)	164	733	193		1220			
12 mg (N=7)	131	667	328		1942			

Source: RPL554-007-2014 CSR, Table 13, Table 14.2.3.1 and Table 14.2.3.2
Abbreviations: AR, arithmetic ratio; AUC_{0-12}, area under the concentration-time curve from time 0 to 12 hours; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; max, maximum; min, minimum; N, number of subjects in treatment group; NA, not applicable; T_{max}, time to peak concentration; $t_{1/2,}$ half-life

Figure 9. Mean Ensifentrine Plasma Concentration-Time-Curve Following Multiple Dosing to COPD Subjects (Part C) (Study RPL554-007-2014)



Source: RPL554-007-2014 CSR, Figure 9
Abbreviation: COPD, chronic pulmonary obstructive disease

Study RPL554-PK-101 (Formulation: High Phosphate Suspension)

This study was a phase 1, randomized, complete block, two-way crossover study to investigate the contribution of oral absorption to total systemic exposure as measured by the relative bioavailability and PK of nebulized ensifentrine with and without oral ingestion of active charcoal block (Actidose Aqua®, total of 50 g in a 240 mL aqueous suspension) in healthy adult male subjects. All subjects received two single 6 mg doses of ensifentrine (high phosphate formulation), one without charcoal block (Treatment A) and one with charcoal block (Treatment B), in a randomized sequence (A:B or B:A), one at Visit 2 and one at Visit 3, respectively.

Following a single nebulized dose of 6 mg ensifentrine alone (Treatment A) or in combination with a total of 50 g of activated charcoal (Treatment B), the plasma ensifentrine concentrations were above the lower limit of quantitation (LLOQ; 5 pg/mL) through 48 hours post-dose in all subjects and following both treatments. The mean ensifentrine plasma concentration-time profiles were characterized by a rise in concentration within 0.25 to 2.0 hours post-dose followed by a slower terminal phase. Following Treatment B (ensifentrine + charcoal), ensifentrine exposure parameters were slightly lower than Treatment A (ensifentrine alone) as shown by geometric mean $AUC_{0\text{-inf}}$, $AUC_{0\text{-last}}$, and C_{max} values in Table 52. This was due to absorption of swallowed (not inhaled) ensifentrine to the orally administered activated charcoal in Treatment B in the gut, thereby reducing equivalent concentrations of ensifentrine in the systemic circulation. Ensifentrine elimination $t_{1/2}$ ranged from 10.8 to 11.7 hours and appeared to be independent of the two treatments. The median T_{max} was earlier following Treatment B (ensifentrine + charcoal) than Treatment A (0.39 hour versus 0.95 hour). Mean difference of bioavailability fraction between two treatments in 11 subjects included in the statistical comparison who completed both treatments was 10.4%, indicating that only a small portion of a nebulized inhaled dose of ensifentrine was absorbed in the gastrointestinal tract.

Table 52. Summary Statistics of Ensifentrine Plasma PK Parameters Following a Single Nebulized Dose Alone (Treatment A) and in Combination With Activated Charcoal (Treatment B) (Study RPL554-PK-101)

	Parameter: Geometric Mean (GCV%)							
Treatment	$AUC_{0\text{-inf}}$ (pg*h/mL)	$AUC_{0\text{-last}}$ (pg*h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	C_{max} (pg/mL)	T_{max} [b] (h)	V_z/F (L)	R [c] (%)
A (n=11) [a]	15600 (24.4)	14800 (24.2)	11.7 (16.9)	385 (24.4)	1780 (42.1)	0.95 (0.25, 2.00)	6520 (28.4)	10.4
B (n=12)	14000 (15.3)	13300 (15.3)	10.8 (15.1)	430 (15.3)	1570 (40.1)	0.39 (0.25, 1.95)	6730 (20.3)	

Source: RPL554-PK-101 CSR Table 5
Abbreviations: $AUC_{0\text{-inf}}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to last quantifiable time point; CL/F, apparent clearance; C_{max}, maximum concentration; GCV, geometric coefficient of variation; h, hours; n, number of subjects in treatment group; PK, pharmacokinetic; R, ratio of victim AUC in the presence and absence of perpetrators (inhibitors or inducers); T_{max}, time to peak concentration; $t_{1/2}$, half-life, V_z/F, apparent volume of distribution

The interpretation of the results from this study is limited due to a not-to-be-marketed formulation and a higher-than proposed ensifentrine dose were used in this study.

Study RPL554-CO-205 (Formulation: Low Phosphate Suspension)

This was a phase 2b, randomized, double-blind, placebo-controlled dose ranging study to assess the effect of ensifentrine *(low phosphate suspension [to-be-marketed] formulation)* over 4 weeks in subjects with moderate to severe COPD (post-bronchodilator FEV_1 30 to 70% predicted) on a stable background therapy of open-label tiotropium (Spiriva® Respimat®). Following a 2-week run-in on tiotropium, subjects were randomized to receive one of five treatments, each administered BID: 0.375, 0.75, 1.5, and 3.0 mg ensifentrine or placebo in addition to the background tiotropium. Ensifentrine or placebo were administered via PARI LC Sprint® jet nebulizer attached to a PARI Vios® PRO compressor. Subjects received two puffs of tiotropium 2.5 mg once daily, prior to ensifentrine administration. 413 subjects were treated, and 373 subjects completed the study.

The primary efficacy endpoint was met by all four doses of ensifentrine tested; administration of ensifentrine in addition to tiotropium provided dose-dependent statistically significant improvements in peak FEV_1 (over 3 hours) at Week 4 compared with placebo in addition to tiotropium (Table 53).

Table 53. Change From Baseline FEV_1 to Peak FEV_1 (Over 3 Hours) at Week 4 (Study RPL554-CO-205)

	RPL554 0.375 mg (N=83)	RPL554 0.75 mg (N=83)	RPL554 1.5 mg (N=81)	RPL554 3 mg (N=82)	Placebo (N=84)
Change from baseline FEV_1 (L)					
Mean (SD)	0.209 (0.241)	0.217 (0.205)	0.220 (0.241)	0.247 (0.241)	0.121 (0.227)
Placebo-corrected treatment effect – MMRM[c] (L)					
LS mean difference vs placebo	0.078	0.091	0.107	0.124	
95% CI	(0.005, 0.150)	(0.018, 0.164)	(0.034, 0.180)	(0.052, 0.197)	
p-value	0.0368	0.0148	0.0040	0.0008	

Source: RPL554-CO-205 page 81, CSR Table 16
[c] MMRM model was used to model the change from baseline FEV_1 to peak FEV_1 using treatment, visit and treatment by visit interaction as fixed effect, subject as random effect and baseline FEV_1 as the covariate. Estimates refer to the Week 4 from treatment by visit interaction.
Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume over 1 second; L, liters; LS, least squares; MMRM, mixed model of repeated measures; N, number of subjects in treatment group; SD, standard deviation

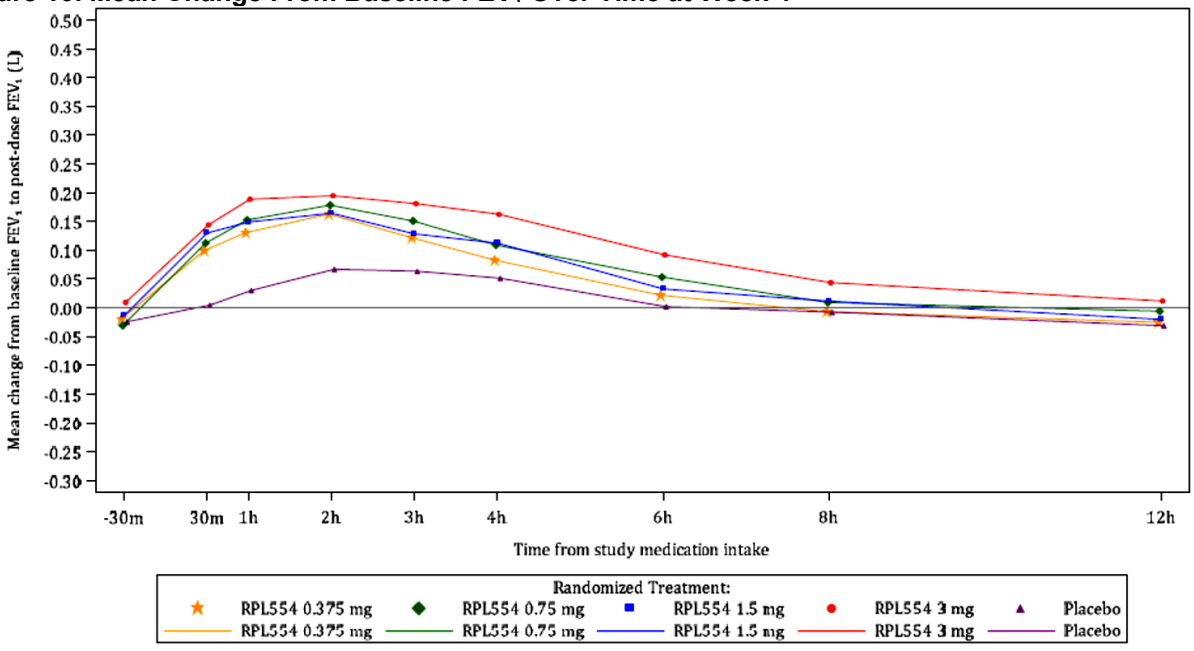
In addition, dose-dependent numerical improvement was also observed in average FEV_1 $AUC_{0\text{-}12h}$ at Week 4, which was one of the secondary endpoints (Table 54). The post-dose FEV_1 time profile of ensifentrine 3 mg dose showed a separation from lower doses at all time points over the 12-hour dosing interval (Figure 10).

Table 54. Change From Baseline FEV_1 to AUC_{0-12h} FEV_1 at Week 4 (Study RPL554-CO-205)

	RPL554 0.375 mg (N=83)	RPL554 0.75 mg (N=83)	RPL554 1.5 mg (N=81)	RPL554 3 mg (N=82)	Placebo (N=84)
Change from baseline FEV_1 (L)					
Mean (SD)	0.040 (0.249)	0.064 (0.191)	0.064 (0.215)	0.100 (0.221)	0.012 (0.204)
Placebo-corrected treatment effect – MMRM[c] (L)					
LS mean difference vs placebo	0.026	0.053	0.054	0.087	
95% CI	(–0.041, 0.094)	(–0.015, 0.121)	(–0.013, 0.122)	(0.020, 0.155)	
p-value	0.4455	0.1244	0.1149	0.0111	

Source: RPL554-CO-205 CSR page 87, Table 18
[c]MMRM model was used to model the change from baseline FEV_1 to average AUC0-3h FEV_1 using treatment, visit and treatment by visit interaction as fixed effect, subject as random effect and baseline FEV_1 as the covariate. Estimates refer to the Week 4 from treatment by visit interaction.
Abbreviations: AUC_{0-12}, area under the concentration-time curve from 0-12 hours; CI, confidence interval; FEV_1, forced expiratory volume over 1 second; L, liters; LS, least squares; MMRM, mixed model of repeated measures; N, number of subjects in treatment group; SD, standard deviation

Figure 10. Mean Change From Baseline FEV_1 Over Time at Week 4



Source: Study RPL554-CO-205 CSR, Figure 2
Abbreviation: FEV_1, forced expiratory volume over 1 second; h, hours; L, liters

In addition, the safety and tolerability of ensifentrine 3 mg BID over 4 weeks was similar to placebo and there was no apparent dose-response trend observed for treatment-emergent adverse events in Study RPL554-CO-205 (Table 55).

Table 55. Summary of Treatment-Emergent Adverse Events (Study RPL554-CO-205)

TEAE Category	Number of Patients, n (%)				
	RPL554 0.375 mg (N=82)	RPL554 0.75 mg (N=83)	RPL554 1.5 mg (N=81)	RPL554 3 mg (N=83)	Placebo (N=84)
Any TEAE	12 (14.6)	15 (18.1)	14 (17.3)	18 (21.7)	17 (20.2)
Any TEAE leading to study medication discontinuation	2 (2.4)	3 (3.6)	0	0	1 (1.2)
Any TEAE related to study medication	1 (1.2)	3 (3.6)	2 (2.5)	2 (2.4)	4 (4.8)
Any serious TEAE	0	1 (1.2)	0	2 (2.4)	0
Any serious TEAE leading to study medication discontinuation	0	1 (1.2)	0	0	0
Any TEAE classified by maximum severity					
Mild	3 (3.7)	6 (7.2)	9 (11.1)	10 (12.0)	8 (9.5)
Moderate	9 (11.0)	8 (9.6)	5 (6.2)	7 (8.4)	9 (10.7)
Severe	0	1 (1.2)	0	1 (1.2)	0
Any serious TEAE classified by maximum severity					
Moderate	0	1 (1.2)	0	1 (1.2)	0
Severe	0	0	0	1 (1.2)	0

Source: RPL554-CO-205 CSR Table 24
Abbreviations: N, number of subjects in treatment group; n, number of subjects in subset; TEAE, treatment-emergent adverse event

Table 56 summarizes the steady-state plasma concentrations of ensifentrine after 14 days (week 2) of dosing, prior to dose administration. A dose-dependent increase in mean trough plasma ensifentrine concentrations was observed over the 0.375 to 3 mg ensifentrine dose range following BID administration for 2 weeks.

Table 56. Summary of Steady-State Plasma Concentrations of Ensifentrine (pg/mL) at Week 2 (Study RPL554-CO-205)

Ensifentrine Dose	Pre-dose Ensifentrine Concentration at Week 2 C_{trough} (pg/mL)	
	n	Geometric Mean (%CV)
0.375 mg (N=82)	59	22.5 (157.81)
0.75 mg (N=82)	57	36.4 (96.28)
1.5 mg (N=81)	63	74.3 (86.19)
3 mg (N=83)	70	106.6 (90.45)

Source: RPL554-CO-205 CSR Table 22
Abbreviations: $C_{trough,}$ concentration at trough level; CV, coefficient of variation; N, number of subjects in treatment group; n, number of subjects in subset

Overall, the 3 mg BID dosing with to-be-marketed formulation provided a numerically greater improvement in lung function than the lower doses at Week 4 in Study RPL554-CO-205. Therefore, it was reasonable for the Applicant to carry the 3 mg dose with BID regimen to the pivotal phase 3 trials.

Study RPL554-CO-302 (Formulation: Low Phosphate Suspension)

This was a phase 3, multicenter, double-blind, placebo-controlled study to evaluate the efficacy and safety of ensifentrine 3 mg BID over 24 weeks in subjects with moderate to severe COPD (post-bronchodilator FEV_1 30 to 70% predicted). Subjects were randomized to receive ensifentrine 3.0 mg BID or placebo. Ensifentrine or placebo were administered via PARI LC Sprint® jet nebulizer attached to a PARI Vios® PRO Aerosol Delivery System (PARI Boy compressor or equivalent).

Blood samples for the determination of plasma concentrations of ensifentrine were collected across visits at Weeks 6, 12, 24 and early termination visit. A sparse sampling collection scheme was followed with sample collection times from predose to 12 hours postdose being different between even (n=240) and odd (n=199) numbered sites. Ensifentrine plasma PK concentrations at Weeks 6, 12, and 24 are shown in Table 57. The highest mean/median concentrations across sites and visits were at 1 to 1.5 hours with a decline in mean/median concentrations thereafter.

Table 57. Summary for Ensifentrine Plasma Pharmacokinetics Concentrations (pg/mL) by Scheduled Time for Each Treatment (Study RPL554-CO-302)

Site, and	Scheduled Week and Time (hours)												
	Week 6				Week 12						Week 24		
Statistic	1	1.5	2.5	4	Pre-dose	0.5	2	4-6	6-8	8-12	Pre-dose	1	1.5
Even (N=240)													
n	229		227		191			216		209	172		191
n<LLOQ	6		3		26			2		4	40		5
Mean	408.1		382.0		155.9			283.5		199.7	121.6		385.4
SD	282.8		268.5		174.7			204.9		176.8	161.4		269.9
CV%	69.3		70.3		112.1			72.3		88.5	132.7		70.0
Odd (N=199)													
n		185		182		169	174		168		128	151	
n<LLOQ		2		2		2	1		1		30	9	
Mean		456.9		356.3		411.0	358.9		227.3		126.6	409.4	
SD		329.1		314.3		359.9	347.3		210.0		169.9	324.4	
CV%		72.0		88.2		87.6	96.8		92.4		134.3	79.2	

Source: RPL554-CO-302 CSR page 139, Table 27
Abbreviations: CV, coefficient of variation; LLOQ, lower limit of quantitation (5.00 pg/mL); n, number of observations; N, total number; SD, standard deviation

Study RPL554-PK-102 (Formulation: Low Phosphate Suspension)

This was a phase 1, open-label, non-randomized, 3-period study to assess the effect of moderate CYP2C9 inhibitor fluconazole on the PK of ensifentrine in healthy subjects. The primary objective was to assess ensifentrine systemic exposure (C_{max} and AUC_{0-inf}) when administered as a single dose alone and in combination with steady state fluconazole in healthy subjects. This clinical study was conducted to determine whether treatment with the known moderate CYP2C9 inhibitor fluconazole results in a clinically significant interaction with ensifentrine, which was determined to be a substrate for CYP2C9 in in vitro experiments. Subjects received a single dose of 3 mg ensifentrine on Days 1 and 11 by nebulization of a 2.5 mL unit dose via the reusable PARI LC Sprint® jet nebulizer attached to a PARI Vios® PRO compressor. A single dose of

ensifentrine was given on Day 1. Fluconazole administration commenced after the final ensifentrine PK sample collection on Day 4, with a loading dose of 400 mg followed by 200 mg once daily on all other dosing days. This dosing schedule was used to facilitate attainment of steady state exposures within 7 days. An additional blood sample was taken predose on Day 1 for DNA analysis for genotyping (CYP2C9 and CYP2D6 polymorph genotyping).

A summary of the plasma PK parameters in the absence (Day 1) and presence (Day 11) of fluconazole are shown in Table 58. In the majority of subjects, ensifentrine plasma concentrations were above the lower limit of quantification (LLOQ; 5 pg/mL) up to 48 hours postdose for both treatments. At the 60- and 72-hour sampling times, ensifentrine was not quantifiable in the majority of subjects, resulting in mean ensifentrine concentrations below 5 pg/mL. In the presence of fluconazole, the exposure of ensifentrine was higher compared to ensifentrine treatment alone. Both geometric mean apparent clearance and apparent volume of distribution (Vz/F) of ensifentrine decreased when subjects were treated with fluconazole. The median T_{max} was 1.28 hours longer when compared to ensifentrine treatment alone. Plasma ensifentrine $t_{1/2}$ was not affected by fluconazole coadministration.

The steady-state levels for fluconazole were reached by Day 10. There was no apparent relationship between CYP2D6 or CYP2C9 polymorph and exposure parameters in this study.

Table 58. Summary Plasma Pharmacokinetic Parameters for Ensifentrine by Treatment (Study RPL554-PK-102)

	C_{max} (pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	$AUC_{0\text{-}inf}$ (h*pg/mL) Geometric Mean (%CV)	$AUC_{0\text{-}last}$ (h*pg/mL) Geometric Mean (%CV)	$t_{½}$ (h) Geometric Mean (%CV)	CL/F (L/h) Geometric Mean (%CV)	Vz/F (L) Geometric Mean (%CV)	C_{max}/Dose[a] (pg/mL/mg)	$AUC_{0\text{-}inf}$/Dose[a] (h*pg/mL/mg)	$AUC_{0\text{-}12}$/Dose[a] (h*pg/mL/mg)
Ensifentrine 3 mg (N=29); Day 1	772.8 (51.3)	0.58 (0.3, 2.08)	5361 (53.6)	5253 (55.1)	8.078 (30.9)	559.6 (53.6)	6522 (49.6)	258	1787	1751
Ensifentrine 3 mg + Fluconazole 200 mg (N=29); Day 11	1109 (43.3)	1.86 (0.73, 4.07)	8800 (41.3)	8708 (41.5)	8.085 (25.3)	340.9 (41.3)	3976 (36.6)	370	2933	2903

Source: RPL554-PK-102 CSR, Table 15
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}last}$, area under the concentration-time curve from time 0 to last measured time; $AUC_{0\text{-}12}$, area under the concentration-time curve from 0 to 12 hours; CL/F, apparent clearance; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; max, maximum; min, minimum; N, number of subjects in treatment group; T_{max}, time to peak concentration; $t_{1/2}$, half-life, V_z/F, apparent volume of distribution

Table 59 shows the urine PK parameters for ensifentrine calculated over the 72-hour collection period. Less than 0.3% of the inhaled ensifentrine dose was recovered in urine irrespective of treatment. Urinary excretion of ensifentrine can be considered negligible either in the presence or absence of fluconazole.

Table 59. Summary of Ensifentrine Urine Pharmacokinetic Parameters for Each Treatment (Study RPL554-PK-102)

	$Ae_{0\text{-last}}$ Geometric Mean (pg) (GeoCV%)	$fe_{0\text{-last}}$ Geometric Mean (%) (GeoCV%)	CLr Geometric Mean (L/h) (GeoCV%)
Ensifentrine 3 mg (N=29); Day 1	4709000 (62.5)	0.157 (62.5)	0.896 (35.7)
Ensifentrine 3 mg + Fluconazole 200 mg (N=29); Day 11	7593000 (46.8)	0.253 (46.8)	0.872 (34.9)

Source: RPL554-PK-102 CSR, Table 18 and 14.2.6
Abbreviations: $Ae_{0\text{-last}}$, amount of ensifentrine excreted in urine from time 0 to last measured time; $fe_{0\text{-last}}$, cumulative fraction of ensifentrine excreted unchanged in urine from time 0 to last measured time; CLr; renal clearance; GeoCV, geometric coefficient of variation; N, number of subjects in treatment group

In the presence of fluconazole, the exposure of ensifentrine increased by 63% for $AUC_{0\text{-inf}}$ and 41% for C_{max} when compared to ensifentrine alone (Table 60).

Table 60. Statistical Evaluation of Drug-Drug Interaction (Study RPL554-PK-102)

						Ensifentrine+Fluconazole versus Ensifentrine [a]	
Parameter	Treatment		n	Geometric LS Mean [a]	95% CI [a]	Ratio (%)	90% CI
Primary Analysis							
$AUC_{(0\text{-inf})}$ (pg*h/mL)	Ensifentrine 3 mg + Fluconazole 200 mg		26	8800	7364, 10520	162.97	136.85, 194.07
	Ensifentrine 3 mg		26	5400	4519, 6452		
C_{max} (pg/mL)	Ensifentrine 3 mg + Fluconazole 200 mg		26	1109	927.8, 1325	141.19	120.03, 166.09
	Ensifentrine 3 mg		26	785.2	657.1, 938.2		
Secondary Analysis							
$AUC_{(0\text{-last})}$ (pg*h/mL)	Ensifentrine 3 mg + Fluconazole 200 mg		26	8708	7265, 10440	164.51	137.63, 196.64
	Ensifentrine 3 mg		26	5293	4416, 6345		

Source: RPL554-PK-102 CSR, page 63, Table 16
[a] Results based on a linear mixed model with terms treatment as fixed effect and subject as random effect.
Abbreviations: $AUC_{0\text{-inf}}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to last quantifiable time point; CI, confidence interval; C_{max}, maximum concentration; LS, least squares; n, number of subjects in subset

Study RPL554-PK-102 also genotyped the CYP2C9 and CYP2D6 alleles for enrolled subjects. Genotyping identified two CYP2D6 poor metabolizers. Visual inspection of data did not find any obvious relationship between individual exposure and CYP2D6 genotype. No CYP2C9 poor metabolizer was identified from the study.

Overall, co-administration of a moderate CYP2C9 and CYP3A4 inhibitor, fluconazole, increased the systemic bioavailability of oral inhaled ensifentrine by less than 2-fold. This increase is not expected to be clinically relevant because doses of ensifentrine up to 6 mg BID (at least 2-fold

higher than a clinically relevant dose) over 4 weeks have been shown to be well tolerated in clinical studies with a safety profile similar to placebo.

Study RPL554-PK-103 (Formulation: Low Phosphate Suspension)

This was an open-label, non-randomized, PK and safety study of single inhaled dose of ensifentrine 3 mg in subjects with moderate and severe HI and healthy matched control subjects. The primary objective was to evaluate the effect of moderate and severe HI on the plasma exposure parameters C_{max} and $AUC_{0\text{-}inf}$ following a single inhaled dose of ensifentrine (3 mg). The secondary objectives were to further characterize the PK of ensifentrine in subjects with moderate and severe HI, and to evaluate the safety of inhaled ensifentrine in subjects with moderate and severe HI. Twenty-three subjects were enrolled and received a dose of ensifentrine. Subjects were enrolled into the following groups:

- Group A: Moderate HI, Child-Pugh B – 10 subjects
- Group B: Severe HI, Child-Pugh C – 3 subjects
- Group C (Matched Controls): Normal healthy subjects – 10 subjects

Ensifentrine was administered as 3 mg single dose by inhalation of an aerosol generated by a reusable PARI LC Sprint® jet nebulizer attached to a PARI PRONEB® Max Aerosol Delivery System (compressor). Blood samples were collected at the following timepoints: predose, 15 minutes, 30 minutes, 45 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 6 hours, 9 hours, 12 hours, 24 hours, 36 hours, 48 hours, and 56 hours.

In total, 23 subjects were dosed and completed this study. The primary analysis set (n=21) excluded two subjects due to study deviations [the 2 hour-postdose blood sample was delayed for Subject (b) (6) (severe HI) and the 6-hour PK sample was inadvertently missed by staff for Subject (b) (6) (control)]. During the analysis, two additional subjects (Control Subject (b) (6) and Subject (b) (6) with severe HI) demonstrated very low C_{max} value (~1/6 value when compared to other subjects). The Applicant excluded these two "outliers" and created a secondary analysis set (n=19). The Applicant also justified that the observed exposures in these two subjects were lower than previously observed in other controlled studies with ensifentrine 3 mg, as well as those observed in healthy subjects and subjects with COPD at 1.5 mg. The terminal phase parameters could not be determined for these two subjects.

Primary Analysis Set

A summary of the PK parameters for the primary analysis set are shown in Table 61. C_{max}, $AUC_{0\text{-}inf}$, and $AUC_{0\text{-}last}$ were higher in subjects with moderate HI and overall HI (moderate and severe) versus control subjects. In subjects with severe HI, C_{max} and $AUC_{0\text{-}last}$ were lower than in control subjects while $AUC_{0\text{-}inf}$ was higher than control subjects. The control and severe HI subjects with very low exposures impacted (decreased) the respective group mean C_{max} and $AUC_{0\text{-}last}$ values as well as the natural log-transformed data in the statistical analyses. As noted above, these two subjects with unexpectedly lower exposure did not contribute data for the $AUC_{0\text{-}inf}$ comparison.

T_{max} was longer in subjects with HI compared to control subjects. The geometric mean apparent clearance and Vz/F of ensifentrine was higher for control subjects than hepatic impaired groups.

Clearance and Vz/F were similar in moderate and severe HI groups. The geometric mean $t_{1/2}$ of ensifentrine was similar between hepatic impaired and control subjects.

Inter-subject variability in systemic exposure to ensifentrine was generally high across groups and was very high in controls and subjects with severe HI due to inclusion of very low exposure subjects ([(b) (6)] control and [(b) (6)] severe HI).

Table 61. Summary of Ensifentrine Plasma Pharmacokinetic Parameters (Primary Analysis Set, Study RPL554-PK-103)

	Ensifentrine Treatment			
Parameter (unit)	Moderate HI (N=10)	Severe HI (N=3)	Matched Controls (N=8)	Overall HI (N=13)
C_{max} (pg/mL)	651 (62.9)	162 (921.3)	285 (302.0)	472 (170.9)
$AUC_{(0\text{-inf})}$ (pg•h/mL)	6300 (80.4)	6630 (5.4) [2]	2840 (101.3) [7]	6350 (71.0) [12]
$AUC_{(0\text{-last})}$ (pg•h/mL)	6180 (80.6)	1270 (5698.2)	1550 (494.4)	4290 (284.3)
T_{max} (h)	1.58 (0.62, 3.08)	1.58 (0.83, 6.08)	0.71(0.33, 1.08)	1.58 (0.62, 6.08)
$t_{1/2}$ (h)	8.39 (22.6)	9.33 (17.3) [2]	7.74 (12.7) [7]	8.54 (21.5) [12]
CL/F (L/h)	476 (80.4)	452 (5.4) [2]	1060 (101.3) [7]	472 (71.0) [12]
V_z/F (L)	5770 (61.6)	6090 (11.8) [2]	11800 (82.5) [7]	5820 (55.1) [12]

Source: RPL554-PK-103 CSR, Table 15
Abbreviations: $AUC_{0\text{-inf}}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to last measured time; CL/F, apparent clearance; C_{max}, maximum concentration; h, hours; HI, hepatic impairment; N, number of subjects in treatment group; T_{max}, time to peak concentration; $t_{1/2}$, half-life; V_z/F, apparent volume of distribution

The ratios for the geometric least squares (LS) means for C_{max} were 2.29 for subjects with moderate HI, 0.57 for subjects with severe HI, and 1.66 for the HI population combined when compared with the control group (Table 62). The ratio geometric means for $AUC_{0\text{-inf}}$ (Subjects [(b) (6)] and Subject [(b) (6)] non-calculable) were 2.22 in moderate HI subjects, 2.33 in severe HI subjects, and 2.24 in the overall HI population when compared to controls (Table 62).

The ratios for the geometric LS means for $AUC_{0\text{-last}}$ were 3.99 in moderate HI, 0.82 for subjects with severe HI, and 2.77 for the overall HI population when compared to matched controls. Both C_{max} and $AUC_{0\text{-last}}$ ratios were impacted by the very low exposure subjects ([(b) (6)] control and [(b) (6)] severe HI) as evident by the wide 90% CIs, which lowered the respective mean C_{max} and $AUC_{0\text{-last}}$ values for control and severe HI groups. This is reflected in apparent higher ratios for moderate and overall HI and the lower geometric LS mean ratio of $AUC_{0\text{-last}}$ for severe HI when compared to control, whereas the ratios observed for $AUC_{0\text{-inf}}$ where these subjects did not contribute values to the analysis were similar across HI categories.

Table 62. Comparison of Geometric Mean Ratios: Primary and Secondary Analyses

			----------Ratio of Geometric LS Mean---	
Parameters (units)	**Group**	**n/n***	**Primary Analysis Set Ratio (90% CI)**	**Secondary Analysis Set Ratio (90% CI)**
C_{max} (pg/mL)	Moderate Control	10 8/7	2.29 (0.92, 5.67)	1.44 (0.79, 2.61)
	Severe Control	3/2 8/7	0.57 (0.07, 4.51)	1.16 (0.33, 4.05)
	All HI Control	13/12 8/7	1.66 (0.60, 4.59)	1.39 (0.79, 2.43)
$AUC_{(0\text{-}inf)}$ (h*pg/mL)	Moderate Control	10 7	2.22 (1.15, 4.28)	N/A
	Severe Control	2 7	2.33 (0.72, 7.61)	N/A
	All HI Control	12 7	2.24 (1.24, 4.05)	N/A
$AUC_{(0\text{-}last)}$ (h*pg/mL)	Moderate Control	10 8/7	3.99 (1.36, 11.75)	2.26 (1.16, 4.41)
	Severe Control	3/2 8/7	0.82 (0.06, 10.78)	2.39 (0.71, 8.08)
	All HI Control	13/12 8/7	2.77 (0.79, 9.67)	2.28 (1.25, 4.17)

Source: RPL554-PK-103 CSR, Table 17
* n/n denotes the sample size for the Primary and Secondary PK analysis sets, respectively.
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}last}$, area under the concentration-time curve from time 0 to last measured time; CI, confidence interval; C_{max}, maximum concentration; h, hours; HI, hepatic impairment; LS, least squares; n/n, overall number/number in subset; N/A, not applicable; PK, pharmacokinetic

Secondary Analysis Set

A summary of C_{max}, $AUC_{0\text{-}last}$, and T_{max} for the secondary analysis set (parameters which exclude outlier control subject 002-304 and severe HI subject (b) (6) because these were substantially impacted by the low-exposure values) are shown in Table 63.

Table 63. Secondary Analysis of Ensifentrine Plasma Pharmacokinetic Parameters (Secondary Analysis Set, Study RPL554-PK-103)

	C_{max} (pg/mL) Geometric Mean (%CV)	$AUC_{0\text{-last}}$ (h*pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	$t_{1/2}$ (h) Geometric Mean (%CV)	C_{max}/Dose [a] (pg/mL/mg) Geometric Mean	$AUC_{0\text{-last}}$/Dose[a] (h*pg/mL/mg) Geometric Mean
Group A Moderate HI (N=10)	651 (62.9)	6180 (80.6)	1.58 (0.62, 3.08)	8.39 (22.6)	217	2060
Group B Severe HI (N=2)	525 (88.1)	6540 (5.2)	3.83 (1.58, 6.08)	9.33 (17.3)	175	2180
Group C Matched Controls (N=7)	453 (100.2)	2730 (105.5)	0.58 (0.33, 1.08)	7.74 (12.7)	151	910
Overall HI (N=12)	628 (62.8)	6240 (71.2)	1.58 (0.62, 6.08)	8.54 (21.5)	209	2080

Source: RPL554-PK-103 CSR, Table 14.2.4
Abbreviations: $AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to last measured time; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; HI, hepatic impairment; N, number of subjects in treatment group; T_{max}, time to peak concentration; $t_{½}$, half-life

When comparing moderate HI to controls, exclusion of the two low-exposure subjects resulted in a reduction of the ratio of the geometric LS mean for C_{max} from 2.29 to 1.44 for primary and secondary analysis, respectively, as well as a reduction in the CI bounds.

Similarly, exclusion of the two low-exposure subjects resulted in a reduction of the ratio of the geometric LS mean for $AUC_{0\text{-last}}$ from 3.99 to 2.26 for primary and secondary analysis, respectively, as well as a reduction in the CI bounds. The latter is consistent with that for $AUC_{0\text{-inf}}$ (2.22) in the primary analysis.

Comparison of the ratio of exposure parameters for the two severe HI subjects and controls led to similar treatment ratios for C_{max} and $AUC_{0\text{-last}}$ as those observed for the moderate HI group.

Overall, ensifentrine exposure, as measured by C_{max} and $AUC_{0\text{-inf}}$, was approximately 2.3-fold higher in subjects with moderate or severe HI when compared to matched controls. Ensifentrine exposure appeared to be similar between moderate and severe HI subjects. Given the free drug C_{max} (<0.1 ng/mL or <0.2 nM) of ensifentrine following 3 mg inhalation is lower than the IC_{50} values for PDE inhibition (subnanomolar IC_{50} for PDE3 and >50 nM for PDE4), and population PK analysis did not identify markers of liver function (ALT, AST, bilirubin, and ALP) as a significant covariate for exposure of ensifentrine, the Applicant proposes that no dosage adjustment in patients with hepatic impairment is needed. The clinical pharmacology review team agrees with this approach.

Study RPL554-CV-101 (Formulation: Low Phosphate Suspension)

This was a phase 1, randomized, double-blind, placebo- and moxifloxacin-controlled, 4-period crossover study to evaluate the effect of inhaled nebulized ensifentrine on 12-lead ECG parameters in healthy adult subjects. The primary objective was to evaluate the effect of a single therapeutic dose (3 mg) and supratherapeutic dose (9 mg) of ensifentrine on the QTc interval. Ensifentrine was administered at therapeutic and supratherapeutic dose levels to achieve peak

plasma concentrations greater than those expected after administration of the highest dose planned for efficacy. Additionally, the study also assessed the effect of a single therapeutic dose (3 mg) and supratherapeutic dose (9 mg) of ensifentrine on other ECG parameters (hazard ratio [HR], PR interval and QRS interval, treatment-emergent T-wave morphology, and appearance of U-waves), PK, safety, and tolerability of a single therapeutic or supratherapeutic dose of ensifentrine. Thirty-two subjects were randomized and received at least one dose of study drug in the following treatments:

- Treatment ST: A supratherapeutic dose of ensifentrine (9 mg)
- Treatment T: The maximum therapeutic dose of ensifentrine (3 mg)
- Treatment P: Placebo inhalation (0 mg)
- Treatment M: Moxifloxacin 400 mg oral tablet as the positive control (open-label)

Ensifentrine and placebo were administered by a PARI LC Sprint® jet nebulizer attached to a PARI PRONEB® Max compressor. Each treatment was separated by a drug-free washout period of 7±2 days. Twenty-nine subjects completed the study.

The PK parameters of plasma ensifentrine are shown in Table 64. Based on the ratio of geometric mean, following the administration of a single inhaled nebulized dose of ensifentrine, a 3-fold increase in ensifentrine dose from 3 mg to 9 mg was associated with an approximately 4.3-fold increase in C_{max} and $AUC_{0\text{-}inf}$, thus achieving the targeted supratherapeutic exposure. Variability for both C_{max} and $AUC_{0\text{-}inf}$ was higher for the 3 mg dose (>80% CV) than for the 9 mg dose (42 to 51% CV, respectively).

Table 64. Summary of Pharmacokinetic Parameters of Plasma Ensifentrine (Study RPL554-CV-101)

Ensifentrine Dose	C_{max} (pg/mL) Geometric Mean (%CV)	$AUC_{0\text{-}last}$ (h*pg/mL) Geometric Mean (%CV)	$AUC_{0\text{-}inf}$ (h*pg/mL) Geometric Mean (%CV)	T_{max} (h) Median (Min, Max)	$t_{1/2}$ (h) Mean (%CV)	C_{max}/Dose (pg/mL/mg)[a]	$AUC_{0\text{-}inf}$/Dose (h*pg/mL/mg)[a]
3 mg (N=30)	551 (82.8)	4270 (87.6)	4500 (85.8)	1.50 (0.50, 3.0)	9.84 (21.5)	184	1500
9 mg (N=30)	2330 (51.0)	16500 (45.6)	19600 (41.6)	0.775 (0.50, 3.05)	10.2 (18.8)	259	2178

Source: RPL554-CV-101 CSR, Table 13 and Table 14.2.2
Abbreviations: $AUC_{0\text{-}inft}$, area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}last}$, area under the concentration-time curve from time 0 to last quantifiable time point; C_{max}, maximum concentration; CV, coefficient of variation; h, hours; N, number of subjects in treatment group; T_{max}, time to peak concentration; $t_{½}$, half-life

The primary endpoint in this study was the placebo corrected change from baseline corrected QT interval for heart rate using the Fridericia method (ΔΔQTcF). No significant relationship was found between ΔΔQTcF and ensifentrine plasma concentrations. The estimated slope of the ensifentrine plasma concentration in the concentration corrected QT interval (C-QTc) relationship was not statistically significant (0.0012 ms per ng/mL [90% CI: -0.00012 to 0.00260]). Model predictions of ΔΔQTcF and the upper limit of the 90% CI corresponding to the geometric mean of the C_{max} at the supratherapeutic dose of 9 mg (2341.8 pg/mL) were well below 10 ms (Figure 11). This supports the conclusion that ensifentrine does not have a clinically relevant impact on cardiac repolarization at either dose. Based on this C-QTc analysis

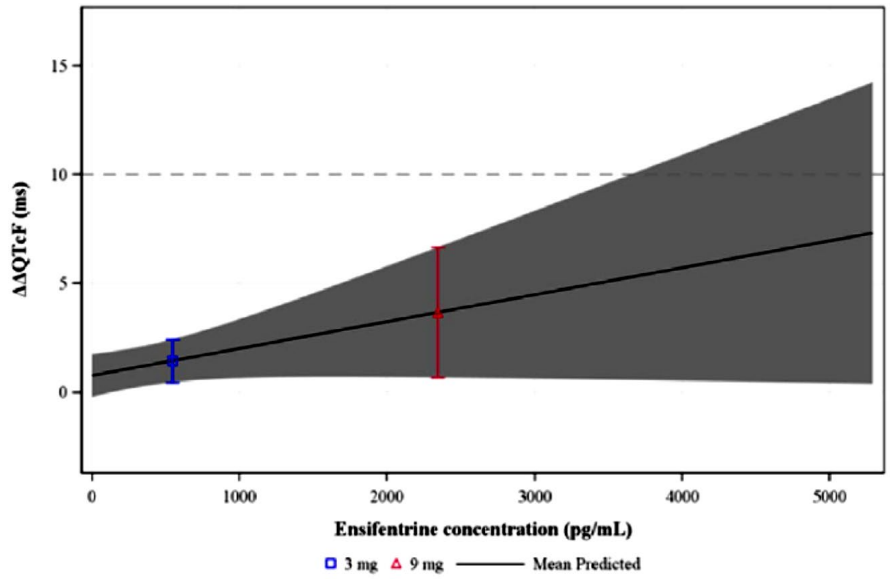
(Table 65), the effect on ΔΔQTcF larger than 10 ms can be excluded up to ensifentrine plasma concentration of 3,650 ng/mL.

Table 65. Predicted ΔΔQTcF Interval at Geometric Mean Peak Concentrations for Ensifentrine (Pharmacokinetic/QTc Population, Study RPL554-CV-101)

Ensifentrine Treatment	Geometric Mean (pg/mL) C_{max} of Ensifentrine	ΔΔQTcF Estimate (ms) (90% CI)
3 mg	545.8	1.43 (0.44, 2.43)
9 mg	2341.9	3.66 (0.66, 6.66)
10 ms Threshold	3650	5.28 (0.55, 10.01)

Source: RPL554-CV-101 CSR, Table 14 and Table 14.1.12.1
Abbreviations: C_{max}, maximum concentration; CI, confidence interval; ΔΔQTcF, placebo-corrected change from baseline-corrected QT interval for heart rate using the Fridericia method; ms, milliseconds; QTc, corrected QT interval; QTcF, QT corrected for heart rate by Fridericia's cube root formula

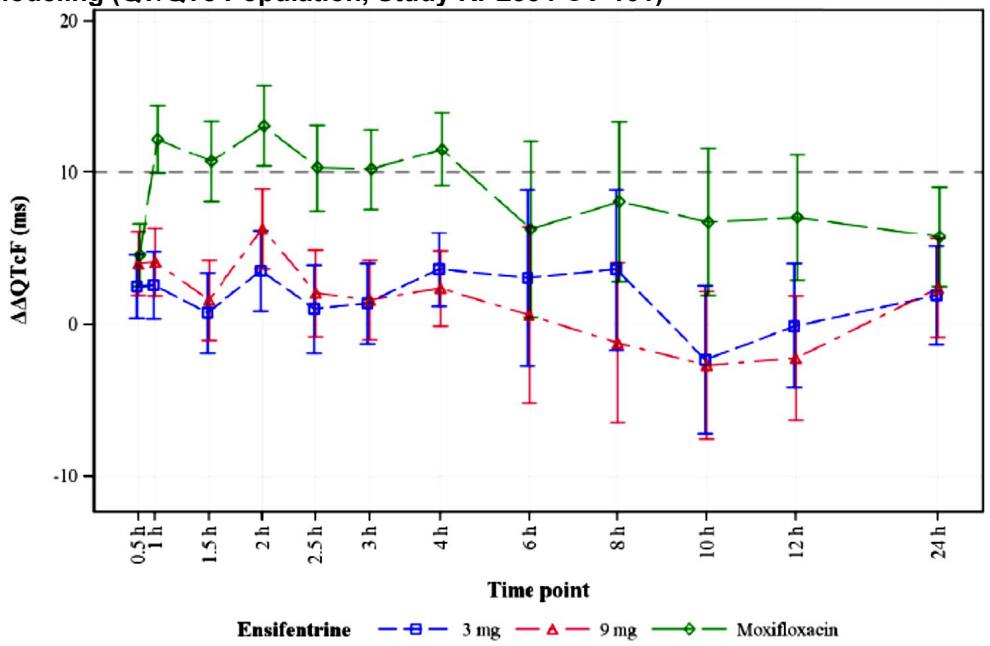
Figure 11. Graphical Representation of Predicted ΔΔQTcF Interval at Geometric Mean Peak Plasma Concentrations for Ensifentrine (Pharmacokinetic/QTc Population, Study RPL554-CV-101)



Source: RPL554-CV-101 CSR, Figure 4
Abbreviations: ΔΔQTcF, placebo-corrected change from baseline-corrected QT interval for heart rate using the Fridericia method; ms, milliseconds; QTc, corrected QT interval

The by-timepoint analysis of ΔΔQTcF is presented graphically in Figure 12. The LS mean ΔΔQTcF across postdose timepoints in the active treatment periods ranged from -2.7 ms (at 10 hours postdose) to 6.3 ms (at 2 hours postdose), both with ensifentrine 9 mg. The upper bound of the 90% CI was below 10 ms across both doses and at all timepoints. Because a substantial HR effect was not observed, other correction methods were not explored.

Figure 12. Placebo-Corrected Change From Baseline QTcF Across Timepoints With Statistical Modeling (QT/QTc Population, Study RPL554-CV-101)



Source: RPL554-CV-101 CSR, Figure 6
Abbreviations: ΔΔQTcF, placebo-corrected change from baseline -corrected QT interval for heart rate using the Fridericia method; QTc, corrected QT interval; QTcF, QT corrected for heart rate by Fridericia's cube root formula

In summary, exposure to ensifentrine increased 4.3-fold from 3 mg to 9 mg. No significant relationship was found between ΔΔQTcF and ensifentrine plasma concentrations. The estimated slope of the ensifentrine plasma concentration in the C-QTc relationship was not statistically significant (0.0012 ms per ng/mL [90% CI: -0.00012 to 0.00260]). Model predictions of ΔΔQTcF and the upper limit of the 90% CI corresponding to the geometric mean of C_{max} at the supratherapeutic dose of 9 mg (2341.8 pg/mL) were well below 10 ms. This supports the conclusion that ensifentrine does not have a clinically relevant impact on cardiac repolarization at therapeutic doses. Ensifentrine 3 mg and 9 mg did not have a clinically relevant effect on HR (mean ΔΔHR was less than 10 bpm for both ensifentrine 3 mg and 9 mg) or on cardiac conduction parameters (PR and QRS intervals).

14.3. Bioanalytical Method Validation and Performance

The bioanalytical assays used to quantify ensifentrine in human plasma and urine are summarized in Table 66.

Table 66. Summary of Ensifentrine Bioanalytical Methods Used in Each Ensifentrine Clinical Study Relevant to the Assessment of PK

Bioanalytical Method Number (CRO)	Type of Method	Matrix	Completed Clinical Studies [a]
(b) (4) 12294 (b) (4)	LLE-HPLC-MS/MS	Plasma	VRP120120
RD 681/25705HV (b) (4) (b) (4)	PPT/HPLC-MS/MS	Plasma	RPL554-007-2014 RPL554-009-2015
(b) (4) 281355QB02 (b) (4)	PPT/UPLC-MS/MS	Plasma	RPL554-PK-101 RPL554-CO-202 RPL554-CO-203 RPL554-CO-204 RPL554-DP-201 RPL554-CO-205 RPL554-MD-201
206554-19M027 (b) (4)	PPT/UPLC-MS/MS	Plasma	RPL554-CV-101
191520VHTVL_VCU (b) (4)	PPT/UPLC-MS/MS	Plasma	RPL554-PK-102 RPL554-PK-103 RPL554-CO-302
191523MQHTVL_VCU (b) (4)	Dilution/UPLC-MS/MS	Urine	RPL554-PK-102

Source: Summary of Biopharmaceutical Studies, Table 2
Abbreviations: CRO, contract research organization; HPLC, high-performance liquid chromatography; LLE, liquid-liquid extraction; MS/MS, tandem mass spectrometry; PK, pharmacokinetics; PPT, protein precipitation; UPLC, ultra-performance liquid chromatography

This section provides summaries of the different bioanalytical assay methods utilized in clinical studies where the low phosphate to-be-marketed formulation was used.

Method for the Analysis of Ensifentrine in Human Plasma via PPT/UPLC-MS/MS (b) (4)

A method for the quantitation of ensifentrine in human plasma (lithium heparin) using protein precipitation (PPT) ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) was developed and validated at (b) (4) 281355QB02) using the IS (b) (4) and deuterated ensifentrine (RPL554-d_4). The method was validated for the analytical range of 5.00 to 5,000 pg/mL. The method was accurate, precise, reproducible, and specific. The cumulative precision (%CV) from LLOQ to ULOQ ranged from 2.6 to 5.7%. Ensifentrine remained stable in plasma when stored in polypropylene for up to 26 hours at 22°C, at -20°C for up to 183 days, and in up to four freeze/thaw cycles at 22°C. In the presence of (b) (4) ensifentrine was found to be stable in human plasma for up to 25 hours at 22°C and after four freeze/thaw cycles at 22°C. Ensifentrine did not bind to red blood cells for up to 60 minutes. In the presence of activated charcoal, ensifentrine bound strongly in strong and weak acidic conditions. Additional details on this method are provided in Table 67.

Table 67. Summary of Method Performance [redacted] Method Validation

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of a PPT/UPLC-MS/MS method for the determination of RPL554 in human plasma (lithium heparin) [redacted]281355QB02			
Method description	PPT/UPLC-MS/MS			
Materials used for standard calibration curve and concentration	Biological matrix – human plasma (lithium heparin) Ensifentrine diluted for calibration standards: 5.00, 10.0, 40.0, 200, 700, 2000, 4000, 5000 pg/mL			
Validated assay range	5.00 to 5,000 pg/mL			
Material used for quality controls and concentration	Internal standards used were [redacted] and RPL554-d_4. Biological Matrix for QC's was human plasma (lithium heparin). Concentrations of QC's used were 5.00, 15.0, 250 and 3750 pg/mL			
Minimum required dilutions	1 in 10			
Source and lot of reagents	RPL554: [redacted] Lot number AJO74C3 Retest date 17 Oct 2017 RPL554: [redacted] Lot Number: - 131112M Retest Date: - 12-January 2018	[redacted] Lot number MKT8008V Retest date 31 Jan 2017	RPL554-d_4: [redacted] Lot number [redacted]15602 Retest date 05 Dec 2021	[redacted] Lot number 1.1 Retest date: N/A
Regression model and weighting	Linear weighted $1/x^2$			

Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	[redacted]281355QB02, Table 2
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.8 - 3	
	Cumulative precision (%CV) from LLOQ to ULOQ	2.6 – 5.7	
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 4 QCs	5.01: -4.2 15.4: - 0.7 254: -1.2 3790: -2.4	[redacted]281355QB02, Table 8
	Inter-batch %CV in 4 QCs	5.01: 8.0 15.4: 9.3 254: 5.6 3790: 3.2	
	Total Error	N/A	
Selectivity & matrix effect	Selectivity was confirmed for ensifentrine and [redacted] in 6 independent control blank plasma samples. No significant matrix effect was observed across 6 individual control blank human plasma samples at low and high QC levels.		[redacted]281355QB02, Section 12.4
	Selectivity in the presence of [redacted] (20 pg/mL) – there were no significant interfering peaks observed at the retention times of ensifentrine and RPL554-d_4 in 5/6 control blank human plasma samples examined		
Interference & specificity	There were no significant interfering peaks observed at the retention times of ensifentrine and RPL554-d_4 in the presence of 20 pg/mL [redacted] in 5 control black samples.		N/A
Hemolysis effect	QC low: 6.4% CV, 11.3% bias QC high: 3.1% CV, -0.5% bias No significant hemolysis effect was observed on the quantitation of ensifentrine in human plasma.		[redacted]281355QB02, Section 12.6.2
Lipemic effect	QC low: 3.5% CV, -2.0% bias QC high: 1.8% CV, 1.1% bias No significant hyperlipidemic effect was observed on the quantitation of ensifentrine in human plasma.		[redacted]281355QB02, Section 12.6.3
Dilution linearity & hook effect	The precision and accuracy of diluted samples after a one in ten dilution (1:10 or 10-fold dilution) was within ±15% bias of the nominal concentration with a precision within 15% CV. This is considered to validate any dilution up to and including a 1 in 10 dilution. Hook effect is not applicable in MS/MS methods of analysis.		[redacted]281355QB02, Section 12.3.4
Bench-top/process stability	Stable in human plasma stored up to 26 hours at nominally +22°C and [redacted] (20 pg/mL) stored up to 25 hours at nominally +22°C		[redacted]281355QB02, Section 12.7
Freeze-Thaw stability	Stable in human plasma for four cycles at nominally -20°C/+22°C and [redacted] (20 pg/mL) for four cycles at nominally -20°C/+22°C		
Long-term storage	Confirmed up to 183 days at nominally -20°C		
Parallelism	Not applicable for MS/MS-based methods.		N/A
Carry over	For ensifentrine: maximum 13.3% For [redacted] maximum 0.0% For RPL554-d_4: maximum 0.5% These results show acceptable carry over for ensifentrine, [redacted] and RPL554-d_4.		[redacted]281355QB02, Table 30

Source: Summary of Biopharmaceutical Studies, Table 13
Abbreviations: CV, coefficient of variation; [redacted] LLOQ, lower limit of quantitation; MS/MS, tandem mass spectrometry; N/A, not applicable; PPT, protein precipitation; QC, quality control; ULOQ, upper limit of quantitation; UPLC, ultra-performance liquid chromatography

Method for the Analysis of Ensifentrine in Human Plasma via PPT/UPLC-MS/MS (Pharmaron ABS)

A method for the quantitation of ensifentrine in human plasma (lithium heparin) using PPT/UPLC-MS/MS was developed and validated at (b) (4) . The IS was RPL554-d4. The method was validated for the analytical range of 5.00 to 5,000 pg/mL. The method was accurate, precise, reproducible, and specific. Ensifentrine remained stable in plasma when stored in polypropylene tubes for at least 76 days at -70°C, at least 26.5 hours at room temperature, and in up to three freeze/thaw cycles. Additionally, ensifentrine remained stable in whole blood (lithium heparin) when stored in polypropylene for at least up to 2 hours on ice (4°C). No significant carryover was detected for ensifentrine or RPL554-d4. Additional details on this method are provided in Table 68.

Table 68. Summary of Method Performance– (b) (4) Method Qualification

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of a Bioanalytical Method for LC-MS/MS Analysis of Ensifentrine (RPL554) in Human Plasma (Lithium Heparin) 206554-19M027		
Method description	PPT/UPLC-MS/MS		
Materials used for standard calibration curve and concentration	Biological matrix: human plasma (lithium heparin) 5.00, 10.0, 40.0, 200, 700, 2000, 4000, 5000 pg/mL		
Validated assay range	5 to 5,000 pg/mL		
Material used for quality controls and concentration	RPL554-d4 15.0, 250, 3750 pg/mL		
Minimum required dilutions	1 in 100		
Source and lot of reagents	Ensifentrine (b) (4) Lot number 131112M Retest date 03 Nov 2021	RPL554-d4: (b) (4) (b) (4) Lot number 15602 Retest date 05 Dec 2021	
Regression model and weighting	Linear regression (y = ax + b where y=peak area ratio of the analyte to the internal standard and x=concentration of the analyte in the calibration standards) with a $1/x^2$ weighting was applied to the chromatographic peak area ratios (analyte/internal standard) to nominal concentration plot for the construction of calibration curves. This provided information on the slope, the intercept and the coefficient of determination (R^2).		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8 from 5.00 to 5,000 pg/mL	206554-19M07, Table 5
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.8% bias to 2.00 % bias	
	Cumulative precision (%CV) from LLOQ to ULOQ	2.76% to 4.53% CV	
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 5 QCs	7.20% to 12.6% bias	206554-19M07, Table 7
	Inter-batch %CV	2.56% to 7.87% CV	
	Total Error	N/A	
Selectivity & matrix effect	There were ensifentrine interfering peaks in one lot (>20% of the mean LLOQ response), but the remaining lots showed no significant interfering peaks at the retention times of ensifentrine and RPL554-d4.		206554-19M07, Section 5.8
Interference & specificity	Inter lot precision and accuracy was demonstrated using 6 blank matrix lots spiked at the low QC level.		206554-19M07, Section 5.11.2 and Table 15
Hemolysis effect	No significant hemolysis effects were observed on the quantification of ensifentrine.		206554-19M07, Section 5.11.3
Lipemic effect	No significant hyperlipidemic effects were observed on the quantification of ensifentrine.		
Dilution linearity & hook effect	Dilution integrity was assessed by diluting spiked matrix sample with the blank matrix (n=6). The precision and accuracy of 100-fold diluted samples were within 15% CV and within ±15% bias. Hook effect is not applicable to MS/MS methods of analysis.		206554-19M07, Section 5.6
Bench-top/process stability	Demonstrated for 26.5 hours at room temperature		206554-19M07, Section 5.12
Freeze-Thaw stability	Demonstrated for 3 freeze/thaw cycles at -70°C		
Long-term storage	Demonstrated for 76 days at -70°C		
Parallelism	Not applicable for MS/MS-based methods.		N/A
Carry over	No significant carryover was observed for ensifentrine and RPL554-d4		206554-19M07, Section 5.9

Source: Summary of Biopharmaceutical Studies, Table 14
Abbreviations: CV, coefficient of variation; LC, liquid chromatography; LLOQ, lower limit of quantitation; MS/MS, tandem mass spectrometry; N/A, not applicable; PPT, protein precipitation; QC, quality control; R^2, coefficient of determination; ULOQ, upper limit of quantitation; UPLC, ultra-performance liquid chromatography

Method for the Analysis of Ensifentrine in Human Plasma via PPT/UPLC-MS/MS [(b) (4)]

A method for the quantitation of ensifentrine in human plasma (lithium heparin) using UPLC/MS/MS was developed and validated at [(b) (4)] (191520VHTVL_VCU). The IS was RPL554-d4. The method was validated for the analytical range of 5.00 to 5,000 pg/mL. The method was accurate, precise, reproducible, and specific. Ensifentrine remained stable in plasma for 222 days when stored at -70°C, at least 25 hours at room temperature, and in up to five freeze/thaw cycles. Additionally, ensifentrine remained stable in whole blood for up to 2 hours in an ice bath and 2 hours at room temperature. No significant carryover was detected for ensifentrine or RPL554-d4. Additional details on this method are provided in Table 69.

Table 69. Summary of Method Performance– [(b) (4)] Method Validation (Plasma)

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation of RPL554 (Ensifentrine) in Human Plasma by Turbo Ion Spray LC/MS/MS – 191520VHTVL_VCU		
Method description	96-well protein precipitation/UHPLC-MS/MS		
Materials used for standard calibration curve and concentration	RPL554 Calibration Standards were prepared in human plasma (lithium heparin) at concentrations of 5.00, 10.0, 50.0, 200, 500, 2500, 4000 and 5000 pg/mL. The internal standard was RPL554-d_4.		
Validated assay range	5.00 to 5000 pg/mL		
Material used for quality controls and concentration	Quality Controls were prepared in fresh human plasma (lithium heparin) at concentrations of 5.00, 15.0, 1500 and 3750 pg/mL. A Dilution Quality Control of 25000 pg/mL was analysed at a 1 in 10 dilution.		
Minimum required dilutions	1 in 10		
Source and lot of reagents	RPL554 Lot 131112M [(b) (4)] retest dates 30-Nov-2019 and 19-Nov-2020. RPL554-d_4, Lot [(b) (4)] 15602 [(b) (4)] retest date 05-Dec-2021		
Regression model and weighting	Linear, weighted 1/x2		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8 from 5.00 to 5000 pg/mL	191520VHTVL_VCU, Table 3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-0.8 % Bias to 0.6 % Bias	191520VHTVL_VCU, Table 3
	Cumulative precision (%CV) from LLOQ to ULOQ	0.9% CV to 2.7 % CV	191520VHTVL_VCU, Table 3
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 6 QCs	0.6% Bias to 2.7% Bias	191520VHTVL_VCU, Table 6
	Inter-batch %CV	1.8 % CV to 6.0 % CV	191520VHTVL_VCU, Table 6
	Total Error	N/A	N/A
Selectivity & matrix effect	6 Lots, n=18, 5.00 pg/mL, -6.0 % bias to 15.4 % bias – met acceptance		191520VHTVL_VCU, Table 11
Interference & specificity	6 Lots, n=18, no interference was detected in a 15 pg/mL ensifentrine QC plasma sample spiked with 5000 ng/mL fluconazole – met acceptance		191520VHTVL_VCU, Table 15
Hemolysis effect	1 Lot n=3, 5.00 pg/mL, -2.8 % bias to 7.8 % bias, met acceptance.		191520VHTVL_VCU, Table 11
Lipemic effect	1 Lot n=3, 5.00 pg/mL, -4.4 % bias to 9.8 % bias, met acceptance.		191520VHTVL_VCU, Table 11
Dilution linearity & hook effect	Dilution QCs prepared at 25000 pg/mL with 1 in 10 dilutions were assayed in 3 accepted validation runs and gave intra-run bias values between 1.2% bias and 3.2% bias which met acceptance criteria.		191520VHTVL_VCU, Table 5
	The hook effect is not applicable to MS/MS methods of analysis.		
Bench-top/process stability	Processed plasma sample stability at 4°C was 74 hours. Benchtop (room temperature) stability in human plasma was 25 hours		191520VHTVL_VCU, Tables 17 and 18
Freeze-Thaw stability	Stability demonstrated at -20°C and - 70°C over 5 freeze/thaw cycles.		191520VHTVL_VCU, Table 19
Long-term storage	Long term frozen storage stability of ensifentrine in human plasma was demonstrated at -20°C and -70°C for 222 days		191520VHTVL_VCU, Tables 20 and 21
Parallelism	Not applicable to MS/MS analytical methods.		N/A
Carry over	Carryover met acceptance as no MS/MS response was > 20.0% of the mean response ration of the LLOQ standards in the carryover blank.		191520VHTVL_VCU, Section 7.5

Source: Summary of Biopharmaceutical Studies, Table 15
Abbreviations: CV, coefficient of variation; LC, liquid chromatography; LLOQ, lower limit of quantitation; MS/MS, tandem mass spectrometry; n, number; QC, quality control; UHPLC, ultra high-performance liquid chromatography; ULOQ, upper limit of quantitation

Method for the Analysis of Ensifentrine in Treated Human Urine via UPLC/MS/MS [redacted]

A method for the quantitation of ensifentrine in human urine [redacted] polypropylene collection containers) using UPLC/MS/MS was developed and qualified at [redacted]. The IS was RPL554-d_4. The method was qualified for the analytical range of 5.00 to 5,000 pg/mL, which demonstrated acceptable accuracy, precision, and benchtop stability for up to 4 hours and frozen stability at -20°C up to 212 days. Additional details on this method are provided in Table 70.

Table 70. Summary of Method Performance– [redacted] Method Qualification (Urine)

Bioanalytical method validation report name, amendments, and hyperlinks	Method Qualification for the Quantitation of Ensifentrine (RPL554) in Treated Human Urine by Turbo Ion Spray LC/MS/MS. 191523MQHTVL_VCU		
Method description	Human urine samples [redacted] polypropylene collection containers. Dilution with RPL554-d_4 SIL was followed by UPLC-MS/MS using calibration standard covering the range 5.00 to 5000 pg/mL		
Materials used for standard calibration curve and concentration	0.2% Triton X-100 in human urine was spiked with ensifentrine to give standards of 5.00, 10.0, 50, 200, 500, 2500, 4000, and 5000 pg/mL. The stable isotope internal standard was RPL554-d_4.		
Validated assay range	5.00 to 5000 pg/mL		
Material used for quality controls and concentration	0.2% Triton X-100 in human urine was spiked with ensifentrine to give quality controls of 5.00, 15.0, 1500, and 3750 pg/mL. Dilution of Dil. QC 25000 pg/mL was analyzed in a 1 in 10 dilution.		
Minimum required dilutions	1 in 10 dilutions		
Source and lot of reagents	Ensifentrine Lot 131112M [redacted] Retest dates: 30 Nov 2019 and 19 Nov 2020	RPL554-d_4 Lot [redacted] 15602 [redacted] Retest date: 05 Dec 2021	
Regression model and weighting	Linear, $1/x^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8 standard calibrators from 5.00 to 5000 pg/ml	191523MQHTVL_VCU, Table 3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.2 to 1.0 % bias	191523MQHTVL_VCU, Table 3
	Cumulative precision (%CV) from LLOQ to ULOQ	1.5 to 2.6 % bias	191523MQHTVL_VCU, Table 3
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 4 QCs	0.7 to 2.8 % bias	191523MQHTVL_VCU, Table 4
	Inter-batch %CV	2.6 to 6.4% bias	191523MQHTVL_VCU, Table 4
	Total Error	N/A	N/A
Selectivity & matrix effect	Matrix inter-lot selectivity for ensifentrine and RPL554-d_4 both meet acceptance criteria		191523MQHTVL_VCU, Tables 8 and 9
Interference & specificity	Not applicable in method qualification		N/A
Hemolysis effect	N/A in urine		N/A
Lipemic effect	N/A in urine		N/A
Dilution linearity & hook effect	Dilution linearity demonstrated at 25000 pg/mL with 10-fold dilution – 0.8 % bias. Hook effect not relevant to MS/MS methods of analysis		191523MQHTVL_VCU, Table 5
Bench-top/process stability	Benchtop stability demonstrated over 4 hours at room temperature		191523MQHTVL_VCU, Table 10
Freeze-Thaw stability	Not applicable in method qualification		N/A
Long-term storage	Long-term storage stability for Ensifentrine of 212 days at -20°C in treated human urine was demonstrated.		191523MQHTVL_VCU, Table 11
Parallelism	Not applicable to MS/MS methods.		N/A
Carry over	Carry over is typically less than 10%		Analytical Method Summary Appendix to Report 191523MQHTVL_VCU

Source: Summary of Biopharmaceutical Studies, Table 16
Abbreviations: CV, coefficient of variation; LC, liquid chromatography; LLOQ, lower limit of quantitation; MS/MS, tandem mass spectrometry; N/A, not applicable; QC, quality control; UPLC, ultra-performance liquid chromatography; ULOQ, upper limit of quantitation

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Not applicable.

14.5. Pharmacometrics Assessment

Population Pharmacokinetic Analyses

The Applicant has conducted a population PK analysis to characterize ensifentrine PK in healthy subjects and COPD subjects and to evaluate the intrinsic and extrinsic factors that impact variability in PK. All PK data from the clinical studies conducted in healthy volunteers and COPD subjects with the ensifentrine suspension formulations were used to build the popPK model. The analysis included pooled data for 1,070 subjects from the following studies: RPL554-007-2014, RPL554-PK-101, RPL554-PK-102, RPL554-009-2015, RPL554-CO-202, RPL554-CO-204, RPL554-CO-203, RPL554-CO-302, RPL554-CV-101, and RPL554-PK-103. One thousand thirty-six subjects with sufficient dosing and post-dose PK observations were included for PK modeling. These included 293 subjects with full PK profiles, of which 147 subjects were healthy/non-COPD subjects, 146 subjects with moderate to severe COPD, and 743 subjects with moderate to severe COPD with sparse sampling. The covariates tested in the model included the effects of body weight, age, race, ethnicity, sex, creatinine clearance, or estimated glomerular filtration rate, dose on apparent clearance or F1 (relative bioavailability), concomitant medications, CYP2C9 and CYP2D6 polymorphism, renal impairment, hepatic impairment, liver function enzymes (e.g., ALT, AST, bilirubin, ALP, albumin), formulation (high phosphate suspension versus low phosphate suspension formulation), and disease status (COPD versus healthy subjects).

The final population PK model was a two-compartment model with first-order absorption, including inter-individual variability on clearance, V2, Q, V3, and Ka estimated with a full covariance matrix and with inter-occasion variability on F1. Covariate effects included in the final model were effects of disease state (COPD versus non-COPD) and additionally studies RPL554-CO-203 and RPL554-CO-302 on F1, a lower Ka for RPL554-CO-302, an effect of moderate renal impairment on clearance, and a small effect of dose on F1. Residual variability was modeled by a proportional component.

The covariate analyses showed there were no significant effects of body weight, age, sex, race, ethnicity, ALT, AST, bilirubin, ALP, gamma-glutamyl transferase, dose, or concomitant inhibitors of CYP2D6, proton pump inhibitors, short or long acting $beta_2$ adrenergic agonists, muscarinic antagonists, or inhaled corticosteroids on clearance. Parameter estimates from the final population PK model are shown in Table 71.

Table 71. Parameter Estimates and Bootstrap Results for the Final Population PK Model of Ensifentrine

Parameter (Units)	NONMEM Estimate (%RSE) [eta shrinkage]	Bootstrap Estimate (%RSE) [2.5th – 97.5th Percentile]
CL/F (L/h)	430 (0.9)	416 [388-443]
V2/F (L)	2700 (0.6)	2680 [2490-2880]
Q/F (L/h)	227 (1.4)	216 [191-243]
V3/F (L)	1820 (0.9)	1790 [1600-1990]
Ka (/h)	3.57 (5.3)	3.60 [3.17-4.12]
covF1_study_CO-302	0.509 (7.7)	0.520 [0.469-0.578]
covF1_study_CO-203	0.674 (7.7)	0.676 [0.578-0.803]
covKA_study_CO-302	0.628 (21.9)	0.632 [0.508-0.782]
covF1_COPD	0.651 (10.7)	0.618 [0.574-0.664]
covF1_dose_high_phosphate	-0.0913 (18.9)	-0.116 [-0.158 - -0.0715]
covCL_moderate_renal_impairment	0.749 (20.8)	0.752 [0.662-0.861]
IIV CL/F (%CV)	63.2 (5.9) [10.2]	62.7 [58.2-67.1]
IIV V2/F (%CV)	52.5 (12.6) [42.1]	54.0 [46.5-63.0]
IIV Q/F (%CV)	84.2 (15.5) [27.0]	79.6 [66.6-95.1]
IIV V3/F (%CV)	68.0 (13.7) [31.6]	66.6 [58.6-76.8]
IIV Ka (%CV)	109 (13.6) [29.0]	101 [84.2-120]
IOV F1 (%CV)	35.6 (7.3) [40.8]	35.6 [32.3-39.1]
PROPRES (%)	15.5 (2.7)	15.4 [14.6-16.1]
PROPRES_study_CO-203 (%)	48.4 (5.6)	48.4 [44.2-52.7]
PROPRES_study_CO-302 (%)	33.5 (3.1)	33.4 [31.1-35.5]
IIV PROPRES (%CV)	38.2 (9.4) [34.8]	38.3 [34.0-42.0]

Source: 5768-PKDP001 Report, Table 4-5
Abbreviations: CL/F, apparent clearance; cov, covariate; IIV, inter-individual variability; IOV, inter-occasion variability; Ka, acid dissociation constant; NONMEM, non-linear mixed-effects modeling; PK, pharmacokinetics; Q/F apparent intercompartmental clearance; RSE, relative standard error V2/F, apparent central compartment; V3/F apparent peripheral compartment

The results identified 34.9% lower F1 for COPD versus non-COPD subjects, which was also estimated within the rich-PK data subset, and a further 32.6% and 49.1% proportional decrease in F1 for Studies RPL554-CO-203 and RPL554-CO-302, respectively. Rate of absorption was also decreased by 27.2% in Study RPL554-CO-302 and could not be estimated for Study RPL554-CO-203, which only collected samples ~12 hours postdose (predose at Weeks 2 and 4). These changes partly reflect the disease and disease severity, particularly for RPL554-CO-302, the phase 3 study that included subjects with more severe COPD. Additionally, for RPL554-CO-203, the timing of evening dosing prior to the morning trough sample collection was not captured, thus these values may or may not have reflected a true 12-hour postdose trough.

Following the prespecified covariate analysis, the disease status and study effects on F1 appeared as a formulation effect. However, the disease and apparent formulation effects were confounded within COPD studies, as the low phosphate formulation was only used later in Study RPL554-CO-302 (and non-COPD PK studies), while Study RPL554-CO-203 was conducted with the high phosphate formulation; however, Study RPL554-CO-203 contributed very little information for estimation of the extent and rate of absorption due to its highly sparse PK sampling design. Within the richly sampled subset, which included both COPD and non-COPD subjects treated with the high and low phosphate formulations, formulation was not found to be a significant covariate for absorption.

In the final model, a 25.1% reduced clearance of ensifentrine was estimated for subjects with moderate (n=95) or severe (n=2) renal impairment. Continuous covariates of renal function were

statistically weaker or insignificant. Because there were too few subjects with severe renal impairment, further discussion of this effect is limited to moderate renal impairment. Ensifentrine is mainly eliminated through metabolism, and this covariate may reflect the confounding of renal impairment with other associated patient factors in the COPD dataset and that reduced hepatic elimination is associated more generally with renal impairment. The increase in systemic exposure of ensifentrine in subjects with mild or moderate renal impairment is not considered clinically relevant and does not warrant a dose adjustment given the high variability of ensifentrine PK in subjects with COPD, limited renal clearance of ensifentrine, the safety profile for ensifentrine doses up to 6 mg BID for 4 weeks that showed a safety profile (i.e., adverse events, ECGs, Holter monitoring, vital signs and laboratory safety tests) similar to placebo, and the thorough QT results that confirm no effect on the QT interval or other cardiac conduction parameters with a 9 mg dose of ensifentrine.

Overall, a two-compartment model with first-order absorption and linear elimination process adequately described the PK of ensifentrine. Covariate analyses found:

- Formulation (high phosphate or low phosphate suspension) was not a significant covariate for ensifentrine exposure.
- Significant reductions in bioavailability for COPD subjects compared to non-COPD subjects, but no apparent further effects of disease severity within the phase 3 population.
- Moderate renal impairment was associated with a modest (25%) reduction in apparent clearance.
- No significant covariate effects from body weight, sex, race, ethnicity, markers of liver function (ALT, AST, bilirubin, ALP, gamma-glutamyl transferase), concomitant CYP2D6 inhibitors, proton pump inhibitors, or concomitant use of beta2 adrenergic agonists, muscarinic antagonists, or inhaled corticosteroids.

14.6. Pharmacogenetics

Not applicable.

15. Study/Trial Design

Trials RPL554-CO-301 (Trial 301) and RPL554-CO-203 (Trial 302) were phase 3, multicenter, randomized, double-blind, parallel-group, placebo-controlled trials to determine the efficacy and safety of ensifentrine 3 mg twice daily for 24 weeks administered via nebulizer compared to placebo in subjects 40 to 80 years of age with moderate to severe COPD. Trial 301 included an additional placebo-controlled safety cohort that was evaluated over 48 weeks (this cohort was not present in Trial 302). Overall, the two phase 3 trials were similar in design and appear adequate and well controlled. The general trial design will be reviewed together with highlights of the few differences.

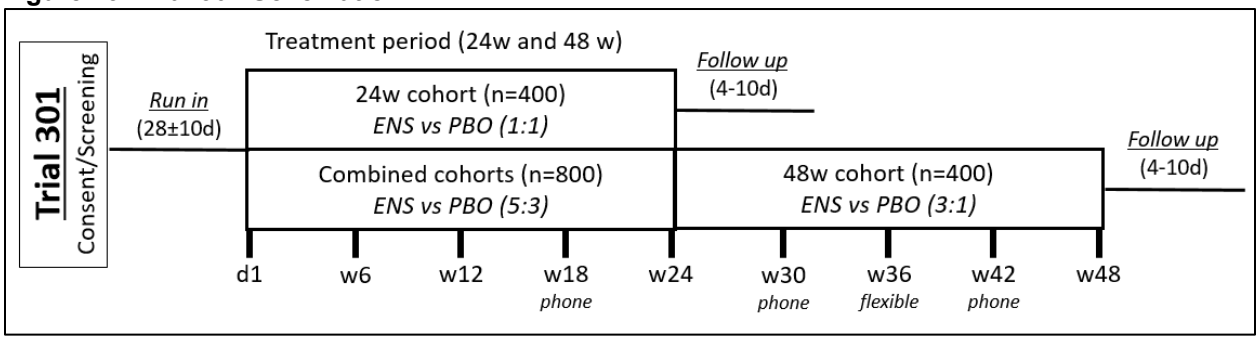
Trial 301 took place from September 29, 2020 (first subject screened) to December 02, 2022 (last subject completed) and included 120 study centers in 12 countries. Trial 302 took place

from September 22, 2020 (first subject screened) to July 6, 2022 (last subject completed) and included 130 study centers in 10 countries.

Subjects were randomized 5:3 active to placebo (in Trial 301, the cohorts were randomized 1:1 active to placebo in the 24-week cohort and 3:1 active to placebo in the 48-week cohort). Randomization was stratified by the following factors: treatment duration (cohort), stable background maintenance long-active muscarinic antagonists (LAMA) or long-acting beta$_2$-agonists (LABA) therapy use (yes or no), and cigarette smoking (current or former). The enrollment goals were 800 subjects per trial.

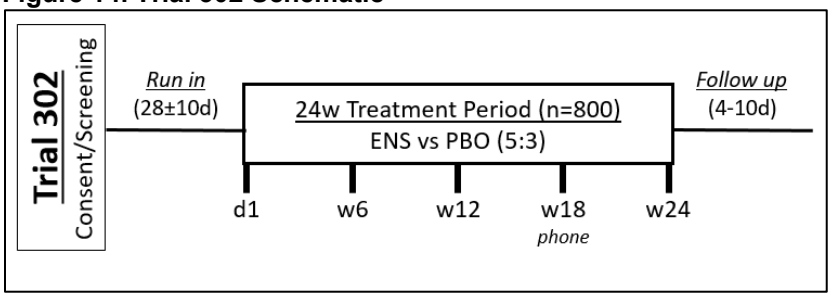
Subjects were screened for eligibility prior to entering a 28-day run-in period to ensure a stable COPD treatment regimen and collect baseline information. Subjects then entered the treatment period (24 or 48 weeks) during which they were assessed every 6 weeks (via either a clinical visit or a phone contact). Following the treatment period, subjects were to complete a Follow-Up Visit within 4 to 7 days. The trial schematics are shown below in Figure 13 and Figure 14.

Figure 13. Trial 301 Schematic



Source: Clinical Reviewer based on information provided by Applicant (Trial RPL554-CO-301 Protocol V6.0, Figure 1.2 Schema, page 637)
Abbreviations: d, days; ENS, ensifentrine; n, target number of subjects to enroll in treatment group; PBO, placebo; w, weeks

Figure 14. Trial 302 Schematic



Source: Clinical Reviewer based on information provided by Applicant (Trial RPL554-CO-302 Protocol V5.0, Figure 1.2 Schema, page 525)
Abbreviations: d, days; ENS, ensifentrine; n, target number of subjects to enroll in treatment group; PBO, placebo; w, weeks

Background Therapy

Trials 301 and 302 aimed to recruit approximately 50% of subjects that using stable background maintenance COPD medication defined as either a LAMA or LABA. LAMA and LABA combination therapy was not permitted. Up to 20% of subjects were permitted to also be on

inhaled corticosteroids (ICS), provided this was in combination with either LABA or LAMA therapy. ICS monotherapy was not permitted.

Study Population

Key Inclusion Criteria

- Age: 40 to 80 years of age inclusive at the time of Screening
- Smoking History: history of ≥10 packyears at Screening
  - Former smokers were defined as those who had stopped smoking for at least 6 months prior to Visit 0
- Established clinical history of COPD as defined by American Thoracic Society/European Respiratory Society guidelines (Celli et al. 2004)
- COPD symptom score ≥2 on the modified Medical Research Counsel dyspnea scale
- Post-SABA FEV_1 ≥30% and ≤70% of predicted normal calculated by NHANES III (Hankinson et al. 1999)
- Maintenance COPD therapy: patients on no maintenance/background therapy or patients on stable maintenance as either LAMA or LABA therapy
  - Must demonstrate stable use of maintenance LAMA or LABA therapy for at least 2 months prior to Screening and agree to continue use of their current permitted LABA or LABA medication
- Capable of withholding the following prior to initiation of any spirometry
  - SABA therapy – 4 hours
  - Twice daily LAMA or LABA therapy – 24 hours
  - Once daily LAMA or LABA therapy – 48 hours

Key Exclusion Criteria

- History of life-threatening COPD including ICU admission and/or requiring intubation
- Hospitalization for COPD, PNA, COVID-19 within 12 weeks prior to Screening
- COPD exacerbation requiring oral or parenteral steroids within 3 months of Screening
- Previous lung resection or lung reduction surgery within 1 year of Screening
- Long term oxygen use (therapy prescribed for >12 hours per day)
- Pulmonary rehabilitation, unless in a stable maintenance phase for 4 weeks prior to Visit 1 and remains stable during the study
- Lower respiratory tract infection within 6 weeks of Screening
- Other respiratory disorders including current diagnosis of asthma, TB, lung cancer, sarcoidosis, lung fibrosis, ILDs, unstable sleep apnea, known A1AT deficiency, cor

pulmonale, clinically significant PH, clinically significant bronchiectasis, or other active pulmonary disease

- History or current evidence of clinically significant cardiovascular disease
- History of or current malignancy or any organ system, treated or untreated with in the past 5 years, except for localized basal or squamous cell carcinoma of the skin
- Current or active drug or alcohol abuse within the past 5 years
- Laboratory or other diagnostic parameters
  - GFR <40 mL/min
  - ALT ≥2x upper limit of normal (ULN)
  - Alkaline phosphatase >1.5x ULN
  - Bilirubin >1.5x ULN (isolated bilirubin >1.5x ULN is acceptable if bilirubin is fractionated and direct bilirubin is <35%)
  - CXR at screening, or within past 12 months, with clinically significant abnormalities not attributable to COPD

Key Prohibited Medications (Time Interval)

- Oral, Systemic, or Parenteral Steroid therapies (3 months)
- Antibiotics for lower respiratory tract infection (6 weeks)
- Inhaled corticosteroids (4 weeks)
- Oral leukotriene inhibitors (48 hours)
- Theophylline and PDE4 inhibitors (48 hours)
- Terbutaline (1 day)
- Ipratropium (6 hours)
- LAMA/LABA combination products (4 weeks)
- Nebulized LAMA or LABA or ICS
- No maintenance therapy (4 weeks)
- In LAMA or LABA stratum (1 week)
- Oral beta2-agoinsts (1 week)

Stable use of ICS was allowed if the patient had been taking the medication for at least 4 weeks prior to Screening and if the patient was also taking maintenance LABA or LAMA therapy. ICS was not to be initiated or discontinued during the study. ICS monotherapy and high dose ICS (e.g., >1000 µg fluticasone propionate or equivalent) were not permitted.

Trial Objectives and Endpoints

The primary objective of Trials 301 and 302 was to evaluate the efficacy of ensifentrine on lung function compared to placebo over a 12-hour dosing interval in subjects with moderate to severe COPD. To evaluate this, the primary endpoint for Trials 301 and 302 was the change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12.

The secondary endpoints included:

- Peak FEV_1 over 4 hours at Week 12 (change from baseline)
- Evaluating-Respiratory Symptoms (E-RS) Total Score at Week 24 (change from baseline as a weekly average)
- St. George's Respiratory Questionnaire (SGRQ) total score at Week 24 (change from baseline)
- Morning trough FEV_1 at Week 12 (change from baseline)
- FEV_1 $AUC_{0\text{-}4h}$ postdose at Week 12 (change from baseline)
- The proportion of SGRQ responders at Week 24
- Rescue medication use at Week 24 (change from baseline)
- Transitional Dyspnea Index (TDI) at Week 24
- Evening trough FEV_1 at Week 12 (change from baseline)
- Peak FEV_1, morning and evening trough FEV_1, FEV_1 $AUC_{0\text{-}4h}$, E-RS Total Score, SGRQ responder analysis, and TDI at other Study Visits (change from baseline)
- Rescue medication use at Weeks 6 and 12 (change from baseline)

Other endpoints outlined by the Application are listed below.

- Moderate/severe exacerbation frequency over 24 and 48 Weeks
- Time to first moderate/severe COPD exacerbations over 24 and 48 Weeks
- Study withdrawal before 24 and 48 Weeks
- EuroQol-5-Domain Questionnaire at Week 12 (change from baseline)
- Healthcare Resource Utilization over 24 and 48 Weeks
- FEV_1 $AUC_{6\text{-}12h}$ postdose at Week 12 (change from baseline)

COPD Exacerbation Definition

The Applicant included the following criteria to define a COPD exacerbation.

A COPD exacerbation was defined as the worsening of two or more major symptoms for at least two consecutive days or the worsening of one major symptoms with one minor symptoms for at least 2 consecutive days.

- Major Symptoms
  - Dyspnea
  - Sputum volume
  - Sputum purulence (color)
- Minor Symptoms
  - Sore throat
  - Colds (nasal discharge and/or nasal congestion)
  - Fever (oral temperature >37.5°C) without other cause
  - Increased cough
  - Increased wheeze

For the grading of severity, a moderate COPD exacerbation was defined as worsening of symptoms of COPD requiring a minimum of 3 days of treatment with oral or systemic corticosteroids and/or antibiotics. A severe COPD exacerbation was defined as the worsening of symptoms COPD requiring in-patient hospitalization. The definition and severity grading of COPD exacerbations is reasonable.

Statistical Analysis Plan

Sample Size Calculation

Approximately 800 subjects were planned to be randomized. The standard deviation for the change in FEV_1 $AUC_{0\text{-}12h}$ was estimated to be 250 mL. With a 2-sided test at a 5% significance level and 500 versus 300 evaluable subjects in the two groups, there would be a 90% power to detect a true difference of 59 mL between the treatments. If the withdrawal rate at 24 weeks exceeds 25% overall, additional subjects were to be randomized to the study to compensate for the loss of data.

Analysis Population Definitions

The Applicant defined the analysis populations used in this review as follows:

- All subjects enrolled set – All subjects who provided consent.
- All subjects randomized set – All subjects who were randomized to study medications. This was the intent-to-treat (ITT) population.
- Modified intent-to-treat (mITT) population – All subjects who were randomized and received at least one dose (or partial dose) of study medication, classified according to randomized treatment.
- Per-protocol population – All subjects in mITT population who did not discontinue the study medication before study Day 74 and did not experience any major/important protocol deviations.
- Safety analysis set – all subjects who received at least one dose (or partial dose) of study medication, classified according to treatment received.

These analysis populations exclude data from the two sites closed for good clinical practice noncompliance and concerns with data reliability (Sites 1721 and 1768 in Trial 301 and Site 1741 in Trial 302; see Section 10 for more information). The Applicant submitted additional datasets that included the subjects that had been enrolled in these sites as part of 'extended' data sets. The review will not include the extended data sets/subjects enrolled at the closed sites unless specifically noted.

Primary Estimand

- Treatments – Ensifentrine 3 mg nebulizer suspension, placebo nebulizer solution
- Population – Subjects with moderate to severe COPD aged 40 to 80 years (inclusive) that are in the mITT population
- Endpoint – Change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12
- Intercurrent events (handling strategy):
  - Premature treatment discontinuation (treatment policy strategy)
  - Major protocol deviation (treatment policy strategy)
- Population-level summary – Treatment difference at Week 12 of the double-blind period

Primary Efficacy Variable & Derivation

The primary efficacy variable was change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12. FEV_1 data captured in the eResearch Technology database were used for the model in determining

baseline value and for AUC calculation. For $AUC_{0\text{-}T}$, spirometry (FEV_1) was performed at Screening and the following time points during, each treatment:

- Day 1: ≤40 minutes predose (2 measurements); 30 minutes, 1, 2, and 4 hours postdose
- Week 6 and Week 24: ≤40 minutes predose; 30 minutes, 1, 2, and 4 hours postdose
- Week 12: ≤40 minutes predose; 30 minutes, 1, 2, 4, 6, 8. and 12 hours postdose
- Early Termination and no Week 12 visit spirometry assessment: ≤40 minutes predose; 30 minutes, 1, 2, 4, 6, 8, and 12 hours postdose
- Early Termination but with Week 12 visit spirometry assessment: ≤40 minutes predose; 30 minutes, 1, 2, and 4 hours postdose

Baseline FEV_1 was the mean of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes predose on Day 1.

The average effect was calculated as the AUC divided by the length of the time interval of interest (for example, 12 hours for $AUC_{0\text{-}12h}$ or 4 hours for $AUC_{0\text{-}4h}$). AUC was calculated using the trapezoidal method, see Figure 15 below.

Figure 15. Trapezoidal Method Equation

$$AUC=\frac{1}{2}\sum_{i=1}^{n-1}(T_{i+1}-T_i)(C_{i+1}+C_i)$$

Source: RPL554-CO-301 SAP page 46

Where T_i is the i^{th} time value, C_i is the i^{th} effect value, n is the number of time values. The change from the baseline in FEV_1 (AUC) was summarized and analyzed. If two last data points were missing the AUC was set to missing, that is for $AUC_{0\text{-}12h}$ the Applicant required at least up to 8 hours of assessments and for $AUC_{0\text{-}4h}$ they required at least up to 2 hours of assessments. If there was no predose measurement available for the calculation of the AUC, then the AUC was not calculated. The predose measurement used in the calculation of AUC is the latest value measured ≤40 minutes predose. The predose value was used as time 0 when computing $AUC_{0\text{-}T}$.

For $AUC_{6\text{-}12h}$, the Applicant required at least the 6-hour and 12-hour timepoint for analysis.

Primary Efficacy Analysis Model

Efficacy analysis was primarily performed on the mITT population. The primary endpoint, change from baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12, was compared between treatments using an analysis of covariance (ANCOVA) model adjusting for treatment, region, background medication strata, and smoking status as factors and baseline FEV_1 as covariate. The estimated treatment difference with 95% confidence interval and associated (two-sided) p-value was presented.

The main analysis for the primary endpoint included estimates from missing data imputation described below. This was done to investigate the influence of missing data due to drop-outs. Only subjects with non-missing baseline were included in the analysis.

The primary analysis was analyzed using multiple imputation (MI) based on the missing data assumption missing at random (MAR). This process followed four steps:

1) Partially impute the data for a monotone missing pattern with 100 as a number of imputations and "20905" as seed number. Employ the Markov Chain Monte Carlo method with treatment, region, background medication strata, smoking strata, baseline FEV_1 and average FEV_1 $AUC_{0\text{-}12h}$ at Week 12.
2) Impute the monotone data using a regression imputation model. Apply by imputation using seed number "20905" with treatment, region, background medication strata, smoking strata, baseline FEV_1, and average FEV_1 $AUC_{0\text{-}12h}$ at Week 12.
3) Each multiple imputed dataset in Step 2 is analyzed separately using the ANCOVA model as described above.
4) The estimates (LS means, SE, CI, and p-value) from the model analysis in Step 3 are combined using Rubin's combination rules for statistical inference.

Missing Data Sensitivity Analyses

The following sensitivity analyses were performed to assess the sensitivity of the missing data assumption of the primary efficacy analysis.

- Observed data analysis was performed by the following subgroups:
  - Analysis with no data imputation, including all subjects with data collected, on treatment or not, and at required timepoint or at EOS visit (excluding subjects with missing data).
  - Analysis with no data imputation, including only subjects with data collected at the required visit while still on randomized treatment at scheduled visit, or at end of study visit within the visit window.
- Average imputation was based on data collected on treatment at required visit, or at early termination visit. This was done at the subject level. If there was no such data available, imputed with average change from baseline for the particular visit in the opposite randomized treatment group.
- Tipping point approach repeated the ANCOVA analysis, under Missing not at Random assumption, to assess the robustness of p-value significance. In case the original analysis produced a p-value <0.05, then the imputed values were to be adjusted by a value k (positive) added or subtracted (depending on what worsens the endpoint) to the endpoint of the subjects in ensifentrine group (worsening results) and added or subtracted (depending on what improves the endpoint) from the endpoint of the subjects in placebo group (improving results). The value for k was determined by adjusting the bias starting with a value of 0.01 and increasing it with increments of 0.01 until p-value obtained from MI method was no longer statistically significant (p-value >0.05). Seed number used were the same as for MI analysis, "20905". If the primary analysis was not significant (p-value >0.05), then the tipping point was reversed by determining value for k by adjusting the bias until p-value ≤0.05. If the p-value <0.05 with more improvement in the placebo group comparably to ensifentrine group, then the tipping point was reversed by adjusting until there were no more treatment difference between groups.

Secondary Efficacy Analysis Model

All variables used in change from baseline analysis (including average FEV_1 AUC_{0-4h}, peak FEV_1, morning trough FEV_1, evening trough FEV_1, mean weekly E-RS^{TM}: COPD, SGRQ) were analyzed using ANCOVA approach, as used for the primary efficacy analysis.

- Peak FEV_1 was computed as the maximum value in the 4 hours after dosing (4 timepoints after dosing: 30 minutes, 1 hour, 2 hours and 4 hours). Baseline FEV_1 was the average of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes predose on Day 1. The change from baseline in peak FEV_1 at Week 12 was analyzed using the ANCOVA approach.
- EXACT-Respiratory Symptoms for COPD (E-RS^{TM}: COPD) data were collected within EXACT-patient reported outcome questionnaire (consisting of 14 questions). E-RS^{TM}: COPD consists of 11 questions, with subdomains of: Breathlessness (scale range: 0 to 17), Cough and Sputum (0 to 11), and Chest Symptoms (0 to12). This data was to be collected everyday throughout the duration of the study. The E-RS Total score was derived as the sum of the raw scores of the 11 items (score range: 0 to 40). Baseline was the mean over the 7 days prior to the first intake of study treatment, using only days where data was recorded. If less than 4 days of data were available for the week, the weekly mean was set to missing. The change from baseline (i.e., mean over the last 7 days of run-in) to the mean weakly value at Week 24 in E-RS^{TM}: COPD was analyzed using the ANCOVA approach.
- The SGRQ is a questionnaire consisting of 17 questions and split into two parts. Part 1 consists of the first eight questions and is related to the Symptoms subdomain. The remaining nine questions are in Part 2, which are related to the Activity (items 11 to 15) and Impacts subdomains (items 9, 10, 12, 13, 14, 16 and 17). Each possible answer to each question has a weight assigned described in the Appendix 5 of the statistical analysis plan. The score for each component is calculated separately by dividing the summed weights by the maximum possible weight for that component and expressing the result as a percentage. The higher the score, the more severe impact of COPD on subject's life. The statistical analysis plan indicates that it was not possible to have missing items in the questionnaire, as the data were collected in an electronic tablet which doesn't allow subjects to skip between items. The change from baseline in the SGRQ total score at Week 24 was analyzed using the ANCOVA approach.
- The change from baseline FEV_1 to morning trough FEV_1 at Week 12 was analyzed using the ANCOVA approach.
- The change from baseline FEV_1 to average FEV_1 AUC_{0-4h} at Week 12 was analyzed using the ANCOVA approach.
- A proportion of responders, those with an improvement from baseline in SGRQ total score of 4 or more, was analyzed using a logistic regression model with fixed effects for treatment, region, actual background medication strata and actual smoking strata at baseline, and baseline SGRQ total score as covariate. For the analysis of proportion of SGRQ, weekly mean total E-RS and Morning trough FEV_1 responders, an analysis was performed using MI based on the missing data assumption MAR. Additionally, in the first two steps the MIN=

and MAX= options will be used to restrict the possible values that can be drawn referred to each endpoint.

For missing data handling for secondary efficacy endpoints, the following imputation methods were applied:

- MI method – continuous data: The analysis for key secondary endpoints (FEV_1 AUC_{0-4h}, peak FEV_1, morning trough FEV_1, SGRQ total score and weekly mean E-RS total score) and for evening trough FEV_1 and weekly rescue medication were performed using MI based on the missing data assumption MAR.
- MI method – binary data: For the analysis of proportion of SGRQ, weekly mean total E-RS and Morning trough FEV_1 responders, an analysis was performed using MI based on the missing data assumption MAR.
- MI method – range data: The sensitivity analysis for key secondary endpoints (SGRQ total score and weekly mean E-RS total score) and the main analysis of TDI were performed using MI based on the missing data assumption MAR as described in MI method – continuous data. Additionally, in the first two steps, the MIN= and MAX= options were used to restrict the possible values that can be drawn referred to each endpoint.

Multiplicity Adjustment

To address multiplicity in the analysis of the endpoints, statistical testing of the primary endpoint and key secondary endpoints was done in the hierarchical order (listed below). Initially, the primary endpoint was checked to see if comparison between active treatment and placebo was statistically significant. If yes, the first key secondary endpoint was evaluated. If yes, the next key secondary endpoint was evaluated. This process continued until either all endpoints were evaluated, or if one of the endpoints' comparisons was not significant.

Order of the endpoints that were used for the hierarchical testing:

(1) Change from baseline FEV_1 to FEV_1 AUC_{0-12h} at Week 12

(2) Change from baseline FEV_1 to Peak FEV_1 at Week 12

(3) Change from baseline (i.e., mean over the last 7 days of run-in) to the mean weekly value at Week 24 in COPD symptoms, as measured by daily diary (E-RS^{TM}:COPD)

(4) Change from baseline in the SGRQ total score at Week 24

(5) Change from baseline FEV_1 to Morning Trough FEV_1 at Week 12

(6) Change from baseline FEV_1 to average FEV_1 AUC_{0-4h} at Week 12

(7) Proportion of SGRQ Responders at Week 24 (defined as an improvement in at least 4 units from baseline in total SGRQ score)

Other Efficacy Analyses

The other efficacy analyses were performed for the mITT population. The rate of and time to first moderate/severe COPD exacerbations were analyzed in three cut-off rules:

1. All subjects (subjects in 24-week and 48-week strata combined) using full study length
2. Period truncated at Week 24 visit (subjects in 24-week and 48-week strata combined)
3. Subjects in 48-week stratum using full study length

For each cut-off rule, the number of moderate/severe COPD exacerbations was analyzed using a negative binomial model adjusting for treatment, region, actual background medication strata and actual smoking strata, as well as using log study time (years) as an offset. If the model did not converge, the covariate that caused the issue was removed from the model. The number of subjects who experienced exacerbations and the number of exacerbations were also presented using the data used to analyze negative binomial model. The logic for time in study used as offset or for censoring purpose is as follows:

- For 24-week completers, use the day of final Week 24 clinic visit. For 48-week completers, use the day of the final Week 48 clinic visit.
- For non-completers who were not lost to follow-up, use the end of study date, which is assumed to be their end of study visit.
  - If no end of study date, use the last site contact out of any assessment (excluding eDiary).
- For subjects who were lost to follow-up, the censoring date was their last reported site contact.

Moderate/severe exacerbations occurring after the day used as offset were not considered in the analysis. The treatment difference from the negative binomial model was expressed as an annualized risk ratio. The data were also summarized by treatment group over duration of the study period. Only moderate/severe COPD exacerbations meeting the protocol-specified criteria were presented in the listing.

Time to first moderate/severe COPD exacerbation during the study period was presented for all subjects using full study length, all subjects using data truncated at the Week 24 visit, and subjects in 48-week stratum. The primary analysis included log-rank test stratified by region, actual background medication strata and actual smoking strata. Summary of number of subjects with events and number of subjects censored was presented. Outcome was to be visualized using a Kaplan-Meier plot. Sensitivity analysis for time to first moderate/severe COPD exacerbation was done for the three data groups used in the main analysis: all subjects, period truncated at the Week 24 visit, and subjects in 48-week stratum. The analysis was done using Cox proportional hazards regression model stratified by region, background medication strata, and smoking strata. Subjects without exacerbations were censored (as described in the logic for time in study above) at last day in the treatment period, including period of recording after possible treatment withdrawal. Moderate/severe COPD exacerbations that happened in the follow-up period were excluded from the analysis since these occur after censoring. The treatment difference in the Cox model was expressed as a hazard ratio.

Adverse Event and Serious Adverse Event Definitions

An adverse event (AE) was defined appropriately as any untoward occurrence in a subject temporally associated with the use of blinded study medication, whether or not considered related to the blinded study medication. This included exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency or intensity of condition or new conditions detected or diagnosed after blinded study medication administration even though it may have been present before the start of the study. "Lack of efficacy" or "failure of expected pharmacological action" were not reported as AEs or serious adverse events (SAEs) and such instances were to be captured in the efficacy assessments.

Specific events that were listed by the Applicant to not meet the AE definition are summarized as follows:

- Any clinically significant abnormal laboratory finding or other abnormal safety assessments associated with the underlying disease, unless judged to by the Investigator to be more severe than expected for the subject's condition
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition
- Anticipated day-to-day fluctuations or pre-existing disease or condition present or detected at the start of the study that do not worsen

An SAE was appropriately defined as any untoward occurrence that, at any dose results in death, is life-threatening, requiring in-patient hospitalization or prolongation of existing hospitalization, results in persistent disability/incapacity, or is a congenital anomaly/birth defect. Additionally, other important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed in the SAE definition were considered SAEs.

The intensity of AEs and SAEs were assigned by the Investigator to the following categories:

- Mild – an event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities
- Moderate – an event that causes sufficient discomfort and interferes with normal everyday activities
- Severe – An event that prevents normal everyday activities.

In addition, causality of the AEs and SAEs was assessed by the Investigator and characterized as follows:

- Probably related – suggestion of causal relationship
- Possibly related – suggestion that the association of the AE with the blinded study medication is unknown
- Unlikely to be related – Suggestion that only a remote connection exists between the blinded study medication and the AE
- Unrelated – There is not a reasonable possibility that the blinded study medication caused the AE

Study Drug Interruption and Stopping Rules

Subjects with a positive COVID-19 test result indicating an active infection were required to discontinue blinded study medication and to withdraw from the trial. Those who otherwise permanently discontinued blinded study medication were not required to withdraw.

Women who were pregnant or breast feeding were not eligible to participate. Women exhibiting a positive pregnancy test during the study were to be discontinued from blinded study medication and followed-up per protocol.

COPD Exacerbation Withdrawal Criteria

Subjects who experience a single COPD exacerbation during the treatment period may remain in the study and should continue to take their blinded study medication if possible. However, subjects who experience a second COPD exacerbation or a severe COPD exacerbation that requires an in-patient hospitalization will be discontinued from study treatment.

Liver Chemistry Stopping Criteria

If any of the following stopping criteria were met, the study drug was immediately discontinued. The event was to be reported and the liver event case report form and SAE data collection tool completed. Liver event follow-up assessments and subject monitoring was to be performed until liver chemistries resolve, stabilize, or return to baseline values.

1. ALT ≥3x ULN and bilirubin ≥2x ULN (35% direct bilirubin) (or ALT ≥3x ULN and INR >1.5, if measured)
2. ALT ≥8x ULN
3. ALT ≥5x ULN but <8x ULN persists for ≥2 weeks
4. ALT ≥3x ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, RUQ pain/tenderness, jaundice) or hypersensitivity (such as fever, rash, or eosinophilia)
5. ALT ≥5x ULN but <8x ULN and cannot be monitored weekly for ≥2 weeks

Electrocardiogram Withdrawal Criteria

Subjects may be discontinued from blinded study mediation if they experience a significant abnormality(ies) on their ECG, including but not limited to the following:

- Sinus tachycardia (≥120 bpm) or sinus bradycardia (<37 bpm)
- Increase in HR ≥40 bpm relative to baseline
- Multifocal atrial tachycardia
- Supraventricular tachycardia (>100 bpm)
- Atrial fibrillation or flutter with rapid ventricular response (rate >120 bpm)
- Increase in QTcF >60 msec from baseline
- Uncorrected QT >600 msec
- For subjects with QRS duration <120 msec: QTc(F) ≥500 msec or ECG that is unsuitable for QT measurements (e.g., poor defined termination of the T-wave)
- For subjects with QRS duration ≥120 msec: QTc(F) ≥530 msec or ECG that is unsuitable for QT measurements

Protocol Amendments

Trial 301 was amended 5 times as summarized:

- Amendment 1.0 (prior to initial regulatory submissions) – Added ECG inclusion criteria, spirometry at Week 24, and optional [redacted] (b) (4) COVID-19 testing.
- Amendment 2.0 (prior to initial regulatory submissions) – Removed [redacted] (b) (4)
- Amendment 3.0 (prior to initial regulatory submissions) – Reordered the statistical endpoint testing hierarchy (raised SGRQ score at Week 24 and lowered the proportion of SGRQ responders at Week 24, reportedly to elevate the continuous variable in the testing hierarchy) and revised 'Events Meeting the AE Definition.'
- Amendment 4.0 (during enrollment) – Reordered the statistical endpoint testing hierarchy (average FEV_1 $AUC_{0\text{-}4h}$ was moved to secondary endpoint #5 and morning trough FEV_1 was moved to secondary endpoint #4) and added additional endpoints (average FEV_1 $AUC_{6\text{-}12h}$ at Week 12), updated handling of missing data in the statistical analysis, allowed some subjects with stable use of inhaled corticosteroids (up to 20% of subjects and only if used in combination with LABA or LAMA).
- Amendment 5.0 (during enrollment) – Clarification to allow flexibility to enroll approximately 50% of subjects on stable background therapy rather than to cap enrollment of subjects on background therapy at 50%.

Trial 302 was amended 4 times. The changes were very similar to those noted in Trial 301 and are summarized below:

- Amendment 1.0 (prior to initial regulatory submissions) – Added ECG inclusion criteria, spirometry at Week 24, and optional [redacted] (b) (4) COVID-19 testing.
- Amendment 2.0 (prior to initial regulatory submissions) – Removed [redacted] (b) (4)
- Amendment 3.0 (prior to initial regulatory submissions) – Reordered the statistical endpoint testing hierarchy (raised SGRQ score at Week 24 and lowered the proportion of SGRQ responders at Week 24, reportedly to elevate the continuous variable in the testing hierarchy) and revised 'Events Meeting the AE Definition.'
- Amendment 4.0 (prior to initial regulatory submissions) – Reordered the statistical endpoint testing hierarchy (average FEV_1 $AUC_{0\text{-}4h}$ was moved to secondary endpoint #5 and morning trough FEV_1 was moved to secondary endpoint #4) and added additional endpoints (average FEV_1 $AUC_{6\text{-}12h}$ at Week 12), updated handling of missing data in the statistical analysis, allowed some subjects with stable use of inhaled corticosteroids (up to 20% of subjects and only if used in combination with LABA or LAMA).

COVID Impact

Trials 301 and 302 were conducted during the COVID-19 pandemic. In both trials, the rates of COVID-19 related rates of study drug discontinuation or study withdrawal and the COVID-19 related treatment-emergent adverse events were low and similar between the ensifentrine and placebo groups.

Protocol Deviations

The Applicant defined major and critical protocol violations as follows:

- Critical deviations – a deviation from Protocol-related procedures that threatens integrity of data, adversely affects subjects, and/or could invalidate acceptability of a product (or part of it).
- Major deviations – a deviation from Protocol-related procedures that could affect integrity of the data or adversely affect subjects.

In Trial 301, 16.8% (128/763) of randomized subjects were noted to have at least one major/critical protocol deviation (ensifentrine: 16.1%, 77/479; placebo: 18.0%, 51/284). One critical protocol deviation was reported where a subject randomized to the ensifentrine treatment arm was dispensed one kit containing placebo at Week 12 (20 ampules were used). The most common major/critical protocol deviations reported were related to the adherence to the timing of the morning trough FEV_1 (25.0%, 32/128) and randomization into the incorrect stratum (background medication: 8.7%, 11/128; smoking: 4.8%, 6/128).

In Trial 302, 31.3% (247/790) of randomized subjects were noted to have at least 1 major/critical protocol deviation (ensifentrine: 30.5%, 152/499; placebo: 32.6%, 95/291). The one critical protocol deviation identified was related to a subject that was enrolled despite not satisfying two aspects of the exclusion criteria (the subject had a reported history of uncontrolled psychiatric

disease of schizophrenia and had a history of ongoing drug abuse). The most common major/critical protocol deviations reported related to adherence to the timing of the morning trough FEV_1 within the 11.5- to 12-hour post-evening dose window (27.3%, 68/249), randomization into the incorrect stratum for background medications (14.9%, 37/247), or smoking stratum (4.8%, 12/247). There was also one reported minor protocol deviation related to blinding (the screening visit and informed consent form was completed by the unblinded coordinator rather than the blinded coordinator). The rates of protocol deviation were small and consistent with other similarly sized trials in this population and are unlikely to impact the overall analysis.

Data Quality and Integrity

No major data quality or integrity issues were identified that would preclude a safety review for this NDA. There were no major issues identified with respect to recording, coding, and characterizing AEs. The Applicant's translations of verbatim terms to the Medical Dictionary for Regulatory Activities preferred terms for the events reported in Trials 301 and 302 were reviewed and found to be acceptable. The definition, grading scheme, and assessment of causality for all AEs and SAEs were reviewed and found to be acceptable.

16. Efficacy

16.1. Subject Demographics

Past Medical History and Concomitant Medications

The past medical history by condition occurring in ≥10% of subjects, listed individually by trial, is reviewed below in Table 72 and Table 73. Overall, the past medical history report was balanced between the treatment group and placebo and consistent with a moderate to severe COPD population.

Table 72. Trial 301, Past Medication History by Condition, Occurring in ≥10% of Subjects (mITT)

Past Medical History	ENS (N=477) n (%)	PBO (N=283) n (%)	Total (N=760) n (%)
Hypertension	285 (59.7)	160 (56.5)	445 (58.6)
Type 2 diabetes mellitus	73 (15.3)	49 (17.3)	122 (16.1)
Hyperlipidemia	68 (14.3)	44 (15.3)	112 (14.7)
Gastroesophageal reflux disease	67 (14.0)	39 (13.8)	106 (13.9)
Hypercholesterolemia	58 (12.2)	34 (12.0)	92 (12.1)
Obesity	53 (11.1)	27 (9.5)	80 (10.5)
Osteoarthritis	53 (11.1)	34 (12.0)	87 (11.4)
Coronary artery disease	44 (9.2)	35 (12.4)	79 (10.4)
Hypothyroidism	40 (8.4)	22 (7.8)	62 (8.2)
Depression	39 (8.2)	31 (11.0)	70 (9.2)

Source: Clinical reviewer calculated/confirmed: JMP 15.0 dataset ADMH with the following parameters: USUBJID, MHSOC, MHDECOD, TRT01A
Abbreviations: ENS, ensifentrine; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

Table 73. Trial 302, Past Medication History by Condition, Occurring in ≥10% of Subjects (mITT)

Past Medical History	ENS (N=498) n (%)	PBO (N=291) n (%)	Total (N=789) n (%)
Hypertension	295 (59.2)	165 (56.7)	460 (58.3)
Gastroesophageal reflux disease	140 (28.1)	72 (24.7)	212 (26.9)
Menopausal symptoms	113 (22.7)	52 (17.9)	165 (20.9)
Depression	109 (21.9)	68 (23.4)	177 (22.4)
Hyperlipidemia	106 (21.3)	67 (23.0)	173 (21.9)
Osteoarthritis	98 (19.7)	63 (21.6)	161 (20.4)
Anxiety	87 (17.5)	54 (18.6)	141 (17.9)
Back pain	77 (15.5)	43 (14.8)	120 (15.2)
Insomnia	76 (15.3)	50 (17.2)	126 (16.0)
Hypercholesterolemia	68 (13.7)	59 (20.3)	127 (16.1)
Obesity	68 (13.7)	59 (20.3)	127 (16.1)
Drug hypersensitivity	65 (13.1)	31 (10.7)	96 (12.2)
Type 2 diabetes mellitus	65 (13.1)	58 (19.9)	123 (15.6)
Seasonal allergy	53 (10.6)	33 (11.3)	86 (10.9)
Rhinitis allergic	44 (8.8)	32 (11.0)	76 (9.6)
Hypothyroidism	38 (7.6)	30 (10.3)	68 (8.6)

Source: Clinical reviewer calculated/confirmed: JMP 15.0 dataset ADMH with the following parameters: USUBJID, MHSOC, MHDECOD, TRT01A
Abbreviations: ENS, ensifentrine; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

Table 74 and Table 75 review the most common concomitant medications used by ≥10% of subjects in Trials 301 and 302. The medications reported were consistent with a known history of moderate to severe COPD, the reported past medical histories, and were balanced between treatment groups.

Table 74. Trial 301, Concomitant Medications Used by ≥10% of Subjects (mITT)

Medications	ENS (N=477) n (%)	PBO (N=283) n (%)	Total (N=760) n (%)
Salbutamol	203 (42.6)	128 (45.2)	331 (43.6)
Tiotropium	112 (23.5)	54 (19.1)	166 (21.8)
Acetylsalicylic Acid	99 (20.8)	59 (20.8)	158 (20.8)
Atorvastatin	86 (18.0)	55 (19.4)	141 (18.6)
Formoterol	86 (18.0)	43 (15.2)	129 (17.0)
Amlodipine	64 (13.4)	34 (12.0)	98 (12.9)
Metformin	49 (10.3)	31 (11.0)	80 (10.5)
Budesonide/formoterol	25 (5.2)	30 (10.6)	55 (7.2)

Source: Clinical reviewed calculated and confirmed: JMP 15.0 dataset ADCM with the following parameters: USUBJID, ATCT1, ATCT4, CMDECOD, TRT01A
Abbreviations: ENS, ensifentrine; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

Table 75. Trial 302, Concomitant Medications Used by ≥10% of Subjects (mITT)

Medications	ENS (N=498) n (%)	PBO (N=291) n (%)	Total (N=789) n (%)
Salbutamol	336 (67.5)	198 (68.0)	534 (67.7)
Tiotropium	130 (26.1)	65 (22.3)	195 (24.7)
Acetylsalicylic Acid	102 (20.5)	53 (18.2)	155 (19.6)
Atorvastatin	77 (15.5)	42 (14.4)	119 (15.1)
Lisinopril	62 (12.4)	32 (11.0)	94 (11.9)
Metformin	60 (12.0)	48 (16.5)	108 (13.7)
Amlodipine	60 (12.0)	35 (12.0)	95 (12.0)
Omeprazole	53 (10.6)	26 (8.9)	79 (10.0)
Levothyroxine	38 (7.6)	29 (10.0)	67 (8.5)
Cholecalciferol	36 (7.2)	29 (10.0)	65 (8.2)
Gabapentin	30 (6.0)	31 (10.7)	61 (7.7)

Source: Clinical reviewed calculated and confirmed: JMP 15.0 dataset ADCM with the following parameters: USUBJID, ATCT1, ATCT4, CMDECOD, TRT01A
Abbreviations: ENS, ensifentrine; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

16.2. Efficacy Results

Statistical Hierarchy – Trials 301 and 302

1. FEV_1 $AUC_{0\text{-}12h}$ at Week 12 (primary endpoint)
2. Peak FEV_1 at Week 12
3. Weekly mean E-RS total symptom score at Week 24
4. SGRQ total score at Week 24
5. Morning trough FEV_1 at Week 12 (Division's preferred efficacy endpoint)
6. FEV_1 $AUC_{0\text{-}4h}$ at Week 12
7. SGRQ Responders at Week 24

All Efficacy Results – Trial 301 and Trial 302

A summary of the efficacy results from both Trials 301 and 302 is included in Table 76. Overall, the secondary endpoints of each trial were generally supportive of the primary endpoint, however, early losses in the statistical hierarchy of Trial 302 limit the interpretation of the later results.

Table 76. Trials 301 and 302, Efficacy Results (mITT)

Parameter	Trial 301	Trial 302
Subjects randomized	N=763	N=790
Subjects treated	Total N=760 ENS N=477 PBO N=283	Total N=789 ENS N=498 PBO N=291
	LS Mean Difference vs. PBO (p-value[1])	
Primary endpoint		
CFB in Week 12 FEV_1 AUC_{0-12h}	87 mL (p<0.0001)	94 mL (p<0.0001)
Key secondary endpoints		
CFB in Week 12 peak FEV_1	147 mL (P<0.0001)	146 mL (p<0.0001)
CFB in Week 24 E-RS total score	-1.0 units (p=0.0111)	-0.6 units (p=0.1344, NS)
CFB in Week 24 SGRQ total score	-2.3 units (p=0.0253)	-0.5 units (p=0.6694, NS)
CFB in Week 12 morning trough FEV_1	35 mL (p=0.0413)	49 mL (p=0.0016, NS[1])
CFB in Week 12 FEV_1 AUC_{0-4h}	139 mL (p<0.0001)	136 mL (p<0.0001, NS[1])
Week 24 SGRQ responders[2]	Odds ratio: 1.49 (p=0.0194)	Odds ratio: 0.92 (p=0.1651, NS)
Secondary endpoints		
CFB in Week 24 rescue medication use (average daily puffs over a week)	-0.454 puffs	-0.139 puffs
CFB in Week 24 transition dyspnea index	1.0 units	0.9 units
Other endpoints		
Moderate/severe COPD exacerbation rate (24w)	RR 0.64 (95% CI: 0.40, 1.00)	RR 0.57 (95% CI: 0.38, 0.87)
Time to first COPD exacerbation (24w)	HR 0.62 (95% CI: 0.39, 0.97)	HR 0.58 (95% CI: 0.38, 0.87)

Source: Statistical reviewer calculated/confirmed
[1] p-value is shown for the primary endpoint and key secondary endpoints in the statistical hierarchy.
[2] Not significant due to earlier losses in the statistical hierarchy.
[3] SGRQ Responder defined as an improvement of at least 4 units from baseline total SGRQ score.
Abbreviations: AUC_{0-4}, area under the concentration-time curve from 0 to 4 hours; AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; CFB, change from baseline; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ENS, ensifentrine; E-RS, evaluating-respiratory symptoms; FEV_1, forced expiratory volume in 1 second; HR, hazard ratio; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; NS, not significant; PBO, placebo; RR, rate ratio; SGRQ, St. George Respiratory Questionnaire; w, weeks

E-RS Total Score at Week 24

The Applicant evaluated the change in mean E-RS at Week 24. There was a statistically significant difference between the change in E-RS score in those in the ensifentrine treated group compared with placebo in Trial 301 but not in Trial 302.

A minimal clinically important difference in the E-RS has not been established for regulatory purposes and we do not recommend the inclusion of the E-RS results in the United States prescribing information (USPI).

FEV_1 AUC_{0-4h} at Week 12

The Applicant also evaluated the average FEV_1 AUC in the first 4 hours after treatment (AUC_{0-4h}). Ensifentrine treatment resulted in a higher FEV_1 AUC_{0-4h} compared to placebo in both Trials 301 and 302. In Trial 302, the difference was not statistically significant due to losses earlier in the statistical hierarchy; although, because the confidence interval excludes null, this is supportive of the primary endpoint. However, we do not recommend the inclusion of the FEV_1

AUC_{0-4h} in the USPI because it does not provide significant additional information for the safe and effective use of ensifentrine above what is demonstrated by the primary endpoint.

Week 24 Rescue Medication Use

The Applicant evaluated the reduction from baseline in mean rescue medication (the mean daily use averaged over 1 week) in both phase 3 trials. The differences noted in the ensifentrine group compared to placebo in both trials are numerically small and are not clinically meaningful. In addition, the endpoint was analyzed outside the statistical hierarchy and, overall, does not support inclusion in the USPI.

Week 12 Transition Dyspnea Index

The TDI, a reported measure of severity of dyspnea, was also evaluated as a secondary endpoint, although the clinical meaning of this differences observed with ensifentrine treatment compared to placebo is not entirely clear. In addition, the endpoint was analyzed outside the statistical hierarchy and, overall, do not support inclusion in the USPI.

16.3. Patient Perception Survey

During the development program for ensifentrine, concerns regarding the color difference between the study drug (off white/yellow) and placebo (clear) were raised given that this may negatively impact the blinding of the trials. Potential options to address this included the development of an alternative placebo that was visually matched. The Applicant did not consider this to be feasible and were encouraged to ensure that measures were taken to maintain blinding and that, following the study, patient perception was assessed. To maintain blinding and minimize bias, the Applicant took the following measures in in their phase 3 trials:

- Pouches and packaging were identical between the study drug and placebo.
- Electronic randomization technology was utilized.
- An unblinded site staff member not associated with the study conduct administered in-clinic doses of the study medication.
- Patients were instructed not to inform site personnel of the appearance of their blinded study medication.
- Study personnel were not to discuss the appearance with patients or other study personnel.

The Applicant also conducted a patient perception survey at the end of the trial period. They were asked: “Do you think you received active study drug, placebo, or are you not sure?” For those patients who answered either that they believed they received active study drug or placebo, they were then asked: “why do you think that?” Responses were provided in free text. The results of the patient perception survey for Trials 301 and 302 are reviewed in Table 77.

Overall, there was an adequate response rate for the patient perception survey, with about 85 to 90% of patients responding. Within the treatment group, the proportion of patients who correctly guessed they were receiving the study medication was 48.9% in Trials 301 and 53.4% in Trial 302. Within the placebo group, the proportion of patients who correctly guessed they

were receiving placebo was 28.5% in Trial 301 and 29.3% in Trial 302. The proportion of patients receiving treatment in Trials 301 and 302 who incorrectly guessed which treatment group they had been in ranged from 16.7 to 17.8%.

Table 77. Trials 301 and 302, Patient Perception Survey Results (mITT)

	Trial 301		Trial 302	
Parameter	ENS (N=477) n (%)	PBO (N=283) n (%)	ENS (N=498) n (%)	PBO (N=291) n (%)
Completed patient perception survey	427 (89.5)	256 (90.5)	425 (85.3)	246 (84.5)
Concordant (correctly guessed treatment group)	209 (48.9)	73 (28.5)	227 (53.4)	72 (29.3)
Discordant (incorrectly guessed treatment group)	76 (17.8)	72 (28.1)	71 (16.7)	84 (34.1)
Unsure of treatment group	142 (33.3)	111 (43.4)	127 (29.9)	90 (36.6)

Source: Statistical Reviewer calculated/confirmed
Abbreviations: ENS, ensifentrine; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo

The primary reasons that patients either correctly guessed they were receiving treatment or placebo in both Trials 301 and 302 were related to their symptoms as reviewed below. In both trials, the vast majority of concordant responses were based on symptoms (e.g., feeling better or symptom improvement for those in the treatment group or due to no improvement in symptoms in the placebo group). Only one subject in each trial [1/477 (0.2%) in Trial 301 and 1/498 (0.1%) in Trial 302] attributed appearance as to why they responded they were in the treatment group.

The reported rationale behind the responses to the patient perception survey were reassuring, and do not suggest that the differences in color resulted in significant levels of patient unblinding. While compared to placebo, a higher proportion of ensifentrine treated patients correctly guessed their treatment arm. This is not unexpected given the effects on FEV_1 were noted early in the dosing interval. Analyses were also performed comparing the primary endpoint (FEV_1 $AUC_{0\text{-}12h}$) between concordant and discordant patients (Table 78). These data did not reveal significant differences between these two groups. Overall, these data provide reassurance that the differences in appearance between treatment and placebo product did not appear to unblind subjects or impact the primary endpoint results.

Table 78. Trials 301 and 302, Subgroup Analysis of the Primary Endpoint Based on Patient Perception Survey (mITT)

	Concordant Subjects		Discordant Subjects		Comparison
Randomized Treatment	n	Estimate (SE)	n	Estimate (SE)	Difference (95% CI)
Ensifentrine					
Trial 301	209	0.068 (0.032)	76	0.053 (0.039)	0.015 (-0.041, 0.072)
Trial 302	227	0.052 (0.013)	71	0.052 (0.024)	0.000 (-0.053, 0.054)
Pooled	436	0.067 (0.028)	147	0.060 (0.033)	0.007 (-0.031, 0.046)
Placebo					
Trial 301	73	-0.035 (0.046)	72	-0.023 (0.47)	-0.012 (-0.071, 0.046)
Trial 302	72	-0.068 (0.021)	84	-0.028 (0.019)	-0.040 (-0.095, 0.015)
Pooled	145	-0.059 (0.044)	156	-0.035 (0.044)	-0.024 (-0.064, 0.017)

Source: Statistical Reviewer calculated/confirmed
Abbreviations: CI, confidence interval; mITT, modified intent-to-treat; n, number of subjects in subset; SE, standard error

16.4. Additional Analyses for the Primary Efficacy Endpoint

This section supplements the analyses and interpretation of the primary efficacy endpoint presented in Section 6.2.2.4 (Trial 301) and Section 6.2.3.1 (Trial 302). There were some missing data for the primary endpoint in both trials. The number of subjects with missing data was 44 (9%) in the ensifentrine group and 21 (8%) in the placebo group in Trial 301 and 74 (15%) in the ensifentrine group and 60 (21%) in the placebo group in Trial 302. The primary analysis utilized multiple imputation based on the missing data assumption of MAR. To examine the robustness of the primary analysis result to the missing data, an average imputation analysis and a tipping point analysis were conducted by the Applicant. Results from the missing data sensitivity analyses for the primary endpoint are presented here. In addition, results from the subgroup analyses based on age, gender and race are presented.

16.4.1. Average Imputation

For the planned missing data sensitivity analysis with average imputation, any missing data postbaseline were to be imputed based on data collected at early termination visit. If no such data were available, imputation was performed using average change from baseline in the opposite randomized treatment group. The sensitivity analyses showed that ensifentrine treatment demonstrated a statistically significant increase compared to placebo in both Trials 301 (0.072 liters [L] [95% CI: 0.042, 0.101], p<0.0001) and 302 (0.062 L [95% CI: 0.037, 0.087], p<0.0001), as shown in Table 79 and Table 80. These analyses indicate that the impact of the MAR assumption on overall missing data are not likely considerable.@

Table 79. Trial 301, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 (mITT)–Average Imputation

Parameter	ENS (N=477)	PBO (N=283)
Baseline[1]		
n	477	282[2]
Mean (SD)	1.420 (0.487)	1.403 (0.468)
Week 12 (imputed[2])		
n	477	282
Mean (SD)	1.482 (0.503)	1.392 (0.496)
Change from baseline at Week 12 (imputed[2])		
n	477	282
Mean (SD)	0.062 (0.199)	-0.011 (0.201)
LS mean change from baseline at Week 12[3]	0.056	-0.016
(95% CI)	(0.023, 0.088)	(-0.051, 0.019)
LS mean difference vs. placebo (95% CI)[3]		0.072 (0.042, 0.101)
p-value		<0.0001

Source: Reviewer calculated/confirmed, SAS 9.4, ADRE and ADREMIAV datasets
[1] Baseline FEV_1 is the mean of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes pre-dose on Day 1.
[2] Number of subjects with observed data is 433 and 261 for ENS and PBO, respectively. Missing data were imputed using average change from baseline at Week 12 in the opposite randomized treatment group (0.069L in ENS; -0.017 L in PBO).
[3] ANCOVA model was used to model the change from baseline in FEV_1 AUC_{0-12h} with treatment, region, background medication strata and smoking strata as fixed effects and baseline FEV_1 as covariate.
Abbreviations: ANCOVA, analysis of covariance, AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine, FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SD, standard deviation ; vs., versus

Table 80. Trial 302, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 (mITT)–Average Imputation

Parameter	ENS (N=498)	PBO (N=291)
Baseline[1]		
n	498	291
Mean (SD)	1.285 (0.451)	1.279 (0.473)
Week 12 (imputed[2])		
n	498	291
Mean (SD)	1.319 (0.465)	1.250 (0.446)
Change from baseline at Week 12 (imputed[2])		
n	498	291
Mean (SD)	0.034 (0.171)	-0.029 (0.182)
LS mean change from baseline at Week 12[3]	0.035	-0.027
(95% CI)	(0.019, 0.050)	(-0.048, -0.007)
LS mean difference vs. placebo (95% CI)[3]		0.062 (0.037, 0.087)
p-value		<0.0001

Source: Reviewer calculated/confirmed, SAS 9.4, ADRE and ADREMIAV datasets
[1] Baseline FEV_1 is the mean of the two measurements taken before study medication on the day of first dosing, i.e., ≤40 minutes pre-dose on Day 1.
[2] Number of subjects with observed data is 424 and 231 for ENS and PBO, respectively. Missing data were imputed using average change from baseline at Week 12 in the opposite randomized treatment group (0.048 L in ENS; -0.049 L in PBO).
[3] ANCOVA model was used to model the change from baseline in FEV_1 AUC_{0-12h} with treatment, region, background medication strata and smoking strata as fixed effects and baseline FEV_1 as covariate.
Abbreviations: ANCOVA, analysis of covariance, AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine, FEV_1, forced expiratory volume in 1 second; L, liters; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; PBO, placebo; SD, standard deviation; vs., versus

16.4.2. Tipping Point Analysis

For the planned missing data sensitivity analysis with tipping point analysis, the Applicant repeated the primary analysis under different conditions of the missing data to assess the robustness of statistical significance. If the unadjusted original analysis produced a p-value <0.05 in favor of ensifentrine then the multiple imputed values of the FEV_1 $AUC_{0\text{-}12h}$ at Week 12 were to be adjusted by the value k, where imputed values for ensifentrine group and placebo were both worsened (k is subtracted) and improved (k is added).

In the tipping point analyses on the primary endpoint, the LS mean difference versus placebo remained statistically significant (p<0.05) under most scenarios after multiple missing data imputations and adding shift variables (-1 L to 1 L in the ensifentrine group; -1 L to 1 L in the placebo group), except under few implausible extreme shifting scenarios Table 81 and Table 82). The resulting tipping points of 0.32 L and 0.18 L are likely implausible changes given that the observed effect sizes were 0.0868 L and 0.0941 L for Trials 301 and 302, respectively.

Table 81. Trial 301, Change From Baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12 (mITT), p-Values–Tipping Point Analysis

E\P	-1.0	-0.8	-0.6	-0.4	-0.2	-0.1	0.0	0.1	0.2	0.4	0.6	0.8	1.0
1.0	<0.0001						<0.0001						<0.0001
0.8		<0.0001					<0.0001					<0.0001	
0.6			<0.0001				<0.0001				<0.0001		
0.4				<0.0001			<0.0001			<0.0001			
0.2					<0.0001		<0.0001		<0.0001				
0.1						<0.0001	<0.0001	<0.0001					
0.0	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	0.0140	0.1222	0.4678
-0.1						<0.0001	<0.0001	<0.0001					
-0.2					<0.0001		<0.0001		0.0013				
-0.4				<0.0001			0.0050			0.0544*			
-0.6			0.0003				0.1107						
-0.8		0.0026					0.5772						
-1.0	0.0129						0.7697						

Source: Reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
* Tipping point occurred at K=0.32 (L) for placebo group (P) and K=-0.32 (L) for ensifentrine group (E).
Proportion of missing data was 9.22% in the ensifentrine group (E) and 7.45% in the placebo group (P) in Trial 301. ANCOVA models were run on 100 imputed datasets and their p-values were combined using Rubin's rule. The p-values were calculated under different conditions of the missing data where the multiple imputed values were adjusted by the shift parameter of K (-1.0 to 1.0 by 0.01).
Abbreviations: ANCOVA, analysis of covariance; $AUC_{0\text{-}12}$, area under the concentration-time curve from 0 to 12 hours; E\P, ensifentrine\placebo; FEV_1, forced expiratory volume in 1 second; K, shift parameter; L, liters; mITT, modified intent-to-treat

Table 82. Trial 302, Change From Baseline in FEV_1 $AUC_{0\text{-}12h}$ at Week 12 (mITT), p-Values–Tipping Point Analysis

E\P	-1.0	-0.8	-0.6	-0.4	-0.2	-0.1	0.0	0.1	0.2	0.4	0.6	0.8	1.0
1.0	<0.0001						<0.0001						0.2102
0.8		<0.0001					<0.0001					0.0593	
0.6			<0.0001				<0.0001				0.0061		
0.4				<0.0001			<0.0001			0.0001			
0.2					<0.0001		<0.0001		<0.0001				
0.1						<0.0001	<0.0001	<0.0001					
0.0	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	0.4768	-	-	-
-0.1						<0.0001	<0.0001	0.0001					
-0.2					<0.0001		<0.0001		0.0583*				
-0.4				<0.0001			0.0488						
-0.6			<0.0001				0.8500						
-0.8		<0.0001					-						
-1.0	<0.0001						-						

Source: Reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
* Tipping point occurred at K=0.18 (L) for placebo group (P) and K=-0.18 (L) for ensifentrine group (E).
Proportion of missing data was 14.86% in ensifentrine group (E) and 20.62% in the placebo group (P) in Trial 302. ANCOVA models were run on 100 imputed datasets and their p-values were combined using Rubin's rule. The p-values were calculated under different conditions of the missing data where the multiple imputed values were adjusted by the shift parameter of K (-1.0 to 1.0 by 0.01).
Abbreviations: ANCOVA, analysis of covariance; $AUC_{0\text{-}12}$, area under the concentration-time curve from 0 to 12 hours; E\P, ensifentrine\placebo; FEV_1, forced expiratory volume in 1 second; K, shift parameter; L, liters; mITT, modified intent-to-treat

This finding, along with the result from average imputation analysis, suggests that the primary analysis result is robust to underlying missing data assumption (MAR). While the demonstrated change in the average FEV_1 $AUC_{0\text{-}12h}$ is statistically significant and robust to missing data based on a tipping point analysis, the effect size is modest but supportive of the benefit of ensifentrine.

16.4.3. Subgroup Analysis

The statistical reviewer conducted subgroup analyses on the primary efficacy endpoint based on gender at birth, age and race. Results for the evaluated subgroups were generally consistent with the overall population for both trials (Table 83, Table 84).

Table 83. Trial 301, Change From Baseline in FEV_1 $AUC_{0\text{-}12h}$ (L) at Week 12 by Subgroups (mITT)

Subgroup	ENS (N=477) n (%)	Placebo (N=283) n (%)	LS Mean Difference[1]	95% CI[1]
Age				
<65	219 (46)	132 (47)	0.070	0.015, 0.125
≥65	258 (54)	150 (53)	0.102	0.067, 0.138
Gender				
Male	274 (57)	166 (59)	0.085	0.039, 0.131
Female	203 (43)	116 (41)	0.091	0.051, 0.131
Race				
White	435 (91)	249 (88)	0.088	0.055, 0.122
Non-White[2]	42 (9)	33 (12)	0.069	-0.016, 0.153

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
[1] Based on the primary efficacy analysis by pre-defined subgroups.
[2] Includes Asian, Black or African American, Other and subjects whose race were not reported.
Abbreviations: $AUC_{0\text{-}12h}$, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; L, liters; LABA, long acting beta-agonist; LAMA, long acting muscarinic antagonist; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; N/A, not applicable

Table 84. Trial 302, Change From Baseline in FEV_1 AUC_{0-12h} (L) at Week 12 by Subgroups (mITT)

Subgroup	ENS (N=498) n (%)	Placebo (N=291) n (%)	LS Mean Difference[1]	95% CI[1]
Age				
<65	224 (45)	124 (43)	0.087	0.039, 0.135
≥65	274 (55)	167 (57)	0.100	0.063, 0.136
Gender				
Male	244 (49)	138 (47)	0.114	0.068, 0.161
Female	254 (51)	153 (53)	0.075	0.039, 0.112
Race				
White	471 (95)	276 (95)	0.092	0.062, 0.122
Non-White[2]	27 (5)	15 (5)	0.154	-0.006, 0.314

Source: Statistical reviewer calculated/confirmed, SAS 9.4, ADREMI dataset
[1] Based on the primary efficacy analysis by pre-defined subgroups.
[2] Includes American Indian or Alaska Native, Asian, Black or African American, Other and subjects whose race were not reported.
Abbreviations: AUC_{0-12h}, area under the concentration-time curve from 0 to 12 hours; CI, confidence interval; ENS, ensifentrine; FEV_1, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; L, liters; LABA, long acting beta-agonist; LAMA, long acting muscarinic antagonist; LS, least squares; mITT, modified intent-to-treat; N, number of subjects in treatment group; n, number of subjects in subset; N/A, not applicable

17. Clinical Safety

Electrocardiograms

No clinically significant ECG trends or safety concerns were identified in the phase 3 trials of ensifentrine.

QT

Ensifentrine did not prolong the QTcF interval in the thorough QT study assessment (RPL554-CV-101). This was reviewed by the Interdisciplinary Review Team for Cardiac Safety Studies.

120 Day Safety Update

The Applicant submitted a 120-day safety update that consisted of updates that had become available since the safety data cut off for the NDA submission, summarizing blinding information on deaths, SAEs, and withdrawals due to AEs for the ongoing clinical studies.

The following studies were included in the report:

- Applicant-sponsored: RPL554-CO-207: Phase 2, randomized, double-blind, placebo-controlled, 2-period cross-over study of the effect of ensifentrine on sputum markers of inflammation in patients with COPD. Ongoing. As of September 1, 2023, cutoff: N=17 (blinded).
- Non-Applicant-sponsored: RPL554-AHC001: Phase 1, open-label, single ascending dose/multiple ascending dose study to evaluate the PK, safety, and tolerability of nebulized

ensifentrine in healthy Chinese subjects. Study complete. As of September 1, 2023, cutoff: N=28.

- Non-Applicant-sponsored: RPL554-CPC001: Phase 3, randomized, double-blind, placebo-controlled, trial to evaluate the safety and efficacy over 24 weeks in patients with moderate to severe COPD. Ongoing. As of September 1, 2023, cutoff: N=44 (blinded).

There were no deaths reported in the 120-day safety update. Although the majority of information provided in the report remains blinded, the reported SAEs and AEs leading to discontinuation were consistent with the information previously provided and did not raise additional concerns regarding the safety profile of ensifentrine.

Additional Safety Review from the Overall Ensifentrine Development Program

To further support the safety of ensifentrine, the Applicant provided data from the complete ensifentrine development program. Review of the safety data including the phase 2 dose ranging trials (RPL554-CO-203 and 205) and non-COPD indications (including COVID-19, asthma, and cystic fibrosis).

There were no appreciable trends or large imbalances noted in terms of deaths, SAEs, AEs leading to discontinuation, or common AEs with ensifentrine treatment compared to placebo. The additional safety review was notable for a suicide in Trial RPL554-CO-203 that was previously referenced in the review in the primary safety section.

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action/Drug Resistance

Not applicable.

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

An Office of Scientific Investigation consult evaluated four trial sites and the Contract Research Organization, IQVIA, from the phase 3 program based on high enrollment, efficacy outcome, and safety. The sites chosen for inspection are shown in Table 83. Based on the inspection results, the studies appear to have been conducted adequately, and the data generated by the clinical investigator sites and submitted by the Applicant appear acceptable to support this NDA. See the separately filed Clinical Inspection Summary dated May 9, 2024, for additional information (*DARRTS Reference ID: 5378693*).

Table 85. Office of Scientific Investigation Inspection Sites

Principal Investigator	Address	Trial(s)	Site No.	No. of Subjects
(b) (4)		301 and 302	n/a	n/a
Deckelmann, Regina	Diezmannstrasse 5 04207 Leipzig Germany	301	806	29
Lienert, Thomas	Bismarckstr. 28 10625 Berlin Germany	301	811	23
Diaz, Jose	1038 West North Blvd Suite 101 Leesburgh, FL 34748	302	1733	12
Siler, Thomas	330 1st Capitol Dr. Suite 470 St. Charles, MO 63301	302	1739	19

Source: Clinical Reviewer generated table
Abbreviations: CRO, contract research organization; no., number

23. Labeling: Key Changes and Considerations

This prescribing information (PI) review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant's draft PI (Table 84). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

Table 86. Key Labeling Changes and Considerations

Full PI Sections[1]	Rationale for Major Changes to Finalized PI[2] Compared to Applicant's Draft PI
BOXED WARNING	N/A
1 INDICATIONS AND USAGE	OHTUVAYRE is indicated for the maintenance treatment of chronic obstructive pulmonary disease (COPD) in adult patients.
2 DOSAGE AND ADMINISTRATION	Following the recommended dosage language, this section was edited with format revisions with bulleted Administration Instructions and adding a heading for Drug Compatibility.
4 CONTRAINDICATIONS	N/A
5 WARNINGS AND PRECAUTIONS	Added 'Psychiatric Events Including Suicidality' to Section 5 Warnings and Precautions because of known safety concerns with similar products and numerical increases in psychiatric adverse events, including suicidal behavior, in clinical studies.
6 ADVERSE REACTIONS	In the *Clinical Trials Experience* subsection, the safety of OHTUVAYRE was based on a pooled population from two randomized, double-blind, placebo-controlled trials (Trials 1 and 2) for 24 weeks, and a 48-week cohort that assessed safety in Trial 1.
7 DRUG INTERACTIONS	This section was omitted from labeling because concurrent administration of OHTUVAYRE and other drugs (e.g., long-acting beta2 agonists, long-acting muscarinic antagonists) commonly used for treatment of COPD did not show an increased in adverse reactions during clinical studies. (b) (4) .
8 USE IN SPECIFIC POPULATIONS (e.g., Pregnancy, Lactation, Females and Males of Reproductive Potential, Pediatric Use, Geriatric Use, Renal Impairment, Hepatic Impairment)	**8.1 Pregnancy** Changed "Exposure margin" based on human AUC data from Study RPL554-PK-102.
9 DRUG ABUSE AND DEPENDENCE	N/A
10 OVERDOSAGE	Removed statement that implied or suggested (b) (4) that was not included in the DOSAGE AND ADMINISTRATION section.
12 CLINICAL PHARMACOLOGY	**12.1 Mechanism of Action** Removed (b) (4) to be consistent with other approved PDE inhibitors and avoid promotional language. **12.2 Pharmacodynamics** Removed proposed (b) (4) from Section 12.2 (b) (4) **12.3 Pharmacokinetics** Some of the proposed (b) (4) The Applicant is requested to include accumulation-related PK parameters at steady state from the to-be-marketed formulation or from population PK estimates.
13 NONCLINICAL TOXICOLOGY	Changed "Exposure margin" based on human AUC data from Study RPL554-PK-102.
14 CLINICAL STUDIES	Efficacy of OHTUVAYRE was based on two 24-week, randomized, double-blind, placebo-controlled, parallel-group clinical trials. In addition to the adequate and well-controlled trials, the Applicant proposed to include (b) (4) which was removed (b) (4) . (b) (4)

Full PI Sections[1]	Rationale for Major Changes to Finalized PI[2] Compared to Applicant's Draft PI
	[redacted] (b) (4) However, [redacted] (b) (4) were removed from this section [redacted] (b) (4)
17 PATIENT COUNSELING INFORMATION	Added counseling information for Psychiatric Events Including Suicidality so that the Healthcare Provider would advise patients and caregivers the signs of psychiatric events and/or other mood changes.
Product Quality Sections (i.e., DOSAGE FORMS AND STRENGTHS, DESCRIPTION, HOW SUPPLIED/STORAGE AND HANDLING)	**Section 11 DESCRIPTION** Revised this section to include description of the particle/droplet size distributions that could be expected from the identified nebulizer under specific and defined operating conditions, e.g., fine particle dose defined as ≤5 mcm. **Section 16 HOW SUPPLIED/STORAGE AND HANDLING** This section was revised consistent with the Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products – Chemistry, Manufacturing, and Controls Documentation guidance.

Source: Labeling Discussion Comments dated March 15, 2024 and April 26, 2024
[1] Product quality sections (Sections 3, 11, and 16) are pooled under the last row in this table; Section 15 (REFERENCES) is not included in this table.
[2] For the purposes of this document, the finalized PI is the PI that will be approved or is close to being approved.
Abbreviation(s): AUC, area under the concentration-time curve; PD, pharmacodynamic; PDE, phosphodiesterase; PI, Prescribing Information; PK, pharmacokinetic

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

- Prescribing information
- Patient Package Insert
- Instructions for use (IFU)
- Carton labeling
- Container labeling

24. Postmarketing Requirements and Commitments

Not applicable.

25. Financial Disclosure

Table 87. Covered Clinical Studies: Trial RPL554-CO-301 and Trial RPL554-CO-302

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: Trial 301: 500 investigators; Trial 302: 707 investigators		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0 reported		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0 reported (financial information for 4 sub-investigators could not be obtained for Trial 302)		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: n/a Significant payments of other sorts: n/a Proprietary interest in the product tested held by investigator: n/a Significant equity interest held by investigator: n/a Sponsor of covered study: n/a		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0 reported		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

Abbreviations: CFR, Code of Federal Regulations, FDA, Food and Drug Administration; n/a not applicable

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27. Review Team

Table 88. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Linda Ebonine, PA-C
Chief Project Management Staff	Ladan Jafari
Nonclinical reviewer	Wei Sun, Ph.D., DABT
Nonclinical team leader	Jessica A. Bonzo, Ph.D.
OCP reviewer(s)	Nisha Kwatra Ph.D. (primary) Yunzhao Ren, M.D., Ph.D (secondary)
OCP team leader	Jingyu Yu (pharmacometrics)
Clinical reviewer	Alison Lennox, M.D.
Clinical team leader	Robert Lim, M.D.
Biometrics reviewer	Dong-Hyun Ahn, Ph.D.
Biometrics team leader	Yongman Kim, Ph.D.
Cross-disciplinary team leader	Robert Lim, M.D.
Division director (pharm/tox)	Andrew Goodwin, Ph.D.
Division director (OCP)	Chandrahas Sahajwalla, Ph.D.
Division supervisor (OB)	Weiya Zhang, Ph.D.
Division director (clinical)	Banu Karimi-Shah, M.D.
Office director (or designated signatory authority)	Kathleen Donohue, M.D.

Abbreviations: OCP, Office of Clinical Pharmacology; OB, Office of Biostatistics

Table 89. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Sharon Kelly / Lawrence Perez (Drug Substance) Craig Bertha (Drug Product ATL) / Nina Ni Ju Du / Shu-Wei Yang (Manufacturing) Emma Gimose (OPQ RBPM)
Microbiology	Koushik Paul / Jesse Wells
OPDP	Taylor Burnett / Adewale Adeleye
OSI	Suyoung Tina Chang / Phillip Kronstein
Patient Labeling	Kelly Jackson
OSE/DEPI	Mingfeng Zhang
OSE/DMEPA	Lissa Owens / Idalia Rychlik Cristina Attinello / Nichelle Rashid (OSE Project Management)
OSE/DRISK	Jacqueline Sheppard
OTS/OB	Primary reviewer: Feng Zhou, Ph.D., Team Lead: Karl Lin, Ph.D.
ADL	Jessica Lee, PharmD
Clinical Data Scientist	Setareh Ashkezari

Abbreviations: ADL, Associate Director of Labeling; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OB, Office of Biostatistics; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations; OTS, Office of Translational Sciences

27.1. Reviewer Signatures

Table 90. Signatures of Reviewers

See next page.

Table 90. Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacology/Toxicology Primary Reviewer	Wei Sun, Ph.D., DABT Office of Immunology and Inflammation DPT-II	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.1, 7.1, 8.4, 13, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Wei Sun -S Digitally signed by Wei Sun -S Date: 2024.06.12 08:52:37 -04'00'				
Pharmacology/Toxicology Team Leader	Jessica A. Bonzo, Ph.D. Office of Immunology and Inflammation DPT-II	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.1, 7.1, 8.4, 13, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Jessica A. Bonzo -S Digitally signed by Jessica A. Bonzo -S Date: 2024.06.12 08:57:34 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacology/Toxicology Division Director	Andrew Goodwin, Ph.D. Office of Immunology and Inflammation DPT-II	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.1, 7.1, 8.4, 13, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Andrew C. Goodwin -S Digitally signed by Andrew C. Goodwin -S Date: 2024.06.12 15:28:41 -04'00'				
Clinical Pharmacology Primary Reviewer	Nisha Kwatra Office of Clinical Pharmacology DIIP	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Nisha V. Kwatra -S Digitally signed by Nisha V. Kwatra -S Date: 2024.06.12 09:21:54 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Secondary Reviewer	Yunzhao Ren Office of Clinical Pharmacology DIIP	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Yunzhao Ren -S Digitally signed by Yunzhao Ren -S Date: 2024.06.12 09:37:27 -04'00'				
Clinical Pharmacology/Pharmacometrics Team Leader	Jingyu Yu Office of Clinical Pharmacology DPM	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 14.5	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: (signed by Fang Li on behalf of Jingyu Yu) Frank Li -S Digitally signed by Frank Li -S Date: 2024.06.12 10:00:24 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Tertiary Reviewer	Chandrahas Sahajwalla Office of Clinical Pharmacology DIIP	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Chandrahas G. Sahajwalla -S Digitally signed by Chandrahas G. Sahajwalla -S Date: 2024.06.12 10:21:35 -04'00'				
Biometrics Primary Reviewer	Dong-Hyun Ahn Office of Biostatistics DB III	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Dong Hyun Ahn -S Digitally signed by Dong Hyun Ahn -S Date: 2024.06.12 10:36:25 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biometrics Secondary Reviewer	Yongman Kim Office of Biostatistics DB III	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Yongman Kim -S Digitally signed by Yongman Kim -S Date: 2024.06.12 10:52:46 -04'00'				
Biometrics Tertiary Reviewer	Weiya Zhang Office of Biostatistics DB III	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 6, 16	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Weiya Zhang -S Digitally signed by Weiya Zhang -S Date: 2024.06.12 10:58:52 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Management Regulatory Project Manager	Linda Ebonine Office of Immunology and Inflammation ORO-II/DPACC	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 12	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Linda U. Ebonine -S Digitally signed by Linda U. Ebonine -S Date: 2024.06.12 16:10:17 -04'00'				
Regulatory Project Management CPMS	Ladan Jafari Office of Immunology and Inflammation ORO-II/DPACC	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 12	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Ladan Jafari -S Digitally signed by Ladan Jafari -S Date: 2024.06.13 10:26:06 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Associate Director for Labeling	Jessica Lee Office of Immunology and Inflammation DPACC	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Jessica K. Lee -S Digitally signed by Jessica K. Lee -S Date: 2024.06.12 15:20:17 -04'00'				
Clinical Primary Reviewer	Alison Lennox Office of Immunology and Inflammation DPACC	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 1, 2, 3, 4, 6, 7, 8.3, 10, 11, 15, 16, 17, 22, 24, 25	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Alison T. Lennox -S Digitally signed by Alison T. Lennox -S Date: 2024.06.12 16:17:03 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Cross-Disciplinary Team Lead	Robert Lim Office of Immunology and Inflammation DPACC	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: All	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Robert H. Lim -S Digitally signed by Robert H. Lim -S Date: 2024.06.12 05:45:39 -04'00'				
Clinical Deputy Director	Banu Karimi-Shah Office of Immunology and Inflammation DPACC	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: All	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature/date/time stamp: Banu A. Karimi-shah -S Digitally signed by Banu A. Karimi-shah -S Date: 2024.06.13 10:49:01 -04'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Signatory Authority	Kathleen Donohue Office of Immunology and Inflammation	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: All	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	

Signature/date/time stamp:

Kathleen Donohue -S

Digitally signed by Kathleen Donohue -S
Date: 2024.06.14 08:40:03 -04'00'

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/

ALISON T LENNOX
06/25/2024 02:24:31 PM

KATHLEEN M DONOHUE
06/25/2024 03:48:50 PM