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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	**NDA 208-294**
Supporting document/s:	**Sequences 0000**
Applicant's letter date:	**June 25, 2015**
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Product:	**Bevespi Aerosphere™ (glycopyrrolate and formoterol fumarate inhalation aerosol)**
Indication:	**Chronic Obstructive Pulmonary Disease (COPD)**
Applicant:	**Pearl Therapeutics**
Review Division:	**Pulmonary, Allergy, and Rheumatology Products**
Reviewer:	**Luqi Pei, Ph.D.**
Supervisor:	**Marcie Wood, Ph.D.**
Division Director:	**Badrul Chowdhury, M.D., Ph.D.**
Project Manager:	**Brandi Wheeler, Pharm. D.**

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LABELING RECOMMENDATION

This review recommends the following text for nonclinical sections of the Bevespi Aerosphere labeling: USE in SPECIFIC POPULATION (8.1 – 8.3), OVERDOSAGE (10), CLINICAL PHMARCOLOGY (12.1), and NONCLINICAL TOXICOLOGY (13.1). The OVERDOSAGE (10) section is included because (b) (4)

8 USE IN SPECIFIC POPULATION

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C. There are no adequate and well-controlled trials of BEVESPI AEROSPHERE or its individual components, glycopyrrolate and formoterol fumarate, in pregnant women. Because animal reproduction studies are not always predictive of human response, BEVESPI AEROSPHERE should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physicians if they become pregnant while taking BEVESPI AEROSPHERE

Glycopyrrolate: There was no evidence of teratogenic effects in rats and rabbits at approximately 18,000 and 270 times, respectively, the maximum recommended human daily inhalation dose (MRHDID) in adults (on a mg/m^2 basis at a maternal oral dose of 65 mg/kg/day in rats and at an maternal intramuscular injection dose of 0.5 mg/kg in rabbits).

Single-dose studies in humans found that very small amounts of glycopyrrolate passed the placental barrier.

Formoterol Fumarate: Formoterol fumarate has been shown to be teratogenic, embryocidal, to increase pup loss at birth and during lactation, and to decrease pup weights in rats and teratogenic in rabbits. These effects were observed at approximately 1500 (rats) and 61,000 (rabbits) times the MRHDID, respectively (on a mg/m^2 basis at maternal oral doses of 3 mg/kg/day and above in rats and 60 mg/kg/day in rabbits). Umbilical hernia was observed in rat fetuses at approximately 1500 times the MRHDID (on a mg/m^2 basis at maternal oral doses of 3 mg/kg/day and above). Prolonged pregnancy and fetal brachygnathia were observed in rats at approximately 7600 times the MRHDID (on a mg/m^2 basis at a maternal oral dose of 15 mg/kg/day). In another study in rats, no teratogenic effects were seen at approximately 600 times the MRHDID (on a mg/m^2 basis at maternal inhalation doses up to 1.2 mg/kg/day).

Subcapsular cysts on the liver were observed in rabbit fetuses at approximately 61,000 times the MRHDID (on a mg/m^2 basis at a maternal oral dose of 60 mg/kg/day in rabbits). No teratogenic effects were observed at approximately (b) (4) times the MRHDID (on a mg/m^2 basis at maternal inhalation doses up to 3.5 mg/kg/day).

(b) (4)

8.2 Labor and Delivery

There are no well-controlled human trials that have investigated the effects of BEVESPI AEROSPHERE on preterm labor or labor at term. Because $beta_2$-agonists may potentially interfere with uterine contractility, BEVESPI AEROSPHERE should be used during labor only if the potential benefit justifies the potential risk.

8.3 Nursing mothers

It is not known whether BEVESPI AEROSPHERE is excreted in human milk. Because many drugs are excreted in human milk and because formoterol fumarate, one of the active ingredients in BEVESPI AEROSPHERE, has been detected in the milk of lactating rats, caution should be exercised when BEVESPI AEROSPHERE is administered to a nursing woman. Since there are no data from controlled trials on the use of BEVESPI AEROSPHERE by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue BEVESPI AEROSPHERE, taking into account the importance of BEVESPI AEROSPHERE to the mother.

10 OVERDOSAGE

The following proposed text is to be evaluated by the medical team: No cases of overdose have been reported with BEVESPI AEROSPHERE. BEVESPI AEROSPHERE contains both glycopyrrolate and formoterol fumarate; therefore, the risks associated with overdosage for the individual components described below apply to BEVESPI AEROSPHERE. Treatment of overdosage consists of discontinuation of BEVESPI AEROSPHERE together with institution of appropriate symptomatic and/or supportive therapy. The judicious use of a cardio-selective beta-receptor blocker may be considered, bearing in mind that such medication can produce bronchospasm. Cardiac monitoring is recommended in case of overdosage.

Glycopyrrolate: High doses of glycopyrrolate, a component of BEVESPI AEROSPHERE, may lead to anticholinergic signs and symptoms. However, there were no systemic anticholinergic adverse effects following single inhaled doses up to 144 mcg in (b) (4) subjects with COPD.

Formoterol Fumarate: An overdose of formoterol fumarate would likely lead to an exaggeration of effects that are typical for $beta_2$-agonists: seizures, angina, hypertension, hypotension, tachycardia, atrial and ventricular tachyarrhythmias, nervousness, headache, tremor, palpitations, muscle cramps, nausea, dizziness, sleep disturbances, metabolic acidosis, hyperglycemia, hypokalemia. As with all sympathomimetic medications, cardiac arrest and even death may be associated with abuse of formoterol fumarate.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

BEVESPI AEROSPHERE: BEVESPI AEROSPHERE contains both glycopyrrolate and formoterol fumarate. The mechanism of action described below for the individual components apply to BEVESPI AEROSPHERE. These drugs represent two different classes of medications ((b) (4) and a long-acting selective beta$_2$-adrenoceptor agonist) that have different effects on clinical and physiological indices.

Glycopyrrolate: Glycopyrrolate is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of the M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methylcholine and acetylcholine-induced bronchoconstrictive effects was dose-dependent and lasted more than 12 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

Formoterol Fumarate: Formoterol fumarate is a long-acting beta$_2$-adrenergic agonist (beta$_2$-agonist) with a rapid onset of action. Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. *In vitro* studies have shown that formoterol has more than 200-fold greater agonist activity at beta$_2$-receptors than at beta$_1$-receptors. The *in vitro* binding selectivity to beta$_2$- over beta$_1$-adrenoceptors is higher for formoterol than for albuterol (5 times), whereas salmeterol has a higher (3 times) beta$_2$-selectivity ratio than formoterol.

Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta$_2$-agonists may have cardiac effects.

The pharmacologic effects of beta$_2$-adrenoceptor agonist drugs, including formoterol fumarate, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

In vitro tests show that formoterol fumarate is an inhibitor of the release of mast cell mediators, such as histamine and leukotrienes, from the human lung. Formoterol fumarate also inhibits histamine-induced plasma albumin extravasation in anesthetized guinea pigs and inhibits allergen-induced eosinophil influx in dogs with

airway hyper-responsiveness. The relevance of these *in vitro* and animal findings to humans is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

BEVESPI AEROSPHERE: Long-term studies in animals have not been performed to evaluate the carcinogenic potential of BEVESPI AEROSPHERE which contains glycopyrrolate and formoterol fumarate. The data described below for the individual components apply to BEVESPI AEROSPHERE.

Glycopyrrolate: Long term studies in animals have not been performed to evaluate the carcinogenic potential of inhaled glycopyrrolate or any other formulations of glycopyrrolate.

Glycopyrrolate was not mutagenic in the bacterial reverse mutation assay, the *in vitro* mammalian cell micronucleus assay in TK6 cells, or the *in vivo m*icronucleus assay in rats.

In reproduction studies in rats, dietary administration of glycopyrrolate resulted in diminished rates of conception in a dose-related manner. Other studies in dogs suggest that this may be due to diminished seminal secretion which is evident at high doses of glycopyrrolate.

Formoterol Fumarate: Long-term studies were conducted in mice using oral administration and rats using inhalation administration to evaluate the carcinogenic potential of formoterol fumarate.

In a 24-month carcinogenicity study in CD-1 mice, formoterol fumarate at oral doses of 0.1 mg/kg and above (approximately 25 (b) (4) the MRHDID on a mg/m^2 basis) caused a dose-related increase in the incidence of uterine leiomyomas.

In a 24-month carcinogenicity study in Sprague-Dawley rats, an increased incidence of mesovarian leiomyoma and uterine leiomyosarcoma were observed at the inhaled dose of 130 mcg/kg (approximately 65 times the MRHDID on a mcg/m^2 basis). No tumors were seen at 22 mcg/kg (approximately 10 times the MRHDID on a mcg/m^2 basis).

Other beta-agonist drugs have similarly demonstrated increases in leiomyomas of the genital tract in female rodents. The relevance of these findings to human use is unknown.

Formoterol fumarate was not mutagenic or clastogenic in the Ames *Salmonella*/microsome plate test, mouse lymphoma test, chromosome aberration test in human lymphocytes, and rat micronucleus test.

A reduction in fertility and/or reproductive performance was identified in male rats treated with formoterol at an oral dose of 15 mg/kg (approximately 7600 times the MRHDID on a mcg/m^2 basis). In a separate study with male rats treated with an oral dose of 15 mg/kg (approximately 7600 times the MRHDID on a mcg/m^2 basis), there were findings of testicular tubular atrophy and spermatic debris in the testes and

oligospermia in the epididymides. No such effect was seen at 3 mg/kg (approximately (b) (4) times the MRHDID on a mcg/m^2 basis). No effect on fertility was detected in female rats at doses up to 15 mg/kg (approximately 7600 times the MRHDID on a mcg/m^2 basis).

LABELING REVIEW

Edits to nonclinical sections of the Bevespi Aerosphere (referred as Bevespi hereafter) labeling proposed by the applicant are recommended. They are Sections 8.1 Pregnancy, 8.2 Labor and Delivery, 8.3 Nursing Mothers, 10 Overdosage, 12.1 Mechanism of Action, and 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility, (b) (4) Edits are recommended to ensure labeling consistency between products of the same pharmacologic class.

1. INTRODUCTION

The review evaluates nonclinical sections of the Bevespi labeling proposed by Pearl on June 25, 2015. These sections are 8.1, 8.2, 8.3, 10, 12.1, 13.1 (b) (4) The review finds major edits necessary. (b) (4) (b) (4) and line edits of the remaining sections. The line edits include (b) (4)

Bevespi is a metered-dose inhaler of a combination drug product. Its proposed indication is COPD. Bevespi contains glycopyrrolate (GP) and formoterol fumarate (FF) as the active pharmaceutical ingredients (API). Each actuation of Bevespi releases 9-µg GP and 4.8-µg FF. The maximum recommended human daily inhalation dose (MRHDID) of Bevespi is 2 puffs, twice daily. For an adult patient with a 60-kg body weight, the respective MRHDID for GP and FF is 36 and 19.2 µg on a total daily dose basis, 0.6 and 0.32 µg/kg/day on a body weight basis, and 22.2 and 11.84 µg/m^2 on a body surface area basis.

Both BP and FF are APIs of approved and currently marketed drug products. Glycopyrrolate is the API of Robinul Injection Solution (NDA 17-855) and other products.[1] Formoterol fumarate is the API of Symbicort (FF/budesonide) Inhalation Aerosol (NDA 21-929) and other products. Both Robinul and Symbicort are the reference products of the Bevespi application. Table 1 (next page) presents an overview of Bevespi and its reference products.

Labeling recommendations for Bevespi are not fully PLLR compliant. The Bevespi NDA was submitted prior to June 30, 2015, and thus labeling is not required to be in PLLR

[1] Seebri and Utibron Neohalers (NDAs 207-923 and 207-930) are two products that the Agency approved in October 2015. The APIs are GP and GP/indacaterol maleate in Seebri and Utibron, respectively. The Bevespi application is a 505(b)(2) application but it was developed independent of the Seebri and Utibron applications.

format. The review team discussed this approach in a filing meeting held on August 3, 2015.

This review consists of 4 sections. Section 1 provides background information relevant to the current review. Section 2 discusses methods and rationale used for this review. It also discusses approaches for deriving dose ratios between animals and humans in the nonclinical sections. Section 3 provides line edits of the proposed labeling sections. Section 4 provides review recommendations. A clean copy of the recommended text of the nonclinical sections of the Bevespi label is presented in the LABELING RECOMMENDATION section which is located at the beginning of the document.

Table 1: Overview of Bevespi and Reference Products

Drug product		API	ROA [a]	NDA number	Indication	MRDD (mcg)		Date of approval
Name	Dosage					GP	FF	
Robinul	Solution	GP	IV, IM	17-558	Peptic ulcer, pre-operative aid	400 [b]	NA	2/6/1975
Symbicort	MDI	Bud/FF	IH	21-929	COPD/Asthma	NA	18	7/21/2006
Bevespi	MDI	GP/FF	IH	208-294	COPD	36	19.2	In review

a. ROA = route of administration, MDI = metered dose inhaler, IV = intravenous, IM = intramuscular, COPD = chronic obstructive pulmonary disease, and NA = not applicable, Bud = budesonide.

b. Derived based on the recommended dose of 100 mcg, 4 times/day (IM) for a 60-kg peptic ulcer patient.

2. EVALUATION

2.1 Method of Labeling Review

The review evaluates the proposed labeling based on the available data in the literature and the labeling of approved products of same class. The review uses the labeling of Robinul and Symbicort (NDA 17-855 and 21-929, respectively) as the primary source of nonclinical data because they are the reference products of the Bevespi application. The API(s) in Bevespi, Robinul, and Symbicort are GP/FF, GP, and FF/budesonide, respectively.

The review also uses labeling of similar products for consistency because of the difference in routes of administration between Robinul and Bevespi. Rubinol is a parenteral product and its labeling was written decades ago. Bevespi is an inhalation product. The difference in routes of administration between the two products renders certain information in Rubinol labeling irrelevant or obsolete. Glycopyrrolate is an anticholinergic. The review uses the labeling of other anticholinergic products to construct Bevespi labeling. Examples of inhaled anticholinergics currently on the market are tiotropium, aclidinium, umeclidinium, and GP.

2.2 Exposure Multiples

This review derives API exposure multiples (EM) between animals and humans on a mg/m^2 basis. Both GP and FF doses were reported as mg/kg/day in animal toxicity

studies and mg/day in humans. The review converts the human dose to mg/kg/day by dividing the total daily dose (mg) by 60 kilograms. The conversion of the mg/kg/day dose to the mg/m^2/day dose in a given species was achieved using the following formula:

$$X \text{ mg/kg} \times K_m = Y \text{ mg/m}^2,$$

Where K_m was 3, 6, 12, and 37 for mice, rats, rabbits, and humans, respectively.

For example, an EM of 1500 between daily FF doses of 3 mg/kg in rats and 19.2 mcg in humans was derived as described below.

Rats: 3 (mg/kg) x 6 (kg/m^2) = 18 (mg/m^2)
Human: 0.0192 mg ÷ 60 (kg) x 37 (kg/m^2) = 0.01184 (mg/m^2)
EM $_{(rat/human)}$: 18 (mg/m^2) ÷ 0.01184 (mg/m^2) = 1520 or ~ 1500

Table 2 lists EMs used in Bevespi labeling. Glycopyrrolate and FF doses in animals in Table 2 were taken from the Robinul and Symbicort labeling (NDA 17-588 and 21-929, respectively) which were approved on May 26, 2005, and May 10, 2010, respectively.[2,3] The recommended daily dose in humans is 0.0222 and 0.01184 mg/m^2 for GP and FF, respectively. See INTRODUCTION section for calculations of GP and FF doses in humans.

Table 2: Dose Ratios between Animals and Humans

API	Section	Species	ROA	Dose		Dose Ratio [a]		Reference
				mg/kg /day	mg/m^2/ day	Calcu-lated	Rounded to	
FF	Pregnancy	Rat	PO	3	18	1520	1500	NDA 21-929
				15	90	7601	7600	
			IH	1.2	7.2	608	600	
		Rabbit	PO	60	720	60810	61000	
				3.5	44.2		(b) (4)	
	Fertility	Rat, M	PO	15	90	7601	7600	
			PO	3.0	1.8	1520	1500	
	Carcinog.	Rat	IH	0.13	0.78	66	65	
				0.022	0.132	11	10	
		Mice	PO	0.1	0.3	25	25	
GP	Pregnancy	Rat	PO	65	390	17,568	18,000	NDA 17-558
		Rabbit	IM	0.5	6	270	270	

a. On a mg/m^2 basis unless specified. The human dose was 0.0222 and 0.01184 mg/m^2/day for GP and FF, respectively.

Of note, there are differences in FF EMs between the Bevespi and Symbicort labeling at a given FF dose in animals. For example, the 3-mg/kg/day FF dose in rats gives EMs of 1500 and 1400 in Bevespi and Symbicort labeling, respectively. The differences in EMs were due to two factors: an increase in the recommended clinical dose of FF and the change in human body weight used to derive the EM. The recommended daily FF

[2] http://www.accessdata.fda.gov/drugsatfda_docs/label/ 2005/017558s053lbl.pdf
[3] http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021929s021lbl.pdf

dose in humans is 19.2 and 18 mcg in Bevespi and Symbicort, respectively. Mean human body weights of 50 and 60 kg were used to derive EMs in the Symbicort and Bevespi labeling reviews, respectively.

2.3 Evaluation of the proposed labeling

Contents of the proposed labeling of Bevespi are generally acceptable, but the review finds it necessary to edit the proposed text. The edits are made to ensure that the to-be-approved labeling reflects the Agency's current thinking. See Section 3 for the recommended edits. See the LABELING RECOMMENDATION Section for a clean copy of the recommended text for the nonclinical section of the Bevespi labeling. Below is a brief discussion of the rationale for the recommended edits.

Pearl states that their proposal was based on labeling of the same class of products approved and marketed in the US. Specifically, Pearl used Symbicort, Robinul, and Anoro (Umeclidinium bromide; NDA 203-975, approved on December 18, 2013) to construct their proposal.[4]

The evaluation concludes that the following sections of the proposed text should be deleted: (b) (4) As discussed below, the deletion is to ensure that the to-be-approved labeling reflects the Agency's most current thinking. The evaluation also concludes that the remaining nonclinical sections of the proposed labeling should be edited. This section discusses the general principles for constructing the information for APIs, alone or in combination.

2.3.1 Glycopyrrolate

With the exception of Section 12.1 Mechanism of Action, contents of GP sections of the proposed labeling of Bevespi were generally acceptable. The reason is that they were generally the same as the Robinul labeling. There is no new, significant nonclinical data in the areas of reproductive toxicology and carcinogenesis in the literature that warrant any revisions to the content of these sections.

Significant advances have been made in understanding the mechanism of action of inhaled anticholinergics in COPD over several decades. The to-be-approved labeling should reflect these advances. Table 3 (next page) provides a comparison between the labeling of Robinul and Seebri (also a GP product). The to-be-approved labeling of Bevespi should be consistent with that of the marketed products in the same class: tiotropium, aclidinium, umeclidinium, and GP.

Further, Robinul and Bevespi differ in their routes of administration. Robinul is a parenteral product while Bevespi is an inhalation product. Some information in the Robinul labeling may not be applicable to the Bevespi application. Overall, the Mechanism of Action section should be updated to reflect the current thinking of the drug class.

[4] http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203975s000lbl.pdf

The content of the Mechanism of Action section proposed by Pearl generally reflects the class labeling that the Agency developed for inhaled anticholinergics with two exceptions: 1) Pearl introduced a new statement that read: (b) (4) [redacted] The review recommends rejecting the proposed addition and accepting the modified duration of action time. The nonclinical data submitted by Pearl showed that the duration of GP action lasted for 12 hours in in vivo animal models (see the nonclinical original NDA review completed by Dr. Luqi Pei on March 10, 2016, DARRTS ID#3900432).

Table 3: Pharmacology Section of Labeling of Currently Marketed Inhaled MAs

Robinul Solution (NDA 17-558)	Seebri™ NeoHaler® (NDA 207-923) [a]
Glycopyrrolate, like other anticholinergic (antimuscarinic) agents, inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation. These peripheral cholinergic receptors are present in the autonomic effector cells of smooth muscle, cardiac muscle, the sinoatrial node, the atrioventricular node, exocrine glands and, to a limited degree, in the autonomic ganglia. Thus, it diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal, and bronchial secretions. (Approved on May 26, 2005, See Footnote 2)	Glycopyrrolate is a long-acting, anti-muscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors, M_1 to M_5. In the airways, it exhibits pharmacological effects through inhibition of M_3-receptors at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The bronchodilation following inhalation of GP is predominantly a site-specific effect. (Approved on Dec. 18, 2013)[5]

a. Tiotropium, aclidinium, and umeclidinium (NDAs 21-935, 202-450, and 203-975, respectively) labeling approved on January 30, 2004, July 23, 2012, and December 18, 2013, respectively, have the same content, except for drug names.

It is noted that this review does not rely on the Seebri application for either approval or labeling, although Table 3 presents the Seebri application as an example. The reason is that: 1) Seebri (approved in 2015) contains the same API as Bevespi, and 2) that the Mechanism of Action section of Seebri and other products (i.e., tiotropium, aclidinium, and umeclidinium) that the Agency approved in the last two decades carry the same statements in the labeling, and 3) these statements were developed in 2004 when the Agency approved the Spiriva application (tiotropium, NDA 21-395).

Overall, the evaluation recommends the following for GP portions of the proposed labeling:

1) Delete the (b) (4) [redacted]
2) Delete the (b) (4) [redacted]
3) Rewrite the Pregnancy section (8.1) (b) (4) [redacted]
4) Remove statements (b) (4) [redacted]

[5] http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207923lbl.pdf

5) Use the class labeling for the 12.1 Mechanism of Action for inhaled anticholinergics.
6) Perform line editing of the remaining nonclinical sections of the proposed labeling. See Section 3 for line editing for the proposed labeling.

2.3.2 Formoterol fumarate

Contents of FF sections of the proposed labeling of Bevespi were generally acceptable because they were generally the same as the Symbicort labeling. However, major edits are recommended. These edits are necessary to ensure labeling consistency across products of similar class and to ensure that the labeling reflect the Agency's current thinking. The review has discussed justifications in detail earlier. Specifically, the review recommends the following edits to the FF portions of the proposed labeling:

1) Delete the (b) (4)
2) Rewrite the Pregnancy section (8.1) (b) (4)
3) Perform line editing of the remaining nonclinical sections of the proposed labeling. See Section 3 for line editing for the proposed labeling.

2.3.3 Glycopyrrolate and Formoterol fumarate

The proposed text for this section is acceptable from the nonclinical perspective. Contents of GP and FF interaction sections of the proposed labeling of Bevespi were generally reflective of the clinical discipline. There were no nonclinical interaction studies that affect the Bevespi labeling. The review defers the evaluation of these sections to the clinical team.

3. LINE EDITS TO THE PROPOSED LABELING

This section presents the recommended line editing to the nonclinical sections of the proposed Bevespi labeling (i.e., Sections 8, 10, 12.1, and 13). Highlights indicate the recommended edits. Underline indicates the suggested additions. Strikethrough indicates the suggested deletions. Section 1 presents a clean copy of the recommended labeling for these sections.

8 USE IN SPECIFIC POPULATION

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C. There are no adequate and well-controlled (b) (4) trials of BEVESPI AEROSPHERE or its individual components, glycopyrrolate and formoterol fumarate, in pregnant women. Because animal reproduction studies are not always predictive of human response, BEVESPI AEROSPHERE should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physicians if they become pregnant while taking BEVESPI AEROSPHERE

Glycopyrrolate: There was no evidence of teratogenic effects in rats and rabbits at approximately 18,000 and 270 times, respectively, the maximum recommended human daily inhalation dose (MRHDID) in adults (on a mg/m^2 basis at a maternal oral dose of 65 mg/kg/day in rats and at an maternal intramuscular injection dose of 0.5 mg/kg in rabbits). (b) (4)

(b) (4)

Single-dose studies in humans found that very small amounts of glycopyrrolate passed the placental barrier.

Formoterol Fumarate: Formoterol fumarate has been shown to be teratogenic, embryocidal, to increase pup loss at birth and during lactation, and to decrease pup weights in rats and teratogenic in rabbits. These effects were observed (b) (4) at (b) (4) approximately (b) (4) 1500 (rats) and 61,000 (rabbits) times (b) (4) the MRHDID (b) (4) (on a mcg/m^2 basis at maternal oral doses of 3 mg/kg/day and above in rats and 60 mg/kg/day in rabbits). Umbilical hernia was observed in rat fetuses at ~~oral doses~~ approximately (b) (4) 1500 (b) (4) times the (b) (4) MRHDID (on a mcg/m^2 basis at maternal oral doses of 3 mg/kg/day and above). Prolonged pregnancy and fetal b~~B~~rachygnathia ~~was~~ (b) (4) observed in rats (b) (4) at (b) (4) approximately (b) (4) 7600 (b) (4) times the MRHDID (on a mcg/m^2 basis at a maternal oral dose of 15 mg/kg/day). (b) (4)

(b) (4) (b) (4) In another study in rats, no teratogenic effects were seen at (b) (4) approximately (b) (4) 600 times the MRHDID (b) (4) (on a mcg/m^2 basis at maternal inhalation doses up to 1.2 mg/kg/day).

Subcapsular cysts on the liver were observed in rabbit fetuses at ~~an oral dose~~ approximately (b) (4) 61,000 times the MRHDID (b) (4) (on a mcg/m^2 basis at a maternal oral dose of 60 mg/kg/day in rabbits). No teratogenic effects were observed at (b) (4) approximately (b) (4) times the (b) (4) MRHDID (on a mcg/m^2 basis at maternal inhalation doses up to 3.5 mg/kg/day).

(b) (4)

(b) (4)

8.2 Labor and Delivery

There are no well-controlled human (b) (4) trials that have investigated the effects of BEVESPI AEROSPHERE on preterm labor or labor at term. Because beta$_2$-agonists may potentially interfere with uterine contractility, BEVESPI AEROSPHERE should be used during labor only if the potential benefit justifies the potential risk.

8.3 Nursing mothers

It is not known whether BEVESPI AEROSPHERE is excreted in human milk. Because many drugs are excreted in human milk and because formoterol fumarate, one of the active ingredients in BEVESPI AEROSPHERE, has been detected in the milk of lactating rats, caution should be exercised when BEVESPI AEROSPHERE is administered to a nursing woman. Since there are no data from controlled trials on the use of BEVESPI AEROSPHERE by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue BEVESPI AEROSPHERE, taking into account the importance of BEVESPI AEROSPHERE to the mother

10 OVERDOSAGE

No cases of overdose have been reported with BEVESPI AEROSPHERE. BEVESPI AEROSPHERE contains both glycopyrrolate and formoterol fumarate; therefore, the risks associated with overdosage for the individual components described below apply to BEVESPI AEROSPHERE. Treatment of overdosage consists of discontinuation of BEVESPI AEROSPHERE together with institution of appropriate symptomatic and/or supportive therapy. The judicious use of a cardio-selective beta-receptor blocker may be considered, bearing in mind that such medication can produce bronchospasm. Cardiac monitoring is recommended in case of overdosage.

Glycopyrrolate: High doses of glycopyrrolate, a component of BEVESPI AEROSPHERE, may lead to anticholinergic signs and symptoms. However, there were no systemic anticholinergic adverse effects following single inhaled doses up to 144 mcg in healthy volunteers and subjects with COPD.

Formoterol Fumarate: An overdose of formoterol fumarate would likely lead to an exaggeration of effects that are typical for beta$_2$-agonists: seizures, angina, hypertension, hypotension, tachycardia, atrial and ventricular tachyarrhythmias, nervousness, headache, tremor, palpitations, muscle cramps, nausea, dizziness, sleep disturbances, metabolic acidosis, hyperglycemia, hypokalemia. As with all sympathomimetic medications, cardiac arrest and even death may be associated with abuse of formoterol fumarate.

(b) (4)

(b) (4)

12.1 Mechanism of Action

BEVESPI AEROSPHERE: BEVESPI AEROSPHERE contains both glycopyrrolate and formoterol fumarate. The mechanism of action described below for the individual components apply to BEVESPI AEROSPHERE. These drugs represent two different classes of medications (a cholinergic and a long-acting selective beta$_2$-adrenoceptor agonist) that have different effects on clinical and physiological indices.

Glycopyrrolate: Glycopyrrolate is a long-acting, (b) (4) –antimuscarinic agent, (b) (4) which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of the M3 receptor at the smooth muscle leading to bronchodilation. (b) (4) The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methylcholine and acetylcholine-induced bronchoconstrictive effects was dose-dependent and lasted more than 12 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

Formoterol Fumarate: Formoterol fumarate is a long-acting ~~selective~~ beta$_2$-adrenergic agonist (beta$_2$-agonist) with a rapid onset of action. Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. *In vitro* studies have shown that formoterol has more than 200-fold greater agonist activity at beta$_2$-receptors than at beta$_1$-receptors. The *in vitro* binding selectivity to beta$_2$- over beta$_1$-adrenoceptors is higher for formoterol than for albuterol (5 times), whereas salmeterol has a higher (3 times) beta$_2$-selectivity ratio than formoterol.

Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors. The precise function of these receptors has not been established, but they raise the possibility that even highly selective beta$_2$-agonists may have cardiac effects.

The pharmacologic effects of beta$_2$-adrenoceptor agonist drugs, including formoterol fumarate, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells.

In vitro tests show that formoterol fumarate is an inhibitor of the release of mast cell mediators, such as histamine and leukotrienes, from the human lung. Formoterol

fumarate also inhibits histamine-induced plasma albumin extravasation in anesthetized guinea pigs and inhibits allergen-induced eosinophil influx in dogs with airway hyper-responsiveness. The relevance of these *in vitro* and animal findings to humans is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

BEVESPI AEROSPHERE: Long-term studies in animals have not been performed to evaluate the carcinogenic potential of BEVESPI AEROSPHERE which contains glycopyrrolate and formoterol fumarate. The data described below for the individual components apply to BEVESPI AEROSPHERE.

Glycopyrrolate: Long term studies in animals have not been performed to evaluate the carcinogenic potential of inhaled glycopyrrolate or any other formulations of glycopyrrolate.

Glycopyrrolate was not mutagenic in the ~~B~~bacterial ~~R~~reverse m~~M~~utation a~~A~~ssay, the *in vitro* m~~M~~ammalian c~~C~~ell m~~M~~icronucleus a~~A~~ssay in TK6 cells, or the *in vivo* m~~M~~icronucleus a~~A~~ssay in rats.

In reproduction studies in rats, dietary administration of glycopyrrolate resulted in diminished rates of conception in a dose-related manner. Other studies in dogs suggest that this may be due to diminished seminal secretion which is evident at high doses of glycopyrrolate.

Formoterol Fumarate: Long-term studies were conducted in mice using oral administration and rats using inhalation administration to evaluate the carcinogenic potential of formoterol fumarate.

In a 24-month carcinogenicity study in CD-1 mice, formoterol fumarate at oral doses of 0.1 mg/kg and above (approximately (b) (4) 25 (b) (4) the (b) (4) ~~(~~MRHDID~~)~~ on a (b) (4) $/m^2$ basis) caused a dose-related increase in the incidence of uterine leiomyomas.

In a 24-month carcinogenicity study in Sprague-Dawley rats, an increased incidence of mesovarian leiomyoma and uterine leiomyosarcoma were observed at the inhaled dose of 130 mcg/kg (approximately 65 times the (b) (4) MRHDID on a mcg/m^2 basis). No tumors were seen at 22 mcg/kg (approximately 10 times the (b) (4) MRHDID on a mcg/m^2 basis).

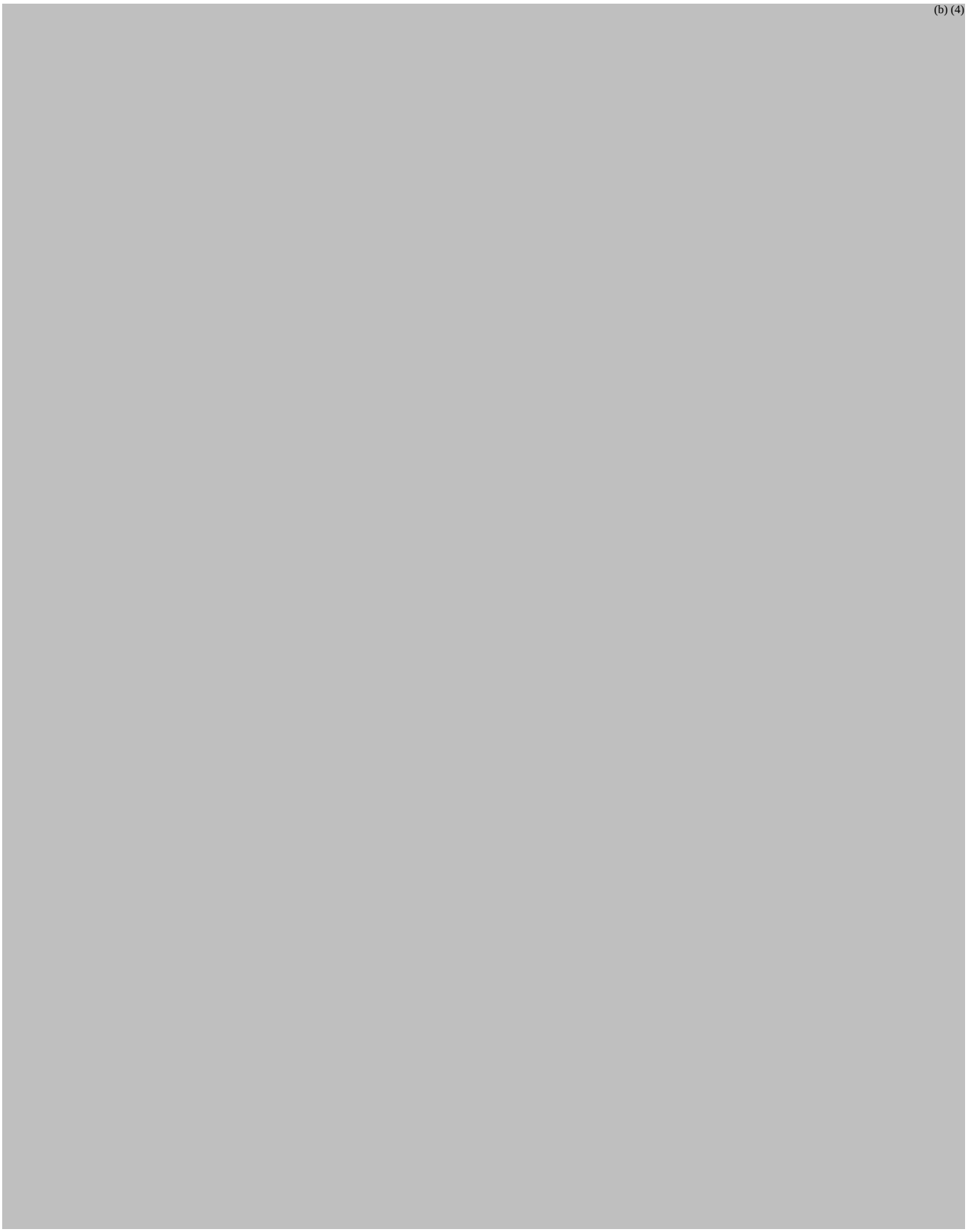
Other beta-agonist drugs have similarly demonstrated increases in leiomyomas of the genital tract in female rodents. The relevance of these findings to human use is unknown.

Formoterol fumarate was not mutagenic or clastogenic in (b) (4) Ames *Salmonella*/microsome plate test, mouse lymphoma test, chromosome aberration test in human lymphocytes, and rat micronucleus test.

A reduction in fertility and/or reproductive performance was identified in male rats treated with formoterol at an oral dose of 15 mg/kg (approximately (b) (4) 7600 times the (b) (4) MRHDID on a (b) (4) $/m^2$ basis). In a separate study with male rats treated with an oral dose of 15 mg/kg (approximately (b) (4) 7600 times the MRHDID (b) (4) on a (b) (4) m^2 basis), there were findings of testicular tubular atrophy and spermatic debris in the testes and oligospermia in the epididymides. No such effect was seen at 3 mg/kg (approximately (b) (4) MRHDID on a (b) (4) $/m^2$ basis). No effect on fertility was detected in female rats at doses up to 15 mg/kg (approximately (b) (4) 7600 times the (b) (4) MRHDID on a (b) (4) $/m^2$ basis).

(b) (4)

(b) (4)



4. RECOMMENDATIONS

1. Delete Section [redacted] (b) (4)
2. Delete [redacted] (b) (4) in Section 10 OVERDOSAGE.
3. Revise dose ratios between animals and humans.
4. Edit the proposed text so that the labeling language is consistent across drug products of the same class. See Section 2.3 EVALUATION OF THE PROPPOSED LABELING for discussions and justifications for the proposed edits.
5. See LABELING RECOMMENDATION for a clean copy of the recommended text for Sections 8, 10, 12, and 13.

Luqi Pei, Ph.D.
Pharmacologist/Toxicologist

Marcie Wood, Ph.D.
Pharmacology Supervisor

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/s/

LUQI PEI
04/18/2016

MARCIE L WOOD
04/18/2016

Secondary Pharmacology and Toxicology Review for NDA 208-294

TO: NDA 208-294 (Pearl Therapeutics)

FROM: Marcie Wood, Ph.D.
Supervisory Pharmacologist
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: March 25, 2016

Overview: I concur with the recommendation of Dr. Luqi Pei (detailed in a nonclinical review dated March 10, 2016) that the pharmacology and toxicology of Bevespi Aerosphere (glycopyrrolate and formoterol fumarate inhalation aerosol) have been adequately studied and the drug product should be approved from a nonclinical perspective.

Background: Bevespi Aerosphere is a fixed dose combination inhalation product containing glycopyrrolate (GP) and formoterol fumarate (FF), an anticholinergic and a $beta_2$-adrenergic agonist, respectively. It is indicated for the treatment of chronic obstructive pulmonary disease (COPD). Each drug product actuation releases 9.0 mcg of GP and 4.8 mcg of FF. The proposed dosage of Bevespi is two actuations twice daily.

Toxicology: The nonclinical program for Bevespi is an abbreviated program, as the applicant is relying, in part, on the Agency's previous findings of safety for GP and FF. Specifically, the applicant is relying on Robinul for GP nonclinical systemic safety information and on Symbicort, an approved inhalation product containing FF, for FF nonclinical safety information. The general toxicology program consisted of GP inhalation studies up to 6 months in rats and dogs, as well as a 3-month inhalation study of the GP and FF combination in dogs to assess for potential toxicological interactions.

In 6-month GP studies, drug-related findings were observed in rats, but not dogs. In the rat study, increased hyaline degeneration of the respiratory and olfactory epithelium and laryngeal squamous metaplasia in the larynx were observed in both sexes at 27.5 (mid-dose) and 54.8 (high-dose) mcg/kg/day (pulmonary deposited dose or PDD). An increased incidence of prostate inflammation was also observed in high-dose males. The rat study NOAEL was identified as the mid-dose due to prostate inflammation at the high dose. Findings in the nasal cavity are not considered relevant to humans who will receive Bevespi via oral inhalation, and findings in the larynx are considered a rat-specific finding. No drug-related findings were observed in the dog study at any dose, up to 18.7 mcg/kg/day (PDD), and the dog study NOAEL was identified as 18.7 mcg/kg/day.

In a 3-month GP and FF study in dogs, drug-related changes were observed in the respiratory tract, liver, and prostate, but there were no indications of significant toxicological interactions between GP and FF. Dr. Pei's review identified the low combination dose (4.3 and 1.1 mcg/kd/day of GP and FF, respectively) as the NOAEL.

See Dr. Pei's review for further details on the toxicological characterization of Bevespi.

Genotoxicity: GP was negative in a bacterial gene mutation assay in vitro, mammalian cell chromosomal aberration in TK6 cells, and in an in vivo micronucleus test in rats. (b) (4) per the Symbicort approved label.

Carcinogenicity: The carcinogenic potential for GP was not evaluated or required, as no pre-neoplastic or neoplastic lesions were observed in chronic inhalation GP studies. Per the Symbicort approved label, (b) (4)

Reproductive and Developmental Toxicology: Per the Robinul approved label, GP was not teratogenic in rats (b) (4) but it did cause reduced pup survival and conception rates in rats. Per the Symbicort label, FF was teratogenic in both rats and rabbits. FF also decreased fertility in male rats.

Labeling: A nonclinical labeling review for Bevespi is pending.

There are no outstanding Pharmacology and Toxicology issues for this product.

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/s/

MARCIE L WOOD
03/28/2016

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	**NDA 208-294**
Supporting document/s:	**Sequences 0000**
Applicant's letter date:	**June 25, 2015**
CDER stamp date:	**June 25, 2015**
Product:	**Bevespi Aerosphere™ (glycopyrrolate and formoterol fumarate inhalation aerosol)**
Indication:	**Chronic Obstructive Pulmonary Disease (COPD)**
Applicant:	**Pearl Therapeutics**
Review Division:	**Pulmonary, Allergy, and Rheumatology Products**
Reviewer:	**Luqi Pei, Ph.D.**
Supervisor:	**Marcie Wood, Ph.D.**
Division Director:	**Badrul Chowdhury, M.D., Ph.D.**
Project Manager:	**Brandi Wheeler, Pharm. D.**

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208-294 are owned by Pearl Therapeutics or are data for which Pearl has obtained a written right of reference. Any information or data necessary for approval of NDA 208-294 that Pearl does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208-294.

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LIST OF ABBREVIATIONS

Acronym	Definition	Acronym	Definition
AD	Achieved dose	ICH	International Conference on Harmonization
ADME	Absorption, distribution, metabolism, and elimination	ID	Inhaled dose
		IND	Investigational new drug
API	Active pharmaceutical ingredient	IIG	Inactive Ingredient Guide
AUC	Area under the curve	IR	Information request
C	Control	ITS	Inhalation toxicity study
CHD	GFF high-dose	IPB	Ipratropium bromide
CLD	GFF low-dose	LABA	Long acting beta-adrenergic antagonist
CMD	GFF mid-dose	LAMA	Long acting muscarinic antagonist
CNS	Central nervous system	LD	Low dose
COPD	Chronic obstructive pulmonary disease	MA	Muscarinic antagonist
DARRTS	Document archiving, reporting, and regulatory tracking system	MD	Mid dose
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine	MDI	Metered-dose inhaler
DMF	Drug master file	MMAD	Mass median aerodynamic diameter
DPARP	Division of Pulmonary, Allergy, and Rheumatology Drug Products	MRHD	Maximum recommended human dose
ECG	Electrocardiogram	MRHDID	maximum recommended human daily inhalation dose
EOP2	End-of-Phase 2	MRS	Muscarinic receptor subtype
FF	Formoterol fumarate	NDA	New drug application
FLD	Formoterol low dose	PDD	Pulmonary deposited dose
FMD	Formoterol mid dose	PK	Pharmacokinetics
FHD	Formoterol high dose	PO	Oral
GFF	Glycopyrrolate and formoterol fumarate	PP	Porous particles
GLP	Good laboratory practice	RS	Respiratory system
GPF	Glycopyrrolate and formoterol fumarate	TIO	Tiotropium bromide
GP	Glycopyrrolate		
GSD	Geometric standard deviation		
HD	High dose		

1 Executive Summary

1.1 Introduction

This review evaluates nonclinically the safety of the Bevespi Aerosphere™ (referred to as Bevespi hereafter) application. The review finds that the applicant has conducted adequate nonclinical characterizations of the product to support the safety of its proposed use.

Pearl Therapeutics (the applicant) proposed to register Bevespi as a therapy for chronic pulmonary obstructive disease (COPD). Bevespi is a metered-dose inhaler (MDI) and a combination product containing glycopyrrolate (GP) and formoterol fumarate (FF) as the active pharmaceutical ingredients (API).[1] The former is a long-acting muscarinic antagonist (LAMA), referred to as an anticholinergic; the latter is a long-acting beta$_2$-adrenergic agonist (LABA). Each actuation of Bevespi releases 9.0-µg GP and 4.8-µg FF, respectively. The recommended human daily dose of Bevespi is two actuations twice daily.

Bevespi is a 505(b)(2) application. It relies, in part, on the Agency's previous determination on the safety and efficacy of GP to support the Bevespi application. Specifically, Pearl relies on the literature and NDA 17-558 for pharmacology and reproductive toxicology data. See Section 2.7 Regulatory Background for additional information.

The review uses pulmonary deposited doses (PDD) in inhalation toxicity studies (ITS) as actual exposures in animals. This approach results in differences in API exposures between the review and the study reports which uses achieved doses (AD). The review derived PDD by multiplying AD by appropriate deposition factors (DF) in a given species: 0.1 and 0.25 in rats and dogs, respectively. Further, the AD was average of males and females reported at the same exposure conditions.[2]

1.2 Brief Discussion of Nonclinical Findings

Daily inhalation exposures to GP for 6 months had no significant adverse effects on the respiratory system in rats and dogs. There was no toxicological drug-drug interaction between GP and FF when inhaled in dogs.

Toxicity of inhaled GP was evaluated in 6-month ITS in rats and dogs. Rats and dogs (15 and 4/sex/dose in rats and dogs, respectively) were exposed to aerosols containing approximately 7.3-µg/L GP for up to 120 and 30 minutes daily, respectively. The respective PDD of GP in the low (LD), mid (MD), and high dose (HD) groups was 6.8, 27.5, and 54.8 in rats; and 4.6, 14.6, and 18.7 mg/kg/day in dogs. The respective mean plasma GP AUC was 2.2, 10.0, and 26.1 µg.h/mL in

[1] Glycopyrrolate and formoterol fumarate are also known as glycopyrronium bromide and formoterol fumarate dihydrate, respectively.

[2] The daily AD was calculated using the following formula: Dose = (C × RMV × T × DF)/BW, where C = aerosol test concentration (µg/L) obtained from the chemical analysis using a HPLC-UV method, T = the duration of exposure (min/day), DF = 1, RMV = $0.499BW^{0.809}$, BW = kg measured in a given week, respectively. The concentration was determined using a HPLC-UV method.

dogs, but not determined in rats. In rats, the mean plasma concentration was 0.035, 0.174, and 0.311 µg/mL for the LD, MD, and HD groups, respectively. Treatment-related findings were observed in rats, but not dogs. Rats in the MD and HD groups showed increases in the hyaline degeneration of respiratory and olfactory epithelium in the nose cavity and laryngeal squamous metaplasia in the larynx in both sexes. The HD males also showed increased incidence of inflammation in the prostate. No changes were observed in the LD group. In dogs, no treatment-related changes were observed in any dose groups.

Potential toxicological interactions between inhaled GP and FF were evaluated in a 3-month ITS of Bevespi clinical formulation (also referred as GFF MDI) in dogs. The PDD of GP/FF was 4.3/1.1, 10.7/2.5, and 14.8/3.5 µg/kg/day in the LD, MD, and HD groups, respectively. Both GP and FF were detected in the plasma. The mean plasma AUC of GP/FF on day 90 (males and females combined) was 3.06/1.34, 10.8/3.06, and 15.3/4.55 µg.h/mL for the LD, MD, and HD groups, respectively. No significant interactions were observed as toxicity observed with the combination product is consistent with the individual monoproducts.

1.3 Recommendations

1.3.1 Approvability

Approval of the application, pending labeling review, is recommended from the nonclinical perspective. The applicant proposed to register Bevespi Aerosphere™ (glycopyrrolate and formoterol fumarate) Inhalation Aerosol for an indication of chronic pulmonary obstructive disease (COPD). The applicant has conducted adequate nonclinical characterization of the API and excipients. There is adequate nonclinical data to support the safety of the proposed use of the product. The review recommends approval of the product from the nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

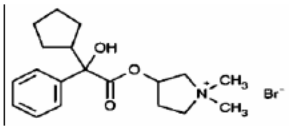
None

1.3.3 Labeling

A labeling review is pending.

2 Drug Information

2.1 Drug

	Glycopyrrolate	Formoterol
Generic Name:	Glycopyrronium bromide (GP)	Formoterol fumarate (FF)
Code Name:	NA, NVA237	PT005
CASRN:	596-51-0	43229-80-7
Chemical Name:	3 [(cyclopentylhydroxyphen-ylacetyl) oxy]-1, 1-dimethyl pyrrolidinium bromide	(±)-2′-Hydroxy-5′-[(*R**)-1-hydroxy-2-[[(*R**)-*p*-methoxy-α-methylphenethyl] amino]ethyl] formanilide fumarate (2:1) (salt), dihydrate
Molecular Formula:	$C_{19}H_{28}BrNO_3$	$C_{42}H_{56}N_2O_{14}$
Molecular Weight:	398.3	840.9
Structure:		
Pharmacologic Class:	Anticholinergic	$Beta_2$ adrenergic agonist

2.2 Relevant INDs, NDAs, and DMFs

(b) (4) INDs, 2 NDAs, and 3 DMFs are relevant to the current application (Table 1). The INDs were in support of the development of (b) (4) combination products of GP and FF. (b) (4) IND 107,739 is for the combination product.

Table 1: Relevant IND, NDA and DMF Applications

Appl. No.	Product	API	Indication	Date[1]
(b) (4)				
IND 107,739	Bevespi (GFF)	GP & FF	COPD	07/08/2010
NDA 17-558	Robinul[2]	GP	Peptic ulcer	02/06/1975
NDA 21-929	Symbicort	Budesonide/FF	COPD/asthma	07/21/2006
DMF	(b) (4)	NA[3]	NA	05/07/2003
DMF		NA	NA	09/08/1992
DMF		NA	NA	05/28/2015

1. Date for application opening and approval dates for INDs and NDAs, respectively. Date for DMFs is the submission date.
2. Parenteral solution for intramuscular and intravenous injection.
3. NA, Not applicable.

The NDAs (#17-558 and #21-929) are the approved and currently marketed products containing one of the APIs. Of the reference NDAs, Pearl is relying on the Agency's previous determination on the safety and efficacy of GP (NDA 17-558). Pearl obtained the right of reference to the FF safety data in the Symbicort MDI (NDA 21-929).

The DMFs are for the propellant (HFA-134a) and excipient (porous particles) of the product. See Section 2.7 Regulatory Background for additional information.

2.3 Drug Formulation

Bevespi Aerosphere™ (also referred as GFF MDI) is a metered-dose inhaler (MDI). It contains GP and FF as the APIs, PP as the excipient, and HFA-134a as the propellant (Table 2). Each canister of Bevespi Aerosphere™ (b) (4) delivers 120 actuations for patient use. Each actuation of Bevespi releases 9.0-µg glycopyrrolate and 4.8-µg formoterol fumarate.

Table 2: Formulation of GFF MDI

Component	Concentration (% w/w)	Quality per actuation [a]		Function
		Metered dose	Delivered dose	
Glycopyrronium bromide (GP)	(b) (4)	8.32 µg	7.2 µg	Active ingredient
Formoterol fumarate (FF)		5.55 µg	4.8 µg	Active ingredient
Porous particle (PP) [b]		(b) (4)		Co-suspending agent
HFA 134a				Propellant

a. Metered and delivered dose are expressed as the active moiety (or free base, i.e. glycopyrronium). Each actuation releases 9.0-µg glycopyrrolate.
b. See Section 2.4 Comments on Novel Excipients for the composition of the porous particles.

2.4 Comments on Novel Excipients

This product contains no novel excipients. Bevespi contains HFA-134a as the propellant and porous particles (PP) as an excipient. Both materials are present in approved and currently marketed products. Porous particles are compromized of (b) (4)% DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) and (b) (4)% $CaCl_2$ (calcium chloride). (b) (4)

(b) (4)

Pearl also evaluated PP safety in their nonclinical program. The evaluation consisted of the vehicle-control group in 6-month inhalation toxicity studies (ITS) of GP in rats and dogs. These studies revealed no porous particles-related effects on the respiratory system.

2.5 Comments on Impurities/Degradants of Concern

There are no safety concerns for any impurities/degradants identified in the product. The applicant has identified GP and FF impurities and degradants (several each), but the levels of the impurities were quite low: not-more-than (NMT) (b) (4)% for any impurity. Table 3 (next page) lists levels of individual impurities and degradants.

None of the identified impurities at the proposed specifications are a safety concern from the nonclinical perspective. GP impurities included glycopentylmandelic acid, 5-nitroisophalic acid, erythro diasteriomer of GP, and unspecified/unidentified compounds.

(b) (4)

Formoterol impurities included Impurities 5, A, F, G, I, and unidentified compounds. The proposed concentration of individual impurities was less than (b) (4)%.

Table 3: Impurities/degradants Specifications for GFF MDI

GP impurities			FF impurities		
	Specification			Specification	
Compound	Proposed	Monograph [a]	Compound	Proposed	Monograph [a]
Glycopentylmandelic acid	(b) (4)	-	Impurity 5	(b) (4)%	-
5-nitroisophalic acid		0.15%	Impurity A	%	0.3%
"Erythro" diasteriomer		≤ 0.4%	Impurity F	%	0.2%
Individual unspecified			Impurity G	%	0.1%
Total unspecified			Impurity I	%	(b) (4)%
			Individual unspecified	%	-
			Total unspecified	%	-

a. Source: Email by Dr. Benjamin Stevens dated October 28, 2015.

The proposed specifications are compliant to the qualification threshold of 1.0% in drug products with a maximum recommended human daily inhalation dose (MRHDID) of less than 10 mg [ICH-Q3B(R2)]. The MRHDID is 36 and 19.2 µg for GP and FF, respectively. The proposed specifications correspond to total daily exposure of (b) (4) and (b) (4) µg/day for GP and FF impurities, respectively. [4] The total daily exposure of each impurity was (b) (4) the qualification specification of 1.5 µg/day for genotoxicity impurities in drug products (ICH M7). However, the FF impurities levels (b) (4). The review recommends the drug product comply with the Monograph specifications.

2.6 Proposed Clinical Population and Dosing Regimen

Adult COPD patients will use Bevespi MDI twice daily. Each dose consists of two actuations. Each actuation of Bevespi releases 9-µg GP and 4.8-µg FF. This dosing regimen corresponds to the maximum recommended human daily inhalation dose (MRHDID) of 36-µg GP and 19.2-µg FF, respectively. This corresponds to nominal doses of 0.6-µg/kg/day glycopyrrolate and 0.32-µg/kg/day formoterol on a unit body-weight basis for a 60-kg patient.

2.7 Regulatory Background

Bevespi Aerosphere™ (glycopyrrolate and formoterol fumarate inhalation aerosol, or GFF MDI) is a 505(b)(2) application.[5] Pearl relies, in part, on the Agency's previous determination on the safety and efficacy of glycopyrrolate to support the nonclinical safety and efficacy of GFF MDI. Specifically, Pearl relies on the Robinul intramuscular solution (glycopyrrolate, NDA 17-558) for nonclinical systemic safety information (i.e., pharmacology and reproductive toxicology data). Approved in 1975, Robinul is given by

[4] Estimated from the total daily dose of 36-µg GP and 19.2-µg per patient, respectively. GP: 36 µg/day x (b) (4)% = (b) (4) µg/day; FF: 19.2 µg/day x (b) (4)% = (b) (4) µg/day.

[5] The Agency granted the trade name on September 15, 2015 (DARTTS ID# 3819018).

intravenous or intramuscular injection and indicated for peptic ulcer and preoperative use in anesthesia procedures to reduce gastric content spill. Pearl also relies on the literature for GP pharmacology information.

Pearl has obtained the right of reference to several other applications to support the safety and/or efficacy of other compounds in Bevespi. These compounds include FF (one of the 2 APIs), HFA-134a (propellant), and PP (excipient). Pearl is referencing Symbicort (NDA 21-929) to support FF safety. Symbicort (budesonide/formoterol) was approved in 2006 and indicated for COPD and asthma. Table 4 lists major characteristics of the reference applications of the APIs. Bevespi is listed for easy reference.

Table 4: Applications Referenced by the Bevespi Aerosphere Application

Reference product		API	ROA [a]	NDA number	Indication	Date of approval
Drug name	Dosage form					
Robinul	Solution	GP	IV, or IM	17-558	Peptic ulcer, pre-operative aid	2/6/1975
Symbicort	MDI	Bud[b]/FF	Inhalation	21-929	COPD and Asthma	7/21/2006
Bevespi	MDI	GP/FF	Inhalation	208-294	COPD	In review

a. ROA = route of administration, MDI = metered dose inhaler, IV = intravenous, IM = intramuscular, COPD = chronic obstructive disease.
b. Bud = budesonide.

Pearl also obtained the right of reference to the drug master file (DMF) of HFA-134a (DMFs (b) (4) HFA-134a is present in many approved and marketed inhalation products. See Table 1 for additional information on the DMFs. Finally, Pearl obtained the right of reference to DMF (b) (4) in support of the parous particles. (b) (4)

Table 5: Key Regulatory Events in INDs in Support of Bevespi Application

Application#	Date	Events	DARRTS ID#
(b) (4)			NA NA NA NA NA
IND 107,739	3/26/2010	Pre-IND meeting for GFF MDI	NA
	7/1/2012	Filing of original IND	NA
	4/19/2012	Written response to Type C meeting in GFF MDI program	NA
	12/21/2012	EOP2 Meeting – Clinical and CMC discipline	3247144
	6/2/2014	Pre-NDA meeting for GFF program	3535312

(b) (4) GFF = glycopyrrolate/formoterol, NA = not available.

(b) (4)

Pearl used (b) (4) INDs to support Bevespi development. These INDs are (b) (4) 107,739 (GFF MDI). (b) (4) (b) (4) Pearl collected additional nonclinical data to support GP use and to bridge the data gap between inhalation and non-inhalation uses of the compound. The additional data included 6-month inhalation toxicity studies of GP in rats and dogs and a 3-month interaction study of the GFF formulation in dogs. Requirements and designs of these studies were discussed under the relevant INDs. Table 5 lists regulatory milestones for these applications.

DPARP and Pearl had a number of meetings (b) (4) to discuss the development of (b) (4) combination products of the APIs. Key nonclinical issues included the rights of reference to NDAs and DMFs and nonclinical requirements for bridging the nonclinical data gap. DPARP requested the rights of reference for the following compounds: GP, FF, HFA-134a, and ingredients of the porous particle. Table 6 summarizes the timeline and applications in which the requests were made.

Table 6: Key Regulatory Events Related to Bevespi Application

Compounds	Key conclusion	Date	IND #
Glycopyrronium	Right of reference needed for data not owned by Pearl		(b) (4)
	6-mo IH study in rats needed for NDA	4/12/2009	107,739
	Carcinogenicity. & reproductive toxicity studies may not be needed pending the 6-mo IH study in rats and literature review	3/10/2010	(b) (4)
Formoterol	Need right of reference	6/4/2014	107,739
HFAa-134	Need right of reference	6/4/2008	(b) (4)
Porous excipient	Need right of reference to DSPC study reports cited	6/4/2008	
	Acceptance of the qualification plan	8/26/2009	
	6-mo ITS in rats sufficient for NDA submission	3/24/2010	
Bevespi MDI	No need for reproductive or genotoxicity studies of either GP or FF	3/26/2010	107,739
Impurities	Qualifying for ICH limits in NDA submission	6/4/2008	(b) (4)

As discussed earlier, the applicant has provided the right of reference to each of the above compounds (Tables 1 and 3). The following section discusses the requirements for glycopyrrolate, porous particles, and the clinical formulation only.

Requirements for glycopyrrolate: Pearl has fulfilled all nonclinical requirements for development of inhaled GP. DPARP determined that a 6-month ITS of glycopyrrolate in rats would be sufficient to evaluate the safety of glycopyrrolate for the nonclinical characterization of inhaled GP, if the study did not reveal any pre-neoplastic changes associated with inhalation of GP. (see *Requirements for GFF Formulation* below) This determination was made after lengthy discussions with the applicant. (b) (4) (b) (4)

Dr. Sancilio evaluated a draft protocol of the 6-month ITS in rats (FY10-120) (b) (4) Pearl submitted the draft protocol on December 1, 2009, proposing to dose rats (15/sex/dose) with 0 (air), 0 (vehicle), 0.05,

0.25, or 0.50-mg/kg/day glycopyrrolate for 6 months. Drs. Sancilio and Shea concurred with the protocol.

Pearl submitted the final report of of the 6-month inhalation toxicity study of GP in rats (Study FY10-120) in the NDA. Pearl also submitted the final report of a 6-month GP ITS in dogs (FY12-073). These studies did not show any pre-neoplastic changes associated with GP inhalation. Pearl has fulfilled all nonclinical requirements for clinical inhalation use of GP.

Pearl also completed a bacterial gene mutation, an in vitro mammalian chromosomal aberration test, and an in vivo micronucleus test of GP

Requirements for formoterol fumarate: Formoterol fumarate is an API of approved and marketed inhalation products. Symbicort MDI (NDA 21-929) is one of the products. Pearl has obtained the right of reference for the Symbicort application. No additional nonclinical data is required to support the current NDA.

Requirements for the GFF formulation: The application has fulfilled nonclinical requirements for the development of the GFF formulation. DPARP agreed in the March 26, 2010, Pre-IND Meeting that a 3-month ITS of the GFF formulation in dogs would be sufficient to support the NDA if the applicant fulfilled the requirements for GP (b) (4) The following text excerpted from the minutes of the March 26, 2010,

"***Nonclinical:***

1. ***Pearl Therapeutics plans to submit a 505(b)(2) NDA for GFF MDI in order to rely on the Agency's finding of safety and the nonclinical studies conducted with the reference listed drugs Robinul, Robinul Forte and Foradil Aerolizer (NDAs 20831, 21279, 17558, and 12827). As such, Pearl Therapeutics does not plan to conduct genotoxicity, reproductive toxicity, or carcinogenicity studies with GFF MDI, assuming no untoward finding in the toxicology studies. In principle, does the Agency concur with this approach?***

   FDA Response: We agree that no additional genotoxicity or reproductive toxicity studies are needed for either glycopyrrolate or formoterol as you will be submitting your NDA under the 505(b)(2) pathway. However, review of the 6-month rat glycopyrrolate study is needed prior to confirming that no carcinogenicity studies are needed (b) (4) *If pre-neoplastic/neoplastic lesions are observed in the 6-month rat study, a carcinogenicity study may be required. However, no additional carcinogenicity studies using the fixed dose combination product will be needed.*

   …

4. ***Can the FDA comment on whether the proposed nonclinical development plan, including a 3-month inhalation study in dogs in GFF MDI and 6-month rat and 3-month dog inhalation toxicology studies for the component products (GP MDI and FF MDI, respectively) and the excipient qualification plan are adequate to support an NDA approval [via the 505(b)(2) pathway] of GFF MDI for the long-term, twice daily (morning and evening) maintenance treatment*** (b) (4) ***with COPD, including chronic bronchitis and emphysema?***

   FDA Response: It appears that your proposed development plan is generally supportive of an NDA; however, (b) (4) *, "Questions regarding support for approval (product registration) cannot be addressed prior to submission and review of your complete application. Pending review, if your studies are sufficient to support required clinical trials, they would generally also be sufficient to support approval."*

The applicant submitted the final report of a 3-month GFF MDI formulation in dogs (Study FY10-129).

Requirements for the excipient: Pearl has fulfilled requirements for porous particle, the excipient in the GFF formulation. DPARP agreed in the March 26, 2010, Pre-IND Meeting that a 6-month PP ITS in an appropriate species would be sufficient to support the NDA. (b) (4) . Also, the 6-month ITS of GP in rats and dogs (one each) which the applicant submitted contained the vehicle-only group to assess the excipients. The applicant also completed a standard battery of genotoxicity testing of DSPC, a PP component.

Others: The Agency approved two GP-containing inhalation drug products on October 29, 2016. The products are Seebri™ and Utibron™ Neohalers® (NDAs 207-923 and 207-930, respectively). The former is a GP monoproduct. The latter is a combination product of GP and indacaterol. Both products are dry powder inhalers indicated for COPD. Both products release 15.6-mcg GP in each actuation. Each actuation of Utibron™ Neohaler® releases 27.5-mcg indacaterol. The recommended clinical dose of both products is one actuation, are twice daily. Bevespi was developed independent of the Seebri™ and Utibron™ applications.

3 Studies Submitted

3.1 Studies Reviewed

Table 7 provides a list of pivotal nonclinical studies reviewed in this document.

Table 7: Pivotal Nonclinical Studies Reviewed

Study No.	Description	Location
FY12-073	6-month IH toxicity study of GP in dogs	4.2.3.2
FY10-129	3-month IH toxicity study of GFF in dogs	4.2.3.2
FY10-120	6-month IH toxicity study of GP in rats	4.2.3.2
1250121CH.BTL	In vivo micronucleus assay of GP in rats	4.2.3.3.1
361ICH.BTL [a]	In vitro mammalian cell micronucleus test of GP in TK6 cells	4.2.3.3.2
502ICH.BTL [a]	Bacterial gene mutation test of GP	4.2.3.3.2

a. The study number has a pre-fix of AD91RM.

3.2 Studies Not Reviewed

Studies listed in Table 8 were not reviewed in this document. These studies were not reviewed because they have been reviewed previously. See footnotes of the table for the previous reviews.

Table 8: Nonclinical Studies Not Reviewed

Treatment duration	Species	Test article and Study Number				EDR location
		Glycopyrrolate	Formoterol	GFF [a]	Porous particle	
Single-dose	Rat	FY08-041a [b]	FY08-041b [c]	FY08-041d [d]	-	4.2.3.1
	Dog	FY08-042a [b]	FY08-042b [c]	FY08-042d [d]	-	4.2.3.1
14-day	Rat	FY09-077 [b]	FY09-039 [c]	FY09-087 [d]	26088 [b]	4.2.3.2
	Dog	FY09-076 [b]	FY09-038 [c]	FY09-086 [d]	26089 [b]	4.2.3.2
Genotoxicity	Ames [e]	-	-	-	P-2002-011 [b]	4.2.3.3
	ChromAb [f]	-	-	-	P-2002-013 [b]	4.2.3.3
	Mouse [g]	-	-	-	0745-1521 [b]	4.2.3.3

a. GFF = glycopyrronium /formoterol, (-) = not applicable.
b (b) (4)
c
d. See nonclinical review completed by Dr. Marcie Wood on July 8, 2010, in IND 107,739.
e. In vitro bacterial gene reverse mutation assay.
f. Mammalian cell chromosomal aberration assay in CHO cells.
g. In vivo mouse micronucleus assay.

3.3 Previous Reviews Referenced

This review references a number of nonclinical reviews completed by Agency staff under relevant IND and NDA applications. See Table 9 for the previous reviews referenced in this review.

Table 9: Previously Completed Reviews Referenced

IND/ NDA #	Date of Completion	Author	Content
(b) (4)			
	3/23/2010	Sancilio	Review of 6-mo ITS of GP in rats
(b) (4)			
IND 107,739	7/8/2010	Wood	Original IND review of GFF MDI
NDA 21-929	11/21/2008	Robison	Symbicort labeling review

4 Pharmacology

Pearl submitted no reports of pharmacology studies. Pear cited Robinul (NDA 17-558) product labeling and the literature to support the pharmacology of GP. See Section 12.3 Pharmacology for the Robinul product labeling. Key literature articles in support of the product labeling are listed below. The section briefly summarizes the findings in the reference articles.

1. Casarosa, et al., Preclinical Evaluation of Long-Acting Muscarinic Antagonists: Comparison of Tiotropium and Investigational Drugs, *J Pharmacol Exp Ther (JPET)*, 2009;330(2):660 - 668
2. Ogoda et al., Comparative Characterization of Lung Muscarinic Receptor Binding after Intratracheal Administration of Tiotropium, Ipratropium and Glycopyrrolate, *J Pharmacol Sci*, 2011;115:374 – 382
3. Sykes et al., The Influence of Receptor Influence of Receptor Kinetics on the Onset and Duration of Action and the Therapeutic Index of NVA237 and Tiotropium, *JPET*, 2012;343(2):520-528
4. Pulido-Rios et al, In Vivo Pharmacological Characterization of TD-4208, a Novel Lung-Selective Inhaled Muscarinic Antagonist with Sustained Bronchoprotective Effect in Experimental Animal Models, *JPET*, 2013;346:241-250
5. Villetti et al., Pharmacological Assessment of the Duration of Action of Glycopyrrolate vs Tiotropium and Ipratropium in Guinea-pig and Human Airways, *Brit J Pharmacol*, 2006;148:291-298

Casarosa et al. (2009) compared affinity of muscarinic antagonists (MA) to recombinant human (rh) muscarinic receptor subtypes (MRS) in vitro (Table 10). The recombinant human MRS (i.e., rhM_{1-5}) was expressed in CHO cell lines. Results showed that GP, like other MA, was non-selective to MRS, as indicated by the lack of the difference in p*K*i values across MRS.

Table 10: Binding Affinities (p*K*i) of MAs against *rhM* Receptors

Compound	pKi				
	rhM_1	**rhM_2**	**rhM_3**	**rhM_4**	**rhM_5**
Atropine	9.77	9.47	6.68	9.97	9.50
NMS [a]	10.31	10.25	10.32	10.42	9.59
Ipratropium	9.40	9.53	9.53	9.65	9.07
Pirenzepine	8.63	< 7.0	7.03	7.91	7.32
Tiotropium	10.80	10.69	11.02	11.02	9.96
Aclidinium	10.78	10.68	10.74	10.74	10.26
Glycopyrrolate	10.09	9.67	10.04	10.26	9.74

a. NMS, N-methyl-scopolamine methyl chloride.

Casarosa et al. also compared potency of 3 MAs in inhibiting acetylcholine (Ach)-induced bronchoconstriction in dogs in vivo. The MAs were tiotropium (TIO), aclidinium, and GP. Anesthetized dogs were dosed with 10-µg/kg Ach to induce bronchoconstriction. They were then treated intratracheally (via Respimat Soft Mist Inhaler) with aerosols containing one of the MAs. Dogs were subsequently challenged with the same dose of Ach at various times after the antagonist treatment. Dose-responses in bronchodilatory effects and duration of action were obtained.

Figure 1 shows time courses of bronchodilatory effects. The maximum bronchodilatory effect was observed at doses of 30, 12, and 3.0-mcg/kg for aclidinium, TIO, and GP, respectively (Panels A – C). At the maximum effective dose, TIO had the longest efficacious duration while GP had the shortest duration (Panel D). At 12 h after dosing, both TIO and aclidinium possessed significant bronchodilatory effects while GP showed no such effect at all. At 24 h after dosing, approximately 35%, 21%, and 0% of effect remained for TIO, aclidinium, and GP, respectively.

Fig. 2

Figure 1: Attenuation of Ach-Induced bronchoconstriction by inhaled MAs

Ogoda et al (2011) compared the binding affinity of TIO, ipratropium (IPR), and GP to muscarinic receptors in 4 rat tissues in vitro. The tissues were the lung, heart, bladder, and submaxillary gland. Rats were dosed by intratracheal instillation with 0.6 – 6.4-nmol/kg TIO, 7.3-nmol/kg IPR, or 7.5-nmol/kg GP. The rats were sacrificed 2 and 24 h after treatment to collect the above tissues. The receptor affinity of the tissue homogenates was determined using radio-labeled [n-methyl-^{3}H]-scopolamine methyl chloride ([^{3}H]NMS, 0.06 – 1.5 nM) at 4°C. The drug concentration was 0.03 – 1, 0.1 – 10, and 0.1 – 10 nM for TIO, IPR, and GP, respectively. Figure 2 showed the competitive bindings curves of these drugs in the lung and heart tissues under the experimental conditions. The IPR and GP curved overlapped while TIO possessed significantly higher affinity than the other two.

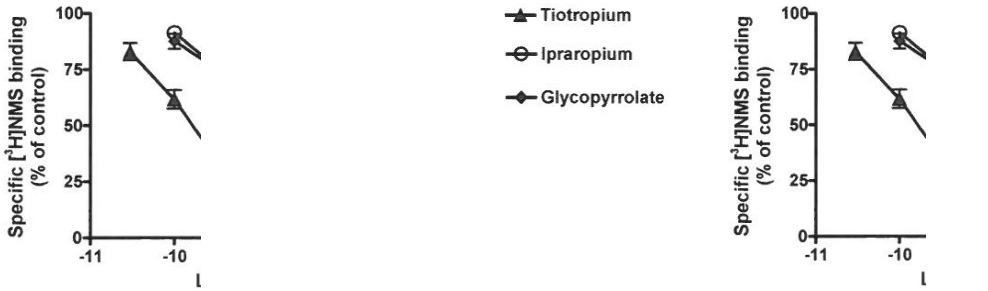


Figure 2: Inhibition of Binding of [^{3}H]-NMS to muscarinic receptors in rat tissues in vitro

Table 11 presents the K_i values and Hill Coefficients in the rat tissues. Hill Coefficient is a parameter used to describe potential binding interactions between two ligands at the same receptor site. A Hill Coefficient of 1 indicates the lack of interactions between the two ligands. A value greater than 1 indicates enhancement of the binding by the other. A value smaller than 1 indicates inhibition of the binding by the other. Hill coefficients for all three compounds were approximately 1 (lack of interaction). The K_i value for the lung tissue was 0.13, 1.46, and 1.26 nM for TIO, IPR, and GP, respectively. Overall, GP behaves more like IPR rather than TIO.

Table 11: K_i and nH of MAs in Rat Tissues

Tissue	Tiotropium		Ipratropium		Glycopyrrolate	
	K*i* (nM)	nH [a]	K*i* (nM)	nH	K*i* (nM)	nH
Lung	0.13 ± 0.02	0.91 ± 0.04	1.46 ± 0.22***	0.89 ± 0.03	1.26 ± 0.14***	0.89 ± 0.06
Heart	0.10 ± 0.03	1.05 ± 0.06	1.13 ± 0.09**	0.88 ± 0.04	1.02 ± 0.08**	0.91 ± 0.06
S gland[b]	0.42 ± 0.12†	0.82 ± 0.08	0.64 ± 0.05††	0.85 ± 0.05	0.70 ± 0.08†	0.86 ± 0.03
Bladder	0.22 ± 0.05	0.96 ± 0.05	1.47 ± 0.10**	0.90 ± 0.05	1.57 ± 0.23**	0.94 ± 0.10

a. nH = Hill Coefficient, ** p < 0.01, *** p < 0.001 vs. tiotropium group; †p< 0.05,††p< 0.01 vs lung group. N = 3 – 5.
b. Submaxillary gland

Sykes et al (2012) determined effect of experimental conditions on binding of TIO and GP to rhM_3 receptors, and their onsets of action at non-physiological and physiological conditions. The authors defined their experimental conditions as non-physiological (10-mM HEPES buffer at room temperature) and physiological (Hanks' balanced salt solution at 37℃) conditions. Binding of GP to rhM3 receptors expressed in CHO membrane and kinetics of the receptor occupancy were determined. Table 12 showed summary results of the receptor binding characteristics in non-physiological and physiological conditions.

Table 12: Binding Characteristics of Tiotropium and GP at Different Conditions

Compound	Non-physiological			Physiological		
	$_pKi$ [a]	K_{off} (/min) [b]	*t* ½	$_pKi$ [a]	K_{off} (/min) [b]	*t* ½
Tiotropium	11.1 ± 0.02	0.0015 ± 0.0002	462	10.37 ± 0.01	0.015 ± 0.002	46.2
Glycopyrrolate	10.30 ± 0.043	0.004 ± 0.002	173	9.59 ± 0.05	0.061 ± 0.002	11.4
[3H]NMS	10.45 ± 0.02	0.017 ± 0.001	41	9.51 ± 0.05	0.31 ± 0.032	2.24

Sykes et al also studied the time course of TIO and GP in receptor binding and time course of the agents in attenuating bethanechol-induced tracheal constriction in rats in vitro. In the functional assay, rat tracheal strips were pre-stimulated with 30-µM bethanechol at 37℃ to induce muscle contraction. After stabilization of contraction, TIO or GP (NVA237) was added to the tissue preparation. Relaxation of contraction was monitored for up to 120 minutes. Results showed that GP had a quicker onset of action than TIO (11.4 vs. 46.2 min).

Pulido-Rios et al (2013) studied the potency of inhaled GP and TIO in inhibiting methacholine-induced bronchoconstriction after single and repeat dose administration of the drug in rats. Rats were treated with GP aerosol in a whole-body exposure chamber for 10 min/day. They were then anesthetized and challenged with methacholine (noncumulative doses of 40 or 80 mcg/kg, IV, for 2.5 min at a rate of 2 mL/kg/min with a 2-min interval between doses). Airway resistance was determined through a respirator. Results showed that GP and TIO showed significant differences in efficacy after a 7-day treatment period in rats. TIO's efficacy remained unchanged while a significant decrease in efficacy was observed in the GP treatment group (Table 13). The mean ID_{50} of GP was 52.9 and 325.8 mcg/mL on days 1 and 7, respectively.

Table 13: Potency of TIO and GP in inhibiting Ach-Induced Bronchoconstriction

Drug	ID 50 (mcg/mL, IH)	
	Day 1	Day 7
Tiotropium	3.2 (2.7 – 3.8)	3.7 (2.6 – 5.2)
Glycopyrrolate	52.9 (43.1 – 65.0)	325.8 (101.3 – 514.1)

Velletti et al (2006) compared the duration of action of GP, TIO, and IPB in guinea pig in vitro and in vivo; and in human airways in vitro. In the in vitro experiment, contractions of strips of guinea pig trachea or human bronchus were induced by carbachol (0.3 – 1.0 µM). Various concentrations of MAs were added to medium to reverse the contraction. IC_{50s} and $t_{½}$ (the time taken for response to carbachol to return to 50% recovery after washout of the test antagonist) were estimated. Table 14 presents the result summary.

Table 14: Potency and Duration of Action of MAs in Guinea Tissues (1)

Compound	Guinea Pig Trachea			Human Bronchus		
	$_{P}IC_{50}$ [a]	t ½ (h) [b]	% Recovery [c]	$_{P}IC_{50}$	t ½ (h)	% Recovery
Tiotropium	9.1 ± 0.002	> 4	10 ± 4	9.5 ± 0.1	> 6	27 ± 3
Glycopyrrolate	9.0 ± 0.07	4.0 ± 0.5	50 ± 8	10.4 ± 0.02	3.7 ± 0.2	101 ± 10
Ipratropium	8.6 ± 0.02	0.5 ± 0.1	70 ± 7	9.5 ± 0.04	3.0 ± 0.2	110 ± 10

a. pIC50 (or –log IC50) represents the -log molar concentration of the test antagonist producing a 50% reversal of carbachol-induced tonic contraction in the guinea-pig isolated trachea or human isolated bronchus.
b. t 1/2 (offset) is the time taken for response to carbachol to return to 50% recovery after washout of the test antagonist in the guinea-pig trachea (tiotropium, glycopyrrolate and ipratropium, 10nM each) or human bronchus (tiotropium, 1 nM; glycopyrrolate, 3 nM and ipratropium, 10nM).
c. Percentage of response to carbachol obtained 4.5–6 h after the washout of the test antagonist. All data are mean7 s.e.m. of at least four observations.

In the in vivo experiment, MAs were intratracheally instilled into the lung of anesthetized guinea pigs. Bronchoconstriction was induced by intravenous administration of Ach (i.e., 20 µg/kg every 3 minutes). Airway resistance was recorded at different times for up to 24 h after MA administration. Table 15 presents the result summary. The maximum efficacy of the MAs was similar (i.e., peak effect of 86 – 88%), but the duration of action varied: approximately 71%, 30% and 14% at 24 hours post dosing for TIO, GP and IPR, respectively.

Table 15: Potency and Duration of Action of MAs in Guinea-Pig Tissues (2)

Compound	ID_{50} [a] (nmol/kg)	Peak effect			Effect remained (%) [d]	
		Dose (nmol/kg)	Time (min) [b]	(%) [c]	3 h	24 h
Tiotropium	0.25 (0.09–0.7)	1.3	90	86.2 ± 75.4	83.5 ± 74.4	70.6 ± 6.0
Glycopyrrolate	0.64 (0.2–1.9)	3	60	88.1 ± 73.9	69.9 ± 74.9*	29.7 ± 76.2**
Ipratropium	0.49 (0.2–1.0)	4.5	15	88.1 ± 79.6	28.3 ± 74.8**	14.2 ± 75.6**

a. ID_{50} is the dose producing 50% inhibition of ACh-induced bronchoconstriction.
b. Time at which the peak effect was observed.
c. The maximal inhibition produced by i.t. administration of each test compound.
d. Inhibitory effect still present at x h after antagonist administration
(*), P < 0.05; (**) P < 0.01.

Pharmacology Summary: The nonclinical information in the literature summarized above indicates that GP is a non-selective muscarinic antagonist. Glycopyrrolate possesses similar pharmacological properties as other marketed muscarinic antagonists. It binds non-selectively to recombinant human MRS (rhM_{1-5}) and natural MRS in rats in vitro. It blocks bronchoconstriction induced by muscarinic agonists in vitro and in vivo. Glycopyrrolate administered by intratracheal instillation or inhalation inhibited MA-induced bronchoconstriction in rats, guinea pigs, and dogs. Pharmacological properties (e.g., receptor affinity, potency and duration of action) of GP were between IPR and TIO.

5 Pharmacokinetics and Toxicokinetics

Plasma levels of both GP and FF increased proportionally with the inhaled dose in rats and dogs. The C_{max} was generally achieved immediately after dosing. Plasma drug levels started to decline post exposure. The mean t½ was 6.3 – 11.7 and 4.9 – 11.5 hr for GP and FF, respectively. There were no pharmacological interactions between GP and FF. Table 16 presents the mean C_{max} and AUC of GP and FF on Day 90 in the 3-month inhalation toxicity study of GFF in dogs.

Table 16: Mean Plasma Drug Levels on Day 90 in the 3-month ITS Study of GFF in Dogs

		Plasma Parameter (mean of males and females, n = 8)				
	Group	**CLD**	**CMD**	**CHD**	**FHD**	**GHD**
	GP (mcg/kg/day, PPD)	**4.3**	**10.7**	**14.8**	**-**	**21.8**
	FF(mcg/kg/day, PPD)	**1.1**	**2.5**	**3.5**	**3.5**	**-**
GP	C_{max} (µg/mL)	1.50	7.51	6.00	-	6.49
	AUC (µg.h/mL)	3.06	10.82	15.25	-	11.40
FF	C_{max} (µg/mL)	0.34	0.95	1.38	0.84	
	AUC (µg.h/mL)	1.34	3.06	4.55	2.87	-

6 General Toxicology

This section reviews 6-month ITS of GP in rats and dogs (one each) and a 3-month ITS of GP and FF (GFF) in combination in dogs. See Section 11.3 for a summary of the study findings.

6.1 6-month ITS of GP MDI in rats

Study title: Glycopyrrolate pMDI: 6-month nose-only inhalation exposure study in SD Rats

Study no.: FY10-120
Study report location: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: January 6, 2011

GLP compliance:	Yes, with a signed statement
QA statement:	Yes, with a signed statement
Drug, lot #, and % purity:	GP MDI: Lot/Batch # N-1046-001A, N-1104-001A, N-1045-001A, N-1047-001A , purity 99% Vehicle: N-1042-000A, N-1103-000A, N-1043-000A, N-1044-000A

Key Study Findings

- Rats treated with 28 and 55 µg/kg/day of pulmonary deposited dose of GP for 6 months showed dose dependent increases in hyaline degeneration of nasal epithelium and laryngeal squamous metaplasia in both sexes.
- Rats treated with 7-µg/kg/day GP showed low incidence (1 – 2/15) of laryngeal squamous metaplasia.
- The vehicle group also showed no or low incidence of laryngeal squamous metaplasia.
- The 55-µg/kg/day males also showed low incidence (1 – 2/15) of chronic or mixed cell inflammation in the prostate.
- The NOAEL for the safety evaluation of the product was the MD (28 µg/kg/day).

Method

Doses:	The respective GP doses in the LD, MD, and HD groups were 67.5, 275, and 548 µg/kg/day in the achieved doses (AD) and 6.8, 27.5, and 54.8 µg/kg/day in the pulmonary deposited doses (PDD).
Frequency of dosing:	Once daily; 15, 60, and 120 minutes/day in the LD, MD, and HD groups, respectively. The air and vehicle groups were exposed for 120 minutes.
Route of administration:	Nose-only inhalation
Dose volume:	Not applicable
Formulation/Vehicle:	GP MDI (30-µg glycopyrrolate/actuation) and vehicle (porous particle/HFA-134a); Aerosols were produced with the (b) (4) pMDI aerosol generator.
Species/Strain:	SD rats
Number/Sex/Group:	15/sex/group
Age:	5 – 6 weeks
Weight:	Respective initial mean weights of approximately 266 and 197 g in males and females, respectively.
Satellite groups:	None
Unique study design:	Dose levels were determined by the duration of exposure (15 – 120 minutes/day).
Deviation from study protocol:	No significant deviation occurred during the study.

Observations and Results

Dosimetry: The mean glycopyrrolate PDD in males and females were 6.8, 27.5, and 54.8 µg/kg/day in the LD, MD, and HD groups. Table 17 summarizes the characteristics of exposure conditions and dosimetry information. The MMAD was about (b) (4)

µm in the GP treatment groups. The aerosol GP concentration was (b) (4) µg/L. The duration of exposure was 15, 60, and 120 minutes for the LD, MD, and HD groups, respectively.

Table 17: Dosimetry of 6-month ITS of Glycopyrrolate in Dogs

	Air	Vehicle	LD	MD	HD
MMAD (µm ± GSD)	-	(b) (4)[c]			
Aerosol mass conc. (mg/L)	-				
Aerosol glycopyrrolate (µg/L)	-				
Duration of exposure (min)	120	120	15	60	120
Glycopyrrolate, AD (µg/kg/day)[a]	-	-	67.5	275	548
PDD (µ/kg/day)[b]	-	-	6.8	27.5	54.8

a. The average between males and females. The respective reported mean GP in the LD, MD, and HD groups was 65, 275, and 523 in males and 70, 286, and 572 in females.
b. Obtained by multiplying AD by a deposition factor (DF) of 0.1.
c. MMAD in the animal breathing zone and released from the inhalers (in parenthesis).

Mortality: No treatment-related effects were observed. Mortality was observed twice daily. One MD male was found dead on day 28. The cause of death in this rat was rupture of the bladder. The death was not considered treatment-related.

Clinical Signs: No treatment-related effects were observed.

Body Weights: No treatment-related effects were observed. Figure 3 presents the body weight – time curve of the study. Table 18 presents the mean body weights at several reference time points (e.g., the baseline, 13 week and 26 week) of the study.

Male **Female**

Figure 3: Mean body weight - time curve of 6-month ITS of GP in Rats

Both males and females in the MD and HD groups showed statistically non-significant decreases ($p < 0.05$) in mean body weights. Also, the decreases in mean body weights lacked any dose-response relationship. The review concludes that there no treatment-related effects on mean body weights.

Table 18: Mean Body Weight and Weight Gains in 6-month GP ITS in Rats

GP (mg/kg/day)		0 (Air)	0 (Veh.)	6.8	27.5	54.8
Male	0 [a]	266 ± 16	266 ± 16	265 ± 18	266 ± 17	266 ± 16
	13	444 ± 33	450 ± 52	446 ± 45	407 ± 37	428 ± 43
	26	483 ±45	490 ± 64	471 ± 53	422 ± 7	455 ± 49
Female	0	192 ± 12	197 ± 15	199 ± 16	196 ± 13	196 ± 12
	13	280 ± 31	272 ± 19	282 ± 33	264 ± 30	261 ± 16
	26	295 ± 37	294 ± 23	309 ± 55	278 ± 37	274 ± 27

a. Baseline value.

Feed Consumption: No treatment-related effects were observed.

Ophthalmoscopy: No treatment-related effects were observed.

Hematology: No treatment-related effects were observed.

Clinical Chemistry: No treatment-related effects were observed.

Urinalysis: No treatment-related effects were observed.

Gross Pathology: No treatment-related effects were observed.

Organ Weights: No treatment-related effects were observed.

Histopathology: Table 19 presents microscopic findings in the study. Dose-related changes in the incidence/severity of microscopic lesions were observed in the nasal cavity, larynx, and prostate in the MD and/or HD groups. In the nasal cavity, nasal turbinates showed hyaline degeneration of the respiratory and olfactory epithelium. In the larynx, epithelial squamous metaplasia was observed in the MD and HD groups in both sexes. In the prostate, low incidence of chronic and mixed cell inflammation was observed in HD males.

Table 19: Histological Findings in 6-Month ITS of GP in Rats

	Male (n=15/group)					Female (n=15/group)				
Tissue/findings	A [a]	V	LD	MD	HD	A	V	LD	MD	HD
Larynx: metaplasia, squamous, M	0	0	1	5	8	0	3	2	5	12
Metaplasia, squamous, S	0	0	0	0	2	0	0	0	0	0
Liver: hyperplasia, biliary, M	0	0	-	-	0	0	0	-	-	1
Nose/turbinate: inflammation, acute, M	0	0	0	0	1	0	0	0	0	1
Turbinate 2: degener. hyaline resp. epith, M	1	0	0	5	10	1	1	1	3	14
Inflammation, acute, M	0	0	0	0	1	0	0	0	0	0
Turbinate 3: degen. hyaline olfact. epith, M	3	6	5	7	11	3	5	2	5	10
Degener., hyaline olfactory epithelium, S	1	0	0	0	2	0	0	0	1	3
Turbinate 4: degen. hyaline olfact. epith, M	5	5	7	9	5	7	7	10	8	10
Degener. hyaline olfactory epithelium, S	1	0	1	0	3	0	0	0	1	3
Prostate: inflammation, chronic, S	0	0	-	-	1	-	-	-	-	-
Inflammation, mix, MO	0	0	-	-	2	-	-	-	-	-
Stomach: cyst, squamous	0	0	-	-	0	0	0	-	-	1

a. A = Air, V = Vehicle, M = minimal, S = slight/mild, MO = moderate.
Dash (-): not examined

Adequate Battery: Microscopic examinations of the tissues and organs were adequate. The study performed extensive examinations of the respiratory system. The examination also included other major organs and tissues in the air-control, placebo (vehicle-control), and HD groups. The Division concurred with the study protocol. (b) (4) (b) (4).

Peer Review: None

Special Evaluation: None

Toxicokinetics: Plasma GP concentrations were determined soon after the last exposure. The mean plasma GP level between males and females was 35.1, 266.7, and 362 pg/mL in the LD, MD, and HD groups, respectively. Table 20 presents the means and range values among groups.

Table 20: TK Result on Day 181 in a 6-month ITS of GP MDI in Rats

	Male			Female		
GP (µg/kg/day, PDD)	4.6	14.6	18.7	4.6	14.6	18.7
Mean GP (pg/mL)	35	173.6	311	35.1	357.7	413
SD	10.5	84.3	58.3	14.7	515.2	208.6
Range (pg/mL)	22.2 - 51.3	131 - 443	239 - 416	20.4 - 77.9	96.9 - 1859	247 - 1075

Dosing Solution Analysis: Not applicable. The (b) (4) (MDI) was used in the study. The amount of GP per spray was analyzed at both the beginning and end of the study, and was found within the specification range.

6.2 6-month ITS of GP MDI in Dogs

Study Title: Glycopyrrolate pMDI: 180-Day Face Mask Inhalation Exposure Study in Beagle Dogs

Study no.:	FY12-073
Study report location:	ECTD 4.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 6, 2011
GLP compliance:	Yes, with a signed statement
QA statement:	Yes, with a signed statement
Drug, lot #, and % purity:	GP MDI: Lot/Batch# N-1257-001A & N-1302-001A, Purity 99.9%; Vehicle: Lot# N-1256-000A

Key Study Findings

- Dogs treated with up to 18.7-µg/kg/day glycopyrrolate (PDD) for 6 months did not show treatment-related effects on the respiratory system or other organs.

- The mean plasma GP AUC was 2.0, 10.1, and 30.7 µg.h/mL in males and 2.5, 9.8, and 21.5 in female dogs in the LD, MD, and HD groups, respectively.
- The NOAEL was 18.7 µg/kg/day.

Method

Doses:	The respective glycopyrrolate doses in the LD, MD, and HD groups were 18.6, 54.2 and 76.4 mcg/kg/day in AD; and 4.6, 14.6 and 18.7 µg/kg/day in PDD.
Frequency of dosing:	Once daily; 10 – 30 minutes/day
Route of administration:	Inhalation (face mask)
Dose volume:	Not applicable
Formulation/Vehicle:	GP MDI (30-µg glycopyrrolate/actuation) and vehicle (porous particle + HFA-134a); Aerosols were produced with the (b) (4) pMDI aerosol generator.
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group
Age:	5 - 7 months
Weight:	Male: 6.7 - 9.1 kg; females: 6.5 - 7.8 kg.
Satellite groups:	None
Unique study design:	Dose levels were determined by the duration of exposure (10 – 30 minutes/day).
Deviation from study protocol:	No significant deviations occurred during the study.

Observations and Results

Dosimetry: The mean glycopyrrolate exposure was 4.6, 14.6, and 18.1 µg/kg/day (PDD) in the LD, MD, and HD groups, respectively. Table 21 summarizes the characteristics of exposure conditions and dosimetry information. The MMAD was about (b) (4) µm in all groups. The aerosol GP concentration ranged between (b) (4) µg/L. The duration of exposure was 10, 20, and 30 minutes for the LD, MD, and HD groups, respectively.

Table 21: Dosimetry of 6-month ITS of Glycopyrrolate in Dogs

	Air	Vehicle	LD	MD	HD
MMAD (µm ± GSD) [a]	-	(b) (4)			
Aerosol mass conc. (mg/L)	-				
Aerosol glycopyrrolate (µg/L)	-				
Duration of exposure (min)	30	30	10	20	30
Glycopyrrolate, AD (µg/kg/day) [b]	-	-	18.6	58.2	74.6
PDD (µ/kg/day) [c]	-	-	4.6	14.6	18.7

a. Particle sizes were measured using NGI Impactors. Aerosols were produced with the (b) (4) pMDI aerosol Generator.
b. The average between males and females. The respective mean glycopyrrolate exposure in the LD, MD, and HD groups was 17.72, 59.05, and 76.5 in males and 19.44, 57.41, and 72.77 in females, respectively. The concentration was determined using a HPLC-UV method.
c. Derived by multiplying the AD by 0.25 (DF).

Mortality: No treatment related effects were observed. No deaths or premature sacrifice occurred in either sex in any groups.

Clinical Signs: No treatment related effects were observed.

Body Weights: No treatment related effects were observed. Figure 4showed mean body weight time-curve changes in the study. Table 22 (next page) showed the mean body changes at key reference time points of the study. There were no statistically significant differences in mean body weights between treated and control animals.

Figure 4: Mean body weight - time curve of the 6-month ITS of GP in dogs

Feed Consumption: No treatment-related effects were observed. Clinical observations were made twice daily. No differences were noted among any groups.

Table 22: Mean Body Weight and Weight Gains in 3-month GFF ITS in Dogs

Time	Mean body weight (kg, n = 4/group)									
	Male					Female				
	Air	Veh	LD	MD	HD	Air	Veh	LD	MD	HD
Day 1	8.65	8.65	8.60	8.00	8.60	7.33	7.58	7.45	7.18	7.30
Day 29	9.23	9.12	8.73	8.78	8.35	8.50	8.15	8.13	7.83	7.38
Day 92	10.05	10.25	9.65	9.40	9.95	8.75	9.05	8.68	8.10	8.40
Day 176	10.23	10.15	9.53	9.58	9.23	9.18	9.38	9.12	8.73	8.40

Ophthalmoscopy: No treatment related effects were observed. Ophthalmic examination was performed at pre-dose and prior to the termination. No differences were noted among any groups.

ECG: No treatment related effects were observed. Six-lead ECG was performed on days 1 and 180. No differences in ECG parameters were noted among any groups.

Hematology: No treatment-related effects were observed.

Clinical Chemistry: No significant treatment-related effects were observed.

Urinalysis: No treatment-related effects were observed.

Pulmonary Function: No treatment-related effects were observed. Pulmonary functions were assessed under anesthesia using a pneumotach on days 1 and 180. Parameters monitored included respiratory frequency, tidal volume, and minute volume. No differences were noted among any groups.

Gross Pathology: No treatment-related effects were observed.

Organ Weights: The HD group showed statistically significant decreases in mean heart weights (Table 23). The significance of this finding is unknown at the present time given the lack of associated functional and microscopic changes.

Table 23: Mean Heart Weight in the 6-month ITS in Dogs

GP (µg/kg/day) [a]	Mean Heart Weight (g, n = 4/group)				
	0 (Air)	**0 (Vehicle)**	4.6	14.6	**18.7**
Male	83.99 ± 4.07	88.20 ± 7.85	79.16 ± 7.56	78.61 ± 11.52	72.83 ± 4.29*
Female	69.93 ± 6.85	70.77 ± 6.78	67.17 ± 8.01	64.41 ± 3.50	64.13 ± 2.47*

a. PPD.
*, $p < 0.05$ (from the vehicle group).

Histopathology: No treatment-related effects were observed. Table 24 presents the microscopic observations in the respiratory system in the study. The male vehicle group showed an increase in the incidence of mixed alveolar inflammation, but the incidence was identical to that of the female air control group. The review does not consider the findings to be treatment-related.

Table 24: Histologic findings in the lung in 6-month ITS of GP MDI in Dogs

Observation	Incidence (n = 4/group)									
	Male					Female				
	A	V	LD	MD	HD	A	V	LD	MD	HD
Inflammation, chronic (peri-bronchovascular)	3	3	4	2	4	4	3	3	4	4
Inflammation, mixed (alveolar)	0	3	2	2	2	3	1	2	3	2
Hypertrophy/hyperplasia, epithelial w/ fibrosis, focal	1	2	2	1	0	1	1	0	0	1
Inflammation, eosinophilic & histiocytic (alveolar)	0	1	0	0	0	0	0	0	0	0
Accumulation, alveolar macrophage	1	0	2	2	1	3	0	2	1	1
Aggregate, alveolar macrophage (Focal, Dense)	0	0	0	1	0	2	3	1	0	2
Adhesion (pleural)	0	1	0	0	0	0	0	0	0	1

Adequate Battery: Yes. Extensive histological examinations of the respiratory system were carried out in every dose group. Other major organs/tissues in the control, vehicle, and HD groups were all examined. A step down approach was used in organs/tissues in the HD group which showed observations of interest.

Peer Review: no.

Toxicokinetics: Table 25 presents the plasma GP levels in the 6-month ITS in dogs. Plasma GP levels generally increased supra-dose proportionally to PDDs. Plasma drug glycopyrrolate levels were determined on days 1 and 180 (prior to and at post exposure hours 0, 0.5, 1, 3, 6, and 24. Both C_{max} and AUC increased in greater proportion to the administered doses in both males and females.

Table 25: TK Result on Day 180 in a 6-month ITS of GP MDI in Dogs

	Male			**Female**		
GP (µg/kg/day, PDD)	4.6	14.6	18.7	4.6	14.6	18.7
C_{max} (µg/mL)	0.74	6.54	22.11	1.41	3.78	10.63
AUC (µg.h/mL)	1.99	10.14	30.65	2.45	9.84	21.48
T_{max} (h)	0.08	0.08	0.19	0.29	0.19	0.08
$t_{½}$ (h)	11.27	8.56	6.10	10.66	7.47	12.45

Dosing Solution Analysis: Not applicable. The (b) (4) (GP MDI) was used in the study. The mount of GP per spray was analyzed at the beginning and the end of the study. The amount of GP was found within the specification range.

6.3 3-month ITS of GPF MDI in Dogs

Study title: Glycopyrrolate and formoterol fumarate pMDI: 90-day Face-Mask Inhalation Exposure Study in beagle dogs

Study no.:	FY10-129
Study report location:	ECTD 4.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 02, 2011
GLP compliance:	Yes, with a signed statement
QA statement:	Yes, with a signed statement
Drug, lot #, and % purity:	GFF MDI: N-1038-003A , N-1040-003A, and N-1039-003A FF MDI: N-1035-005A, N-1036-005A, N-1049-005A GP MDI: N-1102-001A

Key Study Findings

- Beagle dogs (4/sex/group) were dosed with up to 21.8-µg/kg/day GP and 3.5-µg/kg/day FF (PPD), alone or in combination, daily for 90 days.
- Treatment-related changes were observed in the respiratory tract and liver. Changes in the lung included increases in alveolar macrophage accumulation and aggregation, and foci of hypertrophy and hyperplasia. Macrophage increases manifested as loose accumulations and denser, focal aggregates that were up to mild in severity.
- No significant drug-drug interactions between GP and FF were observed.
- NOAEL was 4.3 and 1.1 mcg/kg/day of GP and FF, respectively. These doses corresponded to plasma AUC of 3.1 and 1.3 µg.h/mL

Methods

Doses:	The GP/FF dose (PDD) was 0/0, 0/0, 4.3/1.1, 10.7/2.5, 14.8/3.5, 0/1.1, 0/2.5, 0/3.5, and 21.7/0 for the air, vehicle, CLD, CMD, CHD, FLD, FMD, FHD, and GP groups, respectively. See Table 26 for AD among groups and PDD estimates.
Frequency of dosing:	Daily, 10 – 30 minutes/day
Route of administration:	Inhalation (face mask)
Dose volume:	Not applicable
Formulation/Vehicle:	GFF MDI (30-µg GP and 4.5-µg FF/actuation), GP MDI (30-µg GP/actuation), FF MDI (4.5-µg FF /actuation), and vehicle (porous particle + HFA-134a), and air
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group
Age:	8 – 11 months
Weight:	Male: 7 – 12 kg; females: 6 – 11 kg.

Satellite groups: None
Unique study design: Dose levels were determined by the duration of exposure. Lungs were sectioned extensively, with all major lobes examined over a total of 17 slides.
Deviation from study protocol: No significant deviations occurred during the study.

Observations and Results

Doses: The PDD for GP/FF was 0/0, 0/0, 4.3/1.1, 10.7/2.5, 14.8/3.5, 0/1.1, 0/2.5, 0/3.5, and 21.8/0 µg/kg/day for the air, vehicle, CLD, CMD, CHD, FLD, FMD, FHD, and GP groups, respectively. See Table 26 for dosimetry data.

Table 26: Dosimetry of the 3-Month GPF MDI ITS in Dogs

Grp	Treat-ment	ID	TOE [e] (min/ Day)	Estimated exposure (µg/kg/day)							
				Achieved dose						PPD [c] (mean)	
				Glycopyrrolate (GP)			Formoterol (FF)				
				M [a]	F [a]	Mean [b]	M [a]	F [a]	Mean [b]	GP	FF
1	Air	Air	30	-	-	-	-	-	-	-	-
2	Vehicle	V	30	-	-	-	-	-	-	-	-
3	GFF MDI	CLD	10	17.3	18.0	17.6	4.4	4.5	4.4	4.3	1.1
4		CMD	20	42.7	44.1	43.4	10.1	10.8	10.5	10.7	2.5
5		CHD	30	59.4	62.1	60.7	14.0	14.7	14.4	14.8	3.5
6	FF MDI	FLD	10	- [d]	-	-	4.4	4.5	4.4	-	1.1
7		FMD	20	-	-	-	10.2	10.5	10.3	-	2.5
8		FHD	30	-	-	-	14.1	14.5	14.3	-	3.5
9	GP MDI	GP	30	85.1	89.2	87.2	-	-	-	21.8	-

a. Reported value except for the mean between males and females.
b. Average of the reported value in males and females.
c. Derived by multiplying the mean value by 0.25 (deposition factor).
d. (-), Not applicable.
e. Time of exposure.

Particle characteristics: Aerosol and particle characteristics were determined at the animal's breathing zone every 7 – 14 days. Table 27 summarizes aerosol characteristics of the study. The particle size (MMAD) was (b) (4) µm for the vehicle, GFF, FF, and GP MDI groups, respectively. Aerosol concentrations ranged from 5.06 – 8.10 and 1.1 – 1.17 **µ**g/L for GP and FF, respectively.

Table 27: Aerosol Characteristics of 3-Month GPF ITS in Dogs

Group	Treatment	MMAD (µm ± GSD)	Aerosol Concentration		
			Total mass (mg/L)	Glycopyrrolate (µg/L)	Formoterol (µg/L)
Air	1	- [a]	-	-	-
Vehicle	2	(b) (4)	0.8 ± 0.01	-	-
GFF MDI	3,4,5		0.8 ± 0.02	5.06	1.17
FF MDI	6,7,8		0.8 ± 0.01	-	1.10
GP MDI	9		1.2 ± 0.02	8.10	-

a. (-), not applicable.

Observations and Results

Mortality: No treatment-related effects were observed. No deaths or premature sacrifice occurred in either sex in any groups.

Clinical Signs: No treatment-related effects were observed.

Body Weights: No treatment-related effects were observed. Figure 5 presents mean body weight - time curves for the study. Table 28 shows the mean body weights at several milestones of the study. Body weights were obtained weekly. No significant effects were observed.

Figure 5: **The mean body weight-time curve of the 3-month ITS of GFF in dogs**

Dogs treated with HD DP alone (i.e., GHD) showed no body weight gains in either sex. Dogs receiving FF, alone or in combination with GP, showed greater body weight gain than controls. This is not surprising because formoterol treatment is known to be associated with body weight increase in animals.

Feed Consumption: No treatment related effects were observed.

Ophthalmoscopy: No treatment related effects were observed.

ECG: Dogs receiving FF treatment showed increases in heart rate (Table 29, next page). The degree of increases in heart rates was generally dependent upon FF dose, but this is an expected effect because FF is known for increasing heart rate. No changes in other parameters occurred. ECG monitoring was done at pre-dosing and Weeks 1, 6, and 13 of the treatment period.

Hematology: No treatment-related effects were observed.

Table 28: Mean Body Weight and Weight Gains in 3-month GFF ITS in Dogs

Sex	Time	Mean body weight (kg, n = 4/group)								
		A	V	CLD	CMD	CHD	FLD	FMD	FHD	GH
Male	Baseline	9.35	9.24	9.37	9.32	9.10	9.06	8.93	9.04	9.67
	Week 4	9.90	9.88	10.08	10.04	10.18	10.05	9.62	10.06	9.99
	Week 13	9.66	9.83	10.27	10.06	10.00	9.96	9.88	10.08	9.65
Female	Baseline	7.49	7.39	7.51	7.70	7.54	7.75	7.51	7.57	7.66
	Week 4	7.91	8.14	8.02	8.33	7.96	8.21	8.51	8.52	7.72
	Week 13	7.84	7.96	8.14	8.34	8.15	8.19	8.62	8.53	7.26
		Mean body weight Gain (kg, n = 4/group)								
Male	Wk 0 - 13	0.31	0.59	0.9	0.74	0.9	0.9	0.95	1.04	-0.02
Female	Wk 0 - 13	0.35	0.57	0.63	0.64	0.61	0.44	1.11	0.96	-0.4

Clinical Chemistry: No significant effects were observed. Sporadic and minimal changes (<20%) in a few clinical chemistry parameters were observed. For example, the CHD group showed approximately 20% decrease in serum glucose levels. Dogs receiving FF showed up to 20% increase in serum potassium levels. These effects are known with LABAs.

Table 29: Mean Heart Rate in 3-month GFF Study in Dogs

Time	Mean Heart Rate (bpm, n = 8/group)								
	A	V	CLD	CMD	CHD	FLD	FMD	FHD	GH
Baseline	86	87	87	81	99	98	96	83	81
Week 1	72	69	123	137	150	131	152	153	86
Week 6	90	92	110	120	155	114	125	110	113
Week 13	95	81	111	138	149	122	125	105	119

Urinalysis: No treatment-related effects were observed.

Gross Pathology: No treatment-related effects were observed. The incidence of necropsy findings appear spontaneous and lacked a dose-response relationship. ***Organ Weights:*** No treatment-related effects were observed.

Histopathology

Adequate Battery: Yes, a complete panel of organs and tissues were examined in the study.

Peer Review: No.

The respiratory system and the liver were examined microscopically in all groups. Other organs/tissues were examined in a "read down" fashion: *i.e.* all tissues and gross lesions were examined for Groups 1 (Air), 2 (vehicle), 5 (CHD), 8 (FHD) and 9 (GPH). Lungs were sectioned extensively, with all major lobes examined over a total of 17 slides.

Table 30: Histological Findings in 3-month ITS study of GFF in Dogs

	Sex	Incidence (n = 4/group)								
Tissue/Observation		A [a]	V	CLD	CMD	CHD	FLD	FMD	FHD	GPH
Heart: Degeneration, individual myofiber	M	0	1	-	-	1	-	-	0	1
	F	0	0	-	-	1	-	-	0	1
Inflammation, chronic	M	0	0	-	-	1	-	-	0	0
	F	0	1	-	-	0	-	-	0	0
Kidney: inflammation, chronic	M	0	0	-	-	0	-	-	1	0
	F	0	0	-	0/1	0	-	-	1	0
Larynx: Ulcer w/ acute inflammation, focal	M	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	1	0
Liver: Alteration, hepatocellular (periportal) [b]	M	0	0	1	0	2	0	0	1	0
	F	0	0	1	3	4	0	1	0	0
Lung: alveolar macrophage accumulation	M	0	1	2	2	3	3	2	2	1
	F	2	0	4	2	0	3	3	2	2
Alveolar macrophage aggregation, focal	M	0	1	1	1	4	1	1	3	4
	F	3	2	0	1	3	1	4	3	3
Inflammation, mixed (alveolar)	M	0	0	0	0	1	0	0	0	0
	F	0	0	0	0	0	0	1	0	0
Hypertrophy/hyperplasia, epi w/ fibrosis	M	1	0	1	2	1	1	2	4	0
(focal)	F	0	3	1	0	4	1	2	2	1
Accum, intrabronch material (occlusive)	M	0	0	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	0	0	0
Atelectasis	M	0	0	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	0	0	0
Emphysema	M	0	0	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	0	0	0
Fibrosis, interstitial (centriacinar)	M	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	1
Nose: Turbinate 2: inflammation, chronic	M	0	0	0	0	0	0	0	0	1
	F	0	0	0	0	0	0	0	0	0
Turbinate 4: inflammation, chronic	M	0	0	0	0	0	0	0	0	1
	F	0	0	0	0	0	0	0	0	0

a. A = Air control, V = Vehicle, CLD = GPF low dose, CMD = GPF mid dose, CMD = GPF high dose, FLD = FF low dose, FMD = FF mid dose, FHD = FF high dose, GH = glycopyrrolate high-dose, (-) = Not examined.
b. Swollen cytoplasm.

Table 30 (previous page) presents notable histological findings in the study. Treatment-related changes were observed in the respiratory tract and liver, but there were no indications of significant toxicological interactions between GP and FF as findings in combination groups were consistent with findings in individual monoproducts groups. Changes in the lung included increases in alveolar macrophage accumulation and aggregation, and foci of hypertrophy and hyperplasia. Macrophage increases manifested as loose accumulations and denser, focal aggregates that were up to mild in severity. The incidences were generally similar among the high dose groups of GP, GFF, and FF. The respective incidence of focal alveolar macrophage accumulation in the A, V, CLD, CMD, CHD, FLD, FMD, FHD, and GH (n = 4/group) was 0, 1, 2, 2, 3, 3, 2, 2, and 1 in males; and 2, 0, 4, 2, 0, 3, 3, 2, and 2 in the females. The respective incidence of focal alveolar macrophage aggregation in the A, V, CLD, CMD, CHD, FLD,

FMD, FHD, and GH was 0, 1, 1, 1, 4, 1, 1, 3, and 4 in males; and 3, 2, 0, 1, 3, 1, 4, 3, and 3 in females. The respective incidence of focal hypertrophy and hyperplasia associated interstitial fibrosis (minimal in severity) in the A, V, CLD, CMD, CHD, FLD, FMD, FHD, and GH was 1, 0, 1, 2, 1, 1, 2, 4, and 0 in males; and 0, 3, 1, 0, 4, 1, 2, 2, and 1 in females. There were no consistent trends in the incidence of dose-response relationship between sexes. Liver alterations were defined as swollen cytoplasm of hepatocytes (e.g., hepatocyte hypertrophy) which are typical of enzyme induction associated with drug metabolism. The results indicated no significant toxicological interaction between GP and FF in dogs. The review considers the low dose GFF (4.3 and 1.1 µg/kg/day of BP and FF, respectively) the NOAEL based on the lack of morphologic changes associated with the slight increase in the incidence of focal alveolar macrophage accumulation. It was noted that there were potential treatment-related findings of focal hypertrophy and hyperplasia associated with interstitial fibrosis in the CMD and CHD groups although not consistent across sexes.

Toxicokinetics: Table 31 presents the summary data of the mean C_{max} and AUC values on day 90 of the study. Both C_{max} and AUC were generally dose-proportional in both sexes. C_{max} was reached immediately or within 1 hours post exposure for glycopyrrolate and formoterol. The elimination half-life ranged from 6.34-11.7 and 4.85-11.5 for glycopyrrolate and formoterol, respectively. The mean plasma AUC of GP/FF of males and females combined on day 90 was 0/0, 0/0, 3.06/1.34, 10.8/3.06, 15.3/4.55, 0/1.11, 0/3.00, 0/2.87, and 6.49/0 µg.h/mL for the air, vehicle, CLD, CMD, CHD, FLD, FMD, FHD, and GP groups, respectively. There was no apparent toxicokinetic interaction between GP and FF.[7]

Table 31: Mean Plasma Drug Levels on Day 90 in the 3-month ITS Study of GFF in Dogs

Cmpd	Parameter	Sex	Group means (n = 4)						
			CLD	CMD	CHD	FLD	FMD	FHD	GH
GP	C_{max}	M	1.91	4.02	3.67	-	-	-	5.03
	(µg/mL)	F	1.08	11.0	8.33	-	-	-	7.95
		Mean	1.50	7.51	6.00				6.49
	AUC	M	3.78	12.2	10.3	-	-	-	11.5
	(µg.h/mL)	F	2.34	9.43	20.2	-	-	-	11.3
		Mean	3.06	10.82	15.25				11.40
FF	C_{max}	M	0.43	0.76	0.75	0.41	1.18	0.94	-
	(µg/mL)	F	0.25	1.13	2.00	0.27	0.81	0.74	-
		Mean	0.34	0.95	1.38	0.34	1.00	0.84	
	AUC	M	1.65	3.44	4.61	1.16	2.48	3.30	-
	(µg.h/mL)	F	1.02	2.67	4.49	1.05	3.51	2.44	-
		Mean	1.34	3.06	4.55	1.11	3.00	2.87	-

[7] Plasma concentrations of GP and FF were measured on days 1, 45, and 90 of the study. Blood samples were collected at hours 0 (immediately), 0.5, 1, 3, 6, and 24 post exposure.

Dosing Solution Analysis: Not applicable. The (b) (4) (MDI) was used in the study. The mount of GP and FF concentrations pay spray were analyzed at the beginning and end of the study and were found within the specification range.

7 Genetic Toxicology

Glycopyrrolate was tested in Ames test and mammalian cell micronucleus assay in TK6 cells. Results showed that GP tested negative in both assays.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay

Study no.:	AD91RW.502ICH.BTL
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 18, 2014
GLP compliance:	Yes, there is a signed statement. No significant deviations occurred.
QA statement:	Yes, there is a signed QA statement.
Drug, lot #, and % purity:	GB, Lot #F082, and 99.6% purity

Key Study Findings

- GP tested negative in the Ames test.

Methods

Strains:	TA98, TA100, TA1535, TA1537, and WP2 *uvr*A
Concentrations in definitive study:	6.7 – 5000 mcg/plate
Basis of concentration selection:	The top dose of 5000 mcg/plate was defined by the current testing guidelines.
Negative control:	Water
Positive control:	See Table 33 (next page).
Formulation/Vehicle:	Water and DMSO for GB and positive controls, respectively
Incubation & sampling time:	Incubated for 48-72 hours at 37℃

Study Validity

The study is considered valid. Criteria for a positive response was test article- and dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. The mean increase at the peak response concentration must exhibit at least a 2.0-fold (Strains TA1535 and TA1537) or 3.0-fold (TA98, TA100, and WP2 *uvr*A) increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels

was required to evaluate assay data. Table 32 presents the positive controls used in the study.

Table 32: Positive Controls in the Ames Test

S9 fraction [a]	Strain	Positive control	Concentration (µg/plate)
Yes	TA98, TA1535	2-aminoanthracene	1.0
	TA100, TA1537	2-aminoanthracene	2.0
	WP2 *uvr*A	2-aminoanthracene	15
No	TA98	2-nitrofluorene	1.0
	TA100, TA1535	Sodium azide	1.0
	TA1537	9-aminoacridine	75
	WP2 *uvr*A	Methyl methane-sulfonate	1000

a. The S9 was prepared from male Sprague-Dawley rats dosed with intraperitoneal injection of 500-mg/kg Aroclor™ 1254 (200 mg/mL in corn oil). Rats were sacrificed 5 days later to obtain the S9 fraction.

Results

Glycopyrrolate tested negative in the assay. There was no significant increase in the number of revertants at any of the tested GP concentrations or bacterial strains. The positive controls showed typical and statistically significant increases in the number of revertants. The review concludes that GP tested negative in the Ames test under the conditions of the study.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Micronucleus Assay in TK6 Cells

Study no.:	AD91RW.361ICH.BTL
Study report location:	eCTD 0000, Section 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 21, 2014
GLP compliance:	Yes, there is a signed statement. No significant deviations occurred.
QA statement:	Yes, there is a signed QA statement.
Drug, lot #, and % purity:	GB, Lot #F082, 99.6% purity

Key Study Findings

- GP tested negative in the mammalian cell micronuclei formation assay in TK6 cells in vitro.
- See Table 33 for study design.

Table 33: Design of the Mammalian (TK6) Cell Micronucleus Test in Vitro

Treatment condition	Treatment time (hr)	Recovery Time (hr)	GP Concentration (mcg/mL)
No S9	4	23	0, 50, 100, 200, 300, 398
	27	0	0, 50, 100, 200, 300, 398
S9 [a]	4	23	0, 50, 100, 200, 300, 398

a. The S9 was prepared from male Sprague-Dawley rats dosed with intraperitoneal injection of 500-mg/kg Aroclor™ 1254 (200 mg/mL in corn oil). Rats were sacrificed 5 days later to obtain the S9 fraction.

Methods

Cell line:	TK6 (human lymphoblastoid) cell line
Treatment condition:	Cells were treated with and without the S9 metabolic activation system. See Table 34 for the source of the S9 fraction.
Incubation & sampling time:	Incubation time 4 – 27 hours, sampling time 0 – 27 hours after treatment. See Table 34.
Concentrations in definitive study:	50 – 398 µg/mL
Basis of concentration selection:	0.04 – 398 mcg/mL. The highest concentration was selected based on the culture medium osmolality (~ 20% higher vehicle control).
Negative control:	Water
Positive control:	MMC 0.08 – 0.12 mcg/mL Vinblastine 10 and 12 ng/mL Cyclophosphamide 2.5 – 4.0 mcg/mL
Formulation/Vehicle:	Aqueous solution

Study Validity

The study is considered valid. The criteria for a valid assay include the following: The percent frequency of micronucleated mononucleate cells (MnMNCs) in the concurrent vehicle control should be consistent with the historical control range of the testing laboratory. The percentage of cells with micronucleus induction must be statistically increased ($p \leq 0.05$, Fisher's exact test) in the positive control condition relative to the vehicle control. The result will be considered positive if there was a statistically significant and dose-dependent increase in the frequency of MnMNCs ($p \leq 0.05$). The result may be considered equivocal If only one criterion is met (statistically significant OR dose-dependent increase). The results will be considered to be negative if neither criterion is met.

Results

Glycopyrrolate was tested up to the maximum concentration of 1 mM (± S9) per the ICH S2(R1) Guidance. Substantial cytotoxicity (≥50% relative population doubling to the vehicle control) was not achieved with glycopyrrolate at concentrations up to 398 mcg/mL (±S9). Glycopyrrolate did not cause statistically significant increases in the percentage of MnMNCs in the presence or absence of S9 activation at any dose levels or sampling point ($p > 0.05$, Fisher's Exact one-tailed test). The positive and negative

controls showed typical responses under the test conditions. The review concludes that GP tested negative for the induction of micronuclei in the presence or absence of the S9-activation system in TK6 cells.

7.3 *In Vivo* Clastogenicity Assay in Rodents (Micronucleus Assay)

Study title: *In Vivo* Micronucleus Assay in Rats

Study no.:	AD91RW.1250121ICH.BTL
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 18, 2014
GLP compliance:	Yes, there is a signed statement. No significant deviations occurred.
QA statement:	Yes, there is a signed QA statement.
Drug, lot #, and % purity:	GB, Lot #F082, 99.6% purity

Key Study Findings

- GP tested negative in the in vivo micronuclei assay in rats.
- Male SD rats (5 – 10/dose) were dosed by oral gavage with 500, 1000, or 2000-mg/kg GP. Bone marrow was collected at 24 or 48 hours after dosing to evaluate the number of MnPCE. See Table 34 for study design.
- GP did not cause significant increases in MnPCE at any doses. The HD group showed signs of toxicity such as piloerection and diarrhea.

Table 34: Design of the in vivo Micronucleus Test in Rat

Group	Treatment	Dose (mg/kg)	No. of male rats sacrificed	
			24 hr	48 hr
1	Water	-	5	5
2	GP	500	5	-
3	GP	1000	5	-
4	GP	2000	5	5
5	Cyclophosphamide	40	5	-

b. The S9 was prepared from male Sprague-Dawley rats dosed with intraperitoneally injection of 500-mg/kg Aroclor™ 1254 (200 mg/mL in corn oil). Rats were sacrificed 5 days later to obtain the S9 fraction.

Methods

Doses in definitive study:	0, 500, 1000, 2000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dosing volume:	10 mL/kg
Formulation/vehicle:	Aqueous solution
Species/strain:	Rats, Sprague-Dawley (Hsd:SD)

Number/sex/group:	5 males/time point
Satellite group:	None in the definitive study.
Basis of dose selection:	Maximum dose defined by current guidelines. Dose ranging study at the same doses showed no sex difference in responses.
Negative control:	Water
Positive control:	Cyclophosphamide (40 mg/kg)

Study Validity

The study is considered valid. Criteria for a valid assay include the following: The frequency of MnPCE in the vehicle controls should be consistent with the historical vehicle control range, and must be ≤ 0.4%. The positive control must induce a significant increase ($p \leq 0.05$) in MnPCE frequency as compared to the concurrent vehicle control. The criteria for a positive results is that the test article induced a significant increase in MnPCE frequency ($p \leq 0.05$) at any dose level or sampling time compared to the concurrent vehicle control. A minimum of 2000 PCEs from each animal must be examined and scored for the presence of micronuclei. The GP dose selection was appropriate. The assay criteria was met.

Results

None of the treatment groups showed a significant increase in the number of MnPCE per 1000 PCE over the control. The MnPCE/1000 PCE was 0.2, ≤0.4, and 21.6 in the vehicle, GP, and cyclophosphamide groups, respectively. The high dose group showed piloerection (3/10) and diarrhea (5/5) after receiving GP treatment. The review considers GP tested negative in the in vivo micronucleus assay in rats under the conditions of the study.

8 Carcinogenicity

No carcinogenicity reports were submitted or required.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicity reports were submitted or required.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

This application contained adequate nonclinical information to support the safety of the proposed use of Bevespi® Inhalation Aerosol. Pearl proposes to register Bevespi for a COPD indication. Bevespi is a combination product administered using a metered dose inhaler. It contains glycopyrrolate (GP) and formoterol fumarate (FF) as active pharmaceutical ingredients, HFA-134a as the propellant, and porous particles as an

excipient. There is adequate nonclinical information to evaluate the safety of both active and inactive ingredients of the product.

11.1 Pharmacology

Bevespi Aerosphere™ is a combination product to be marketed as a therapy for COPD. The APIs include GP and FF. The former is a muscarinic antagonist (MA), also known as an anticholinergic, which is the established pharmacologic class. The latter is a long-acting beta$_2$-adrenergic agonist (LABA). Both possess bronchodilatory effects. Inhaled MAs and LABAs, alone or in combination, are efficacious in COPD patients. Table 35 presents examples of approved and currently marketed inhalation products of MAs and LABAs indicated for COPD. There are at least 5 approved and currently marketed MAs: ipratropium (IPR), tiotropium (TIO), aclidinium, umeclidinium, and glycopyrrolate. In the airways, MAs exhibit pharmacological effects by inhibiting M_3-receptors at the smooth muscle leading to bronchodilation.

Glycopyrrolate is a MA, as indicated by the reference product labeling and literature. Robinul Injectable Solution (NDA 17-558, approved on February 6, 1975) is the reference product. The Robinul labeling states: "Glycopyrrolate, like other anticholinergic (antimuscarinic) agents, inhibits the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine but lack cholinergic innervation". [8] Literature data reviewed under Section 4 Pharmacology showed the following:

a. GP binds non-selectively to recombinant human MRS (rhM_{1-5}) and endogenous MRS in rats in vitro.
b. GP administered by intratracheal instillation or inhalation inhibited muscarinic agonist (acetylcholine or methacholine)-induced bronchoconstriction in rats, guinea pigs, and dogs.
c. Pharmacological properties of GP were between ipratropium (IPR) and tiotropium (TIO). The properties included receptor affinity, potency and duration of action.

Formoterol is a LABA and bronchodilator. Bronchodilatory effects of LABAs are well known. Formoterol is currently marketed as a therapeutic agent in COPD. Specifically, FF is an API in Foradil, Symbicort (NDAs 20-831 and 21-929, respectively), and other products. Both Foradil and Symbicort are approved and currently marketed products.

Pearl did not submit any nonclinical pharmacological reports to demonstrate effects of inhaled GP and FF in combination in COPD disease, but there is sufficient evidence in the literature to show that such a combination may be efficacious in COPD. See Table 35 for examples. Finally, the applicant has conducted clinical trials demonstrating the efficacy of Bevespi in COPD patients.

[8] http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/017558s053lbl.pdf.

Table 35: Examples of Marketed Inhalation Products of MRA and/or LABA Indicated for COPD

EPC [a]	Active Component	Representative product	NDA #	Date of Approval
AC	Ipratropium bromide (IPR)	Atrovent HFA	20-394	10/20/1995
	Tiotropium bromide (TIO)	Spiriva HandiHaler	21-395	01/30/2004
	Aclidinium	Tudorza DPI	202-450	07/23/2012
	Umeclidinium bromide (UB)	Incruse Ellipta	205-832	04/30/2014
	Glycopyrrolate (GP)	Seebri Neohaler [b]	207-923	10/29/2015
BA	Formoterol fumarate (FF)	Foradil DPI	20-831	02/16/2001
	Formoterol fumarate	Symbicort MDI [c]	21-929	07/26/2006
	Salmeterol	Serevent DPI	20-962	09/19/1997
	IB/albuterol sulfate	DuoNeb IS	20-950	03/21/2001
AC/BA	UB/vilanterol trifenatate	Anoro Ellipta	203-975	12/18/2013
	GP/indacaterol	Utibron Neohaler [b]	207-930	10/29/2015

a. EPC = established pharmacologic class, AC = anticholinergic, BA = beta-2 adrenergic agonist, DPI = dry powder inhaler, IS = inhalation solution, and MDI = metered-dose inhaler.
b. Not reference products of the current application.
c. Symbicort also contains budesonide, a glucocorticosteroid, as an API.

11.2 Pharmacokinetics

Pharmacokinetic parameters of GP and FF after inhalation in laboratory animals were studied using clinical formulations of GFF (b) (4) The following summary is based on the 3-month clinical formulation (GFF MDI) study in dogs. The pulmonary deposited dose of GP/FF in the study was 4.3/1.1, 10.7/2.5, and 14.8/3.5 µg/kg/day in the LD, MD, and HD GFF groups, respectively. Both GP and FF were detected in the plasma after inhalation exposure to GFF MDI. Plasma concentrations of both compounds generally increased in a dose-dependent manner. The mean plasma AUC of GP/FF on day 90 (males and females combined) was 3.06/1.34, 10.8/3.06, and 15.3/4.55 µg.h/mL for the LD, MD, and HD GFF groups, respectively. The maximum levels were generally observed immediately after inhalation exposures. The respective elimination half-life ($t_{½}$) of GP and FF was 6.3 - 11.7 and 4.9 - 11.5 hours, respectively. There was no apparent toxicokinetic interaction between glycopyrrolate and formoterol.

11.3 General toxicity

The pivotal general toxicity of the GFF MDI application included 6-month ITS of GP MDI in rats and dogs and a 3-month ITS of GFF MDI in dogs. The 6-month studies evaluated the effect of inhaled GP while the 3-month study evaluated potential drug interactions between GP and FF. Each of these studies also included air and vehicle groups (one each). Table 36 provides an overview of the pivotal toxicity studies of the application. Below is a brief summary of each study. See Section 6 General Toxicology for additional information about each study.

In the 6-month rat GP study (FY12-073), SD rats (15/sex/dose) were treated by nose-only with 0 (air), 0 (vehicle), 6.8, 27.5, or 54.8-µg/kg/day GP (PDD) using the clinical GP MDI formulation for 181 days. The MD and HD groups showed increases in the hyaline degeneration of respiratory and olfactory epithelium in the nasal cavity and laryngeal squamous metaplasia in the larynx in both sexes. The HD males also showed increased

incidence of inflammation in the nasal cavity. In the nose, the respective incidence of hyaline degeneration of the respiratory epithelium (e.g., nasal turbinate 2, minimal in severity) in the air, vehicle, LD, MD, and HD groups was 1, 0, 0, 5, and 10 in the males and 1, 1, 1, 3, and 14 in the females. The respective incidence of hyaline degeneration of the olfactory epithelium (e.g., nasal turbinate 3, minimal to slight in severity) in the air, vehicle, LD, MD, and HD groups was 4, 6, 5, 7, and 13 in the males and 3, 5, 2, 6, and 13 in the females. Findings in the nasal cavity may not relevant to humans who will receive the drug by oral inhalation.

Table 36: Overview of Pivotal General Toxicity Studies

Species	Duration (week)	Formulation	GP/FF (PDD, µg/kg/day)	Target organs	Report #
Rat	26	GP MDI	6.8/0, **27.5/0,** 54.8/0	Nose, larynx, prostate	FY12-073
Dog	26	GP MDI	4.6/0, 14.6/0, **18.7/0**	Heart	FY10-129
Dog	13	GP MDI	**21.7/0**	None	FY10-120
		FF MDI	0/1.1, 0/2.5, **0/3.5**	None	FY10-120
		GFF MDI	**4.3/1.1**, 10.7/2.5, 14.8/3.5	None	FY10-120

a. Bold and highlights indicate NOAEL values.

In the larynx, the respective incidence of squamous metaplasia (i.e., minimal in severity) in the air, vehicle, LD, MD, and HD groups was 0, 0, 1, 5, and 10 in the males and 0, 3, 2, 5, and 12 in the females. The findings in the larynx are considered rat specific and not relevant to humans. The HD males showed a low incidence of chronic (1/15) and mixed cell inflammation (2/15) in the prostate. The NOAEL for the safety evaluation of the product was the MD (28 µg/kg/day), corresponding to a mean plasma GP concentration of 267 µg/mL.

In the 6-month dog GP study (FY10-129), beagle dogs (4/sex/dose) were treated by face mask with 0 (air), 0 (vehicle), 4.6, 14.6, or 18.7-µg/kg/day GP (PDD) using the clinical GP MDI formulation for 181 days. No treatment-related effects were observed. The NOAEL was 18.7 µg/kg/day, corresponding to a mean plasma concentration of 16.4 µg/mL.

In the 3-month drug interaction study (FY10-1200), beagle dogs (4/sex/dose) were treated by face mask with GP and FF, alone or in combination, for 90 days to study the potential toxicological drug interactions between the APIs. The study used 3 clinical formulations of GP and FF products: GP MDI, FF MDI and GP/FF MDI. Both FF and GP/FF treatments consisted of 3 different dose groups while there was only one GP treatment group. The GP/FF combination groups were coded as CLD, CMD, and CHD; the FF groups were coded as FLD, FMD, and FHD. The pulmonary deposited dose for glycopyrrolate/formoterol was 0/0, 0/0, 4.3/1.1, 10.7/2.5, 14.8/3.5, 0/1.1, 0/2.5, 0/3.5, and 21.7/0 for the air, vehicle, CLD, CMD, CHD, FLD, FMD, FHD, and GP groups, respectively.

Treatment-related changes were observed in the respiratory tract, liver, and prostate, but there were no indications of significant toxicological interactions between GP and FF at similar doses. Changes in the lung included increases in alveolar macrophage accumulation and aggregation, and foci of hypertrophy and hyperplasia. Macrophage

increases manifested as loose accumulations and denser, focal aggregates that were up to mild in severity. The incidences were generally similar among the high dose groups of GP, GFF, and FF. The respective incidence of focal alveolar macrophage accumulation in the A, V, CLD, CMD, CHD, FLD, FMD, FHD, and GH (n = 4/group) was 0, 1, 2, 2, 3, 3, 2, 2, and 1 in males; and 2, 0, 4, 2, 0, 3, 3, 2, and 2 in females. The respective incidence of focal alveolar macrophage aggregation in the A, V, CLD, CMD, CHD, FLD, FMD, FHD, and GH was 0, 1, 1, 1, 4, 1, 1, 3, and 4 in males; and 3, 2, 0, 1, 3, 1, 4, 3, and 3 in females.

The respective incidence of focal hypertrophy and hyperplasia associated interstitial fibrosis (minimal in severity) in the A, V, CLD, CMD, CHD, FLD, FMD, FHD, and GH was 1, 0, 1, 2, 1, 1, 2, 4, and 0 in males; and 0, 3, 1, 0, 4, 1, 2, 2 and 1 in females. There were no consistent trends in the incidence of dose-response relationship between sexes. Liver alterations were defined as swollen cytoplasm of hepatocytes (e.g., hepatocyte hypertrophy) which are typical of enzyme induction associated with drug metabolism. Low incidence (1 - 2) of inflammation (chronic or mixed cell) was observed in the CHD group.

The review considers the low dose GFF (4.3 and 1.1 µg/kg/day of BP and FF, respectively) the NOAEL, based on the lack of inflammatory response associated with the slight increase in alveolar macrophase accumulation. At higher dose groups, the alveolar macrophage accumulation appeared to be associated with additional findings of hyperplasia and fibrosis in the lung and inflammation in the prostate. Also, the results showed that there was no significant toxicological interaction between the two active drugs in dogs as observed toxicity with the combination was consistent with individual monoproducts

Safety margins: The general toxicity studies of GFF provided adequate safety margin coverage for the proposed clinical dose of Bevespi. As indicated in the Proposed Clinical Population section (2.6), the proposed maximum recommended human daily inhalation dose (MRHDID) of the Bevespi APIs was 0.6 and 0.32-µg/kg for GP and FF, respectively. The above discussions indicate that the NOAEL of GFF formulation in dogs was 4.3 and 1.1 µg/kg/day for GP and FF, respectively. On a unit lung weight basis, these respective doses in dogs and humans correspond to doses of 0.39 and 0.036 mcg/g lung for GP doses and 0.1 and 0.019 mcg/g lung for FF doses. The NOAEL in dogs provide safety margins of approximately 5 and 10 for the proposed MRHDID of FF and GP, respectively. Also, FF is a well-known and currently marketed drug. These safety margins are considered sufficient to support the proposed use of Bevespi from the nonclinical perspective.

Table 37: Safety Margins for the Proposed Clinical Dose

Drug	Species	Dose (PDD)		Safety Margin (Rat/human) [b]
		µg/kg/day	µg/g lung [a]	
Glycopyrrolate	Dog	4.3	0.72	11
	Human	0.6	0.36	-
Formoterol	Dog	1.1	0.183	5.2
	Human	0.32	0.019	-

a. Estimated using the following respective parameters in dogs and humans: 10 and 60 kg in body weights and 110 and 1000 g in lung weights.
b. on a µg/g lung basis.

11.4 Genetic toxicity

GP: Glycopyrrolate tested negative in the following genetic toxicity assays: bacterial gene mutation assay in vitro, mammalian cell chromosomal aberration assay in mouse lymphoma cells in vitro, mammalian cell micronucleus formation assay in TK6 cells, and in vivo micronucleus test in rats.

FF: Formoterol was not mutagenic or clastogenic in Ames Salmonella/microsome plate test, mouse lymphoma test, chromosome aberration test in human lymphocytes, and rat micronucleus test, according to the Symbicort labeling. [9]

11.5 Carcinogenicity

GP: No animal studies were conducted to evaluate the carcinogenicity potential of GP or required, as no pre-neoplastic or neoplastic lesions were observed in the 6-month rat ITS. No carcinogenicity studies of GP/FF were conducted.

FF: Rats and mice treated with FF showed dose-dependent increases in the incidence of leiomyomas in the reproductive system. The carcinogenicity of FF in animals was previously evaluated in 24-month studies rats and mice. According to the Symbicort (NDA 21-929) labeling approved on June 25, 2010, formoterol at oral doses of ≥ 100 µg/kg caused a dose-related increase in the incidence of uterine leiomyomas in CD-1 mice. In Sprague-Dawley rats, an increased incidence of mesovarian leiomyoma and uterine leiomyosarcoma were observed at the inhaled dose of 130 µg/kg/day, but not at 22 µg/kg/day.

11.6 Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies of GP, GFF, or FF were submitted, but the labeling for GP and FF are available.

GP: According to the labeling of Robinul Injection (NDA 17-558), GP was not teratogenic when pregnant rats and rabbits were dosed with 65 (dietary) or 0.5 mg/kg/day (intramuscular), respectively, during the organogenesis period. The labeling also states that dietary administration of glycopyrrolate resulted in diminished survival of rat pups and conceptions rates in a dose-related manner in rats, but there are no mention of NOAEL or the dose at which the aforementioned responses occurred. Furthermore, the labeling states that the decreases in conception rates may be due to diminished seminal secretion.

FF: Reproductive toxicity studies with formoterol fumarate are described in the product labeling with Symbicort.

Formoterol fumarate has been shown to be teratogenic, embryocidal, to increase pup loss at birth and during lactation, and to decrease pup weights in rats when given at oral doses of 3 mg/kg/day and above. Umbilical hernia, a malformation, was observed in rat

[9] Source: http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021929s021lbl.pdf

fetuses at oral doses of 3 mg/kg/day and above. Brachygnathia, a skeletal malformation, was observed in rat fetuses at an oral dose of 15 mg/kg/day. Pregnancy was prolonged at an oral dose of 15 mg/kg/day. In another study in rats, no teratogenic effects were seen at inhalation doses up to 1.2 mg/kg/day. Formoterol fumarate has been shown to be teratogenic in rabbits when given at an oral dose of 60 mg/kg. Subcapsular cysts on the liver were observed in rabbit fetuses at an oral dose of 60 mg/kg. No teratogenic effects were observed at oral doses up to 3.5 mg/kg.

A reduction in fertility and/or reproductive performance was identified in male rats treated with formoterol at an oral dose of 15 mg/kg. In a separate study with male rats treated with an oral dose of 15 mg/kg, there were findings of testicular tubular atrophy and spermatic debris in the testes and oligospermia in the epididymides. No such effect was seen at 3 mg/kg. No effect on fertility was detected in female rats at doses up to 15 mg/kg.

11.7 Impurities

There are no nonclinical safety concerns for the impurity, degradant, extractable, and leachable levels in the product. The impurities levels are set at a (b) (4)% level. This limit corresponds to daily exposure up to (b) (4)mcg/patient. The leachable levels are set according to the PQRI thresholds. See Section 2.7 Comments on Impurities/Degradants on Concern for additional information. The applicant argued that they will not monitor (b) (4) will not be used in the future. The review defer to the chemistry discipline for the evaluation (b) (4).

11.8 Overall Evaluation and Recommendation

The applicant has conducted adequate nonclinical characterization of the active pharmaceutical ingredient. The product contained no novel inactive ingredients. There is adequate nonclinical data to support the safety of the proposed use of the product. The applicant has submitted all studies requested in pre-NDA meetings. No additional nonclinical studies are required for the approval of the NDA. The review recommends approval of Bevespi from the nonclinical perspective, pending the labeling review.

Luqi Pei, Ph.D.
Pharmacologist/Toxicologist

Tim Robison, Ph.D.
Pharmacology Team Leader
for Dr. Marcie Wood

12 Appendices

(b) (4)

(b) (4) Nonclinical Review completed by Dr. Marcie Wood on July 8, 2010 in IND 107,739.

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	107739
Supporting document/s:	SDN 2, SDN 4
Sponsor's letter date:	SDN2: June 8, 2010; SDN4: July 1, 2010
CDER stamp date:	SDN2: June 8, 2010; SDN4: July 1, 2010
Product:	Glycopyrrolate and Formoterol Fumarate (GFF) metered dose inhaler (MDI)
Indication:	Long-term, twice daily maintenance treatment of (b) (4) with COPD, including chronic bronchitis and emphysema
Sponsor:	Pearl Therapeutics, Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Marcie L. Wood, PhD
Supervisor/Team Leader:	Molly Topper, PhD
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Eunice Chung, PharmD

Template Version: December 7, 2009

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1 Executive Summary

1.1 Recommendations

1.1.1 Clinical Study (ies) Safe to Proceed: Yes

The sponsor's proposed clinical study is reasonably safe to proceed from a nonclinical perspective.

1.1.2 If Not Safe to Proceed, Recommendations to Allow Clinical Study (ies) to Proceed

N/A

1.1.3 Additional Recommendation(s) (Non-hold comments/advice to sponsor) *if any.*

a. Urinalysis was not conducted in your 14-day rat GFF pMDI inhalation toxicity study (FY09-086). We recommend that you include urinalysis in future toxicity studies in rat and/or dog.

1.2 Brief Discussion of Nonclinical Findings

GFF pMDI is a glycopyrrolate (quarternary ammonium antimuscarinic agent) and formoterol fumarate (β_2-adrenergic agonist) combination product for metered dose inhalation for the chronic, twice daily treatment [redacted] (b) (4) with COPD. The nonclinical safety assessment of GFF pMDI will be based upon NDA 20-831 and NDA 21-279 (Novartis, Foradil Aerolizer) as well as NDA 12-827 and NDA 17-558 (Robinul and Robinul Forte). GFF will be administered in a mixture of DSPC + $CaCl_2$ with HFA134a as the propellant. DSPC + $CaCl_2$ are regarded as novel excipients for inhalation administration and require appropriate qualification. [redacted] (b) (4)

In the current IND 107,739 submission, the sponsor conducted two pivotal 14-day inhalation toxicity studies of GFF pMDI in rat and dog. In a rat 14-day inhalation toxicity study, rats received the GFF formulation at aerosol concentrations of 4.49-6.33 µg/L GP and 0.89-1.18 µg/L FF for 25, 60, or 90 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 90 min of exposure. An air control group that received filtered room air for

90 min was also included. The resultant average daily glycopyrrolate doses were 72, 226, and 368 µg/kg/day for low-, mid-, and high-dose males, respectively, and 77, 241, and 394 µg/kg/day for low-, mid-, and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 14, 42, and 69 µg/kg/day for low-, mid-, and high-dose males, respectively, and 15, 45, and 73 µg/kg/day for low-, mid-, and high-dose females, respectively. No dose-limiting toxicities were identified. The high-dose was identified as the NOAEL. For the high-dose, pulmonary deposited doses (10% of the delivered dose) for GP/FF calculated on a body weight basis were 36.8/6.9 and 39.4/7.3 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 7.3/1.2 and 6.3/1.2 µg/g lung weight/day for males and females, respectively.

In a dog 14-day inhalation toxicity study, dogs received the GFF formulation at aerosol concentrations of 5.15-7.79 µg/L GP and 1.00-1.34 µg/L FF for 10, 20, or 30 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 30 min of exposure. An air control group that received filtered room air for 45 min was also included. The resultant average daily glycopyrrolate doses were 17.0, 51.3, and 73.8 µg/kg/day for low-, mid-, and high-dose males, respectively, and 17.3, 52.3, and 75.4 µg/kg/day for low-, mid-, and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 3.3, 8.8, and 12.5 µg/kg/day for low-, mid-, and high-dose males, respectively, and 3.4, 9.0, and 12.8 µg/kg/day for low-, mid-, and high-dose females, respectively. Test article-related clinical signs included erythema in 5/12 low-dose dogs (3 M, 2 F), 11/12 mid-dose dogs (5 M, 6 F), and 12/12 high-dose dogs (6 M, 6 F). Mean heart rates were increased for males and females in all dose groups after the first and last exposures. Following the first exposure, HR was increased by 44, 84, and 115% in low-, mid-, and high-dose animals (male and female average) versus placebo control. Following the second exposure, HR was increased by 31, 58, and 87% in low-, mid-, and high-dose animals (male and female average), respectively, versus placebo control. No rhythm abnormalities were observed. The target organs of toxicity were the heart and liver, which are characteristic for β_2 adrenergic agonists. In the heart, minimal to moderate fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 and 2/8 mid- and high-dose animals, respectively, at the end of the treatment period. These findings in dogs are generally attributed to increased heart rate that is monitorable in a clinical setting. At the end of the recovery period, minimal to mild fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 high-dose animals. In the liver, minimal to mild hepatocellular alteration was present in 8/8, 7/8, and 8/8 dogs in the low-, mid-, and high-dose groups. Affected hepatocytes were associated with the portal triad and periacinar regions. This finding resolved at the end of the recovery period. This finding (glycogen deposition) was attributed to the pharmacological action of formoterol and not judged as adverse. A NOAEL was not identified based upon histopathological findings in the liver at all doses; however, findings in the liver were not considered adverse. In addition, findings in the heart at the mid- and high-dose were judged to be monitorable in a clinical setting; therefore, the high-dose was used to determine safety factors. At the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 18.5/3.1 and 18.9/3.2 µg/kg

BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 2.0/0.4 and 2.1/0.4 µg/kg lung weight/day for males and females, respectively. GP systemic exposures for males and females at the high-dose were 17046 and 13437 pg*hr/mL, respectively. FF systemic exposures for males and females at the high-dose were 3633 and 4155 pg*hr/mL, respectively.

In a 14-day toxicology study (b) (4) rats received the DSPC-calcium chloride mixture administered as a dry powder at deposited doses of 2.35 mg/kg DSPC and 0.15 mg/kg calcium chloride. Both sexes showed a decrease in neutrophils; males showed a decrease in WBCs and lymphocytes, and the females showed an increase in WBCs and lymphocytes and a decrease in reticulocytes. The significance of these hematology changes appeared to be minimal. No histopathological findings of the respiratory tract were observed in females, while only 1/6 males showed an indication of inflammation. Chronic progressive nephropathy, a species-specific disease was observed in both sexes. Both sexes showed an increased incidence of hepatic inflammatory cell foci with no change in the clinical chemistry. In the recovery group, focus was on the lung which showed complete recovery. In this study, DSPC-calcium chloride administered at doses of 2.35 and 0.15 mg/kg, respectively, as a dry powder was minimally toxic to the respiratory tract only in males. In both sexes, there was an indication of liver toxicity.

In a 14-day toxicology study (b) (4) dogs received a dry powder formulation of (b) (4) % calcium chloride and (b) (4) % DSPC. The total daily deposited doses were 5.86 mg/kg DSPC and 0.42 mg/kg calcium chloride. Females showed elevated WBCs, neutrophils, monocytes, eosinophils, platelets, ALP and triglycerides. Males showed decreased prostate weight and increased salivary gland, thymus and thyroid gland weight. Females showed decreased spleen and thymus weight. Histopathology was seen only in females; the organs affected were larynx, lungs, trachea and retropharyngeal lymph node. These were reversible. The significance of these findings was unclear given that they were observed in only females.

Formoterol fumarate was negative in the standard battery of genotoxicity tests (Foradil Aerolizer label). Studies to evaluate the genotoxicity of glycopyrrolate have not been conducted. Genotoxicity studies with glycopyrrolate will not be required if the sponsor submits an NDA under the 505(b)(2) pathway (b) (4) . DSPC was negative in the standard battery of genotoxicity tests (b) (4)

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

Glycopyrrolate: 596-51-0

Formoterol fumarate: 43229-80-7

2.1.2 Generic Name

Glycopyrrolate and Formoterol Fumarate (GFF) metered dose inhaler (MDI)

2.1.3 Code Name

PT003

2.1.4 Chemical Name

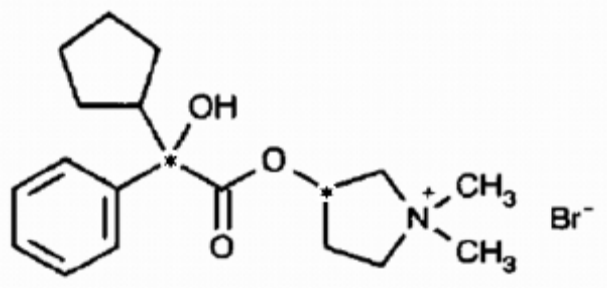
Glycopyrrolate: 3-hydroxy-1,1-dimethylpyrrolidinium bromide α-cyclopentylmandelate
Formoterol fumarate: N[2-Hydroxy-5-[(1RS)-1-hydroxy-2-[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]-amino] ethyl]phenyl]formamide, (E)-2-butenedioate dihydrate

2.1.5 Molecular Formula/Molecular Weight

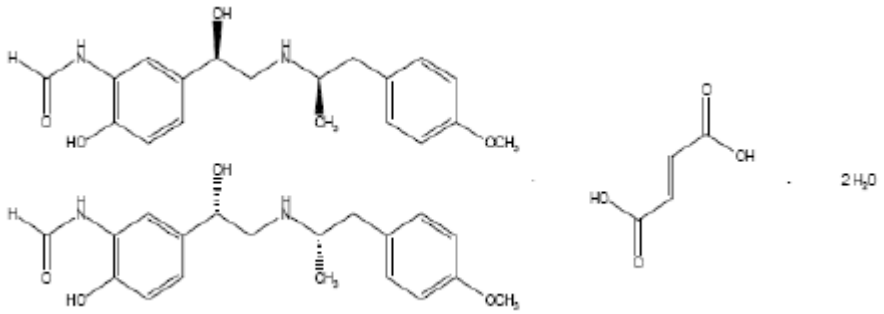
Glycopyrrolate: $C_{19}H_{28}NO_3 \bullet Br$/398.33
Formoterol fumarate: $(C_{19}H_{24}N_2O_4)2 \bullet C_4H_4O_4 \bullet 2H_2O$/ 840.91

2.1.6 Structure

Glycopyrrolate:



Formoterol fumarate:



2.1.7 Pharmacologic class

Glycopyrrolate: Quarternary ammonium antimuscarinic agent
Formoterol fumarate: β_2-adrenergic agonist

2.2 Relevant IND/s, NDA/s, and DMF/s

(b) (4)

NDA 20-831 and NDA 21-279 (Novartis, Foradil Aerolizer)
NDA 12-827 (Shionogi Pharma, Robinul and Robinul Forte) and NDA 17-558 (Baxter Healthcare, Robinul Injection)

2.3 Clinical Formulation

2.3.1 Drug Formulation

The GFF pMDI formulation to be used in proposed clinical study PT0031002 is prepared by cosuspending micronized glycopyrrolate and micronized formoterol fumarate crystals in in HFA propellant with porous particles comprised of 1,2-distearoyl-snglycero-3-phosphocholine (DSPC) and calcium chloride ($CaCl_2$). This is in contrast to prior GFF pMDI formulations, used in 14-day toxicology studies and a Phase I clinical study (PT0030901), in which formoterol fumarate was incorporated in the structure of the porous particle (b) (4) (See CMC review by Dr. Craig Bertha, dated June 15, 2010, for complete details of GFF pMDI reformulation).

The product is formulated to (b) (4) ensure delivery of 120 actuations. After priming, each actuation meters approximately 22 or 44 µg of glycopyrrolate and 6.0 µg of formoterol fumarate from the valve and delivers approximately 18 or 36 µg of glycopyrrolate and 4.8 µg of formoterol fumarate from the actuator. The components and qualitative composition of placebo to match the high strength GFF pMDI, 36 µg GP/4.8 µg FF per actuation, is provided in Table 3.

Table 1: Composition of Glycopyrrolate and Formoterol Fumarate pMDA, 18 µg/ 4.8 µg per actuation, 120 actuations

Component	Quantity per Canister[1,2]	Metered dose (ex-valve)	Delivered dose (ex-actuator)	Reference to Standard
Glycopyrrolate	(b) (4)		18 µg	USP
Formoterol fumarate			4.8 µg	USP
HFA-134a			(b) (4)	Pearl Therapeutics
DSPC				Pearl Therapeutics
Calcium chloride				USP/NF
(b) (4)				

Table 2: Composition of Glycopyrrolate and Formoterol Fumarate pMDA, 36 µg/ 4.8 µg per actuation, 120 actuations

Component	Quantity per Canister[1,2]	Metered dose (ex-valve)	Delivered dose (ex-actuator)	Reference to Standard
Glycopyrrolate	(b) (4)		36 µg	USP
Formoterol fumarate			4.8 µg	USP
HFA-134a			(b) (4)	Pearl Therapeutics
DSPC				Pearl Therapeutics
Calcium chloride				USP/NF
(b) (4).				

Table 3: Composition of placebo MDI, 120 actuations

Component	Quantity per Canister[1,2]	Metered dose (ex-valve)	Delivered dose (ex-actuator)	Reference to Standard
HFA-134a	(b) (4)			Pearl Therapeutics
DSPC				Pearl Therapeutics
Calcium chloride				USP/NF
(b) (4)				
(b) (4)				

(b) (4)

2.3.2 Comments on Novel Excipients

GFF will be administered in a mixture of DSPC + $CaCl_2$ with HFA134a as the propellant. DSPC + $CaCl_2$ are regarded as novel excipients for inhalation administration and require appropriate qualification. The sponsor has completed toxicology studies up to 14 days to support the inhalation route for this novel excipient. Over 40 drugs marketed in the US are formulated with DSPC-containing phospholipids, including 4 products approved for pulmonary routes of administration (b) (4) In addition, $CaCl_2$ is currently a component in an approved product, Pulmozyme, for administration by nebulization. (b) (4)

Therefore, the sponsor is required to qualify DSPC + $CaCl_2$ as an excipient for the chronic inhalation route of administration.

DSPC was negative in the standard battery of genotoxicity tests [redacted].

2.3.3 Comments on Impurities/Degradants of Concern

CMC reviewer Dr. Craig Bertha identified structural alerts for mutagenicity in the following FF-related impurities: A and F [redacted] B, C, D, E, F, H, and I [redacted]. Drug substance specifications for these impurities are as follows: A (NMT 0.3%), B (NMT [redacted]%), C (0.2%), D (0.2%), E (0.1%), F (0.2%), H (0.1%), and I (0.3%). At the highest clinical dose of FF of 19.2 µg/day, the total sum of impurities (A-F, H, I) at the limit is approximately [redacted] µg. Impurities B, D, E, F, H, and I were not detected in drug substance lot P-3M. Impurities A and C were detected at [redacted]% and [redacted]%, respectively. In drug product batch 10MM-004 (18/4.8 µg GP/FF), Impurity A was detected at ~ [redacted]% and Impurity C/D was detected at < [redacted]%. In drug product batch 10MM-007 (36/4.8 µg GP/FF), Impurity A was detected at [redacted]%. Therefore, levels of structurally alerting FF impurities are not a concern at this time.

2.4 Proposed Clinical Population and Dosing Regimen

The IND-opening study, Study PT0031002 is a randomized, double-blind (test products and placebo) chronic dosing (7-days), four-period, eight-treatment, placebo and active-controlled, incomplete block crossover multi-center study that will evaluate the efficacy, safety and PK of two doses of GFF MDI (72 µg/9.6 µg and 36 µg/9.6 µg twice daily), two doses of FF MDI (9.6 µg and 7.2 µg twice daily) and one dose of GP MDI (36 µg twice daily) in patients with moderate to very severe COPD, compared to Foradil Aerolizer (12 µg twice daily, open label) and Spiriva® Handihaler® (18 µg once daily, open label) as active controls. This study is currently underway in Australia and New Zealand, with plans to open sites in the U.S. once IND 107,739 is active. Across these sites, approximately 84 patients (male and female) will be randomized into the study to provide between 32 and 52 evaluations per study treatment.

In part A of the study, 48 patients will be randomized into one of six treatment sequences, as follows:

Selection	*Treatments*			
1	GP MDI 36 µg	GP/FF MDI 36/9.6 µg	Spiriva® 18 µg	FF MDI 9.6 µg
2	GP MDI 36 µg	GP/FF MDI 36/9.6 µg	Spiriva® 18 µg	FF MDI 7.2 µg
3	GP/FF MDI 72/9.6 µg	GP MDI 36 µg	Spiriva® 18 µg	FF MDI 7.2 µg
4	GP/FF MDI 72/9.6 µg	GP MDI 36 µg	Spiriva® 18 µg	FF MDI 9.6 µg
5	GP/FF MDI 72/9.6 µg	GP/FF MDI 36/9.6 µg	Spiriva® 18 µg	Foradil® 12 µg
6	GP/FF MDI 72/9.6 µg	GP/FF MDI 36/9.6 µg	Spiriva® 18 µg	Placebo MDI

FF MDI=Formoterol Fumarate MDI; GP MDI=Glycopyrrolate MDI; GP/FF MDI= Glycopyrrolate/Formoterol Fumarate MDI.

In part B, 36 patients will undergo a 4-period, 4-treatment complete block crossover study in which each patient will receive FF MDI 9.6 µg ex-actuator BID, FF MDI 7.2 ug ex-actuator BID, placebo MDI BID, and Foradil Aerolizer 12 µg BID.

In parts A and B, each treatment is administered BID for one week with a washout period of at least one week (up to 3 weeks) in between treatments. The maximum daily dose of GP/FF to be used in Study PT0031002 is 72/9.6 µg BID. These values were used to calculate safety margins (see Integrated Summary and Safety Evaluation).

Females are eligible to participate in the study if they are of non-child bearing potential, or of child-bearing potential with a negative serum pregnancy test at screening and agreement to use an acceptable method of contraception.

2.5 Regulatory Background

2.5.1 Previous Clinical Experience

Pearl Therapeutics has completed three clinical studies in support of the combination development program for GFF MDI. Study PT0010801 was a single dose, dose-ranging study that evaluated the safety, efficacy and pharmacokinetic (PK) profile of GP MDI in patients with COPD. Study PT0050801 evaluated the safety, efficacy and PK profile of single doses of FF MDI in patients with COPD. A third study, Study PT0030901, evaluated the safety, tolerability and PK profile of GP MDI and FF MDI as single agents and in combination [both as a loose combination from two different MDIs (GP MDI + FF MDI) and as a fixed combination from one MDI (GFF MDI)] in healthy volunteers. The following tables (excerpted from the sponsor's submission) summarize completed clinical studies with GP MDI, FF MDI, and GFF MDI:

Table 4: Summary of Pearl Therapeutic's completed clinical studies with GP MDI and FF MDI

Study Number Study Status	Study Description[1]	Population (N) Males/Females	Treatment/Dose[2]/ Dosing Duration	Observations/Endpoints
Glycopyrrolate MDI				
PT0010801 Completed	MC, R, DB, PC, AC, SD, 4-period, 6-treatment, balanced, incomplete block, cross-over study evaluating 4 doses of GP MDI in patients with mild to moderate COPD, compared to open-label marketed tiotropium (Spiriva Handihaler) as an active control	33 19 M/14 F	GP MDI 18 µg GP MDI 36 µg GP MDI 72 µg GP MDI 144 µg Spiriva Handihaler 18 µg Placebo MDI Single-dose 4-way cross-over, balanced incomplete block	Primary endpoint: Peak change in FEV_1 from Baseline compared to placebo MDI. Secondary endpoints: Change in 12 and 24 hour trough FEV_1, FEV_1 AUC_{0-12}, FEV_1 AUC_{0-24}, time to onset of action, similar analyses for PEFR, FVC, and peak IC; non-inferiority compared to tiotropium. Safety: AEs, vital signs, clinical laboratory assessments, ECGs, physical examination findings dry mouth assessments, paradoxical bronchospasm. PK: To characterize the concentration-time profile and to confirm dose proportionality across the GP MDI doses.
Formoterol Fumarate MDI				
PT0050801 Completed	MC, R, DB, PC, AC, SD, 5-period cross-over study evaluating three doses of FF MDI in patients with moderate-to-severe COPD (reversible to albuterol), compared to open-label marketed formoterol fumarate (Foradil Aerolizer) as an active control	34 18 M/16 F	FF MDI 2.4 µg FF MDI 4.8 µg FF MDI 9.6 µg Foradil Aerolizer 12 µg Placebo MDI Single-dose 5-way cross-over	Primary endpoint: FEV_1 $AUC_{(0-12)}$ for FF MDI compared with placebo MDI. Secondary endpoints: Time to onset of action, change in peak and trough FEV_1, PEFR, FVC, and peak IC; non-inferiority compared to Foradil Aerolizer based on change in FEV_1 $AUC_{(0-12)}$. Safety: AEs, vital signs, clinical laboratory assessments, ECGs, physical examination findings tremor assessments, paradoxical bronchospasm. PK: Define a dose of FF MDI with comparable systemic concentrations to Foradil Aerolizer and to confirm dose proportionality .

Table 5: Summary of Pearl Therapeutic's completed clinical studies with GFF MDI

Study Number Study Status	Study Description[1]	Population (N) Males/Females	Treatment/Dose[2]/ Dosing Duration	Observations/Endpoints
Glycopyrrolate and Formoterol Fumarate MDI				
PT0030901 Completed	SC, R, DB, SD, 4-period cross-over study evaluating 4 inhaled treatments (GP MDI, FF MDI, GFF MDI delivered individually and GP MDI and FF MDI delivered together in separate inhalers) in healthy subjects	16 5 M/11 F	GP MDI 72 µg FF MDI 9.6 µg GFF MDI 72 µg/9.6 µg GP MDI 72 µg + FF MDI 9.6 µg Single-dose 4-way cross-over	Primary endpoint: To demonstrate that the safety of a single dose of GFF MDI is similar to that of a single dose of GP MDI, FF MDI and to GP MDI + FF MDI delivered consecutively from two separate inhalers. Safety assessments included AEs, physical examination findings, symptoms of dry mouth and tremor assessments, vital signs, ECGs, spirometry and clinical laboratory assessments. Secondary endpoints: Plasma concentration-time profiles and PK parameters for glycopyrrolate and/or formoterol fumarate for each treatment.

[1] MC = multi-center; SC = single center; R = randomized; DB = double-blind; PC = placebo-controlled; AC = active-controlled; SD = single dose; FF MDI = Formoterol Fumarate MDI; GP MDI = Glycopyrrolate MDI; GFF MDI = Glycopyrrolate and Formoterol Fumarate MDI.

[2] All MDI doses are ex-actuator; all DPI doses (i.e., Foradil Aerolizer and Spiriva Handihaler) refer to capsule content.

2.5.2 History of Regulatory Submission

A pre-IND meeting was held with the sponsor on March 26, 2010. Refer to meeting minutes dated April 12, 2010 for detailed responses to nonclinical questions and discussion of responses.

During the review of the Original IND 107,739 submission (June 8, 2010) reviewer Marcie Wood noted microscopic findings of potential concern. An Information Request was sent via fax on June 28, 2010 to request justification that a minimal finding in the pancreas in 1/4 placebo males and 2/4 high-dose males were incidental findings. The

sponsor provided reasoning (SDN4, dated July 1, 2010) to support their conclusion that these were incidental findings.

In this general review, the reviewer is providing a detailed evaluation of single- and pivotal repeat-dose toxicology studies (14-day inhalation repeat-dose studies in rat and dog).

3 Studies Submitted

3.1 Studies Reviewed

Study	Title
Single-dose Toxicology	
FY08-042D	Determining the maximum tolerated or maximum feasible dose of glycopyrrolate + formoterol fumarate combination metered dose inhaler in Sprague Dawley rats
FY08-041D	Determining the maximum tolerated or maximum feasible dose of glycopyrrolate + formoterol fumarate combination metered dose inhaler in beagle dogs
Repeat-dose Toxicology	
FY09-086	Glycopyrrolate and formoterol fumarate pMDI: 14-day nose-only inhalation exposure study with recovery in rats
FY09-087	Glycopyrrolate and formoterol fumarate pMDI: 14-day face mask inhalation exposure study with recovery in beagle dogs

3.2 Studies Not Reviewed

(b) (4)

Study	Title
Repeat-dose Toxicology	
LS-2005-048	Summary of 14 day inhalation toxicity study in rats of vehicle dry powder aerosol (DSPC and calcium chloride) compared to air control
LS-2005-049	Summary of 14 day inhalation toxicity study in dogs of vehicle control dry powder aerosol (DSPC and calcium chloride) compared to air control
DSPC Genotoxicity	
P-2002-011	Evaluation of a test article in the Salmonella typhimurium/Escherichia coli plate incorporation mutation assay in the presence and absence of induced rat liver S-9
P-2002-013	Test for chemical induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells with and without metabolic activation
P-2002-012	In vivo test for chemical induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells

3.3 Previous Reviews Referenced

(b) (4)

6 General Toxicology

6.1 Single-Dose Toxicity

Maximum tolerated dose of GFF was determined in single dose studies in rats and dogs, as follows:

Study no. FY08-042D, "Determining the maximum tolerated or maximum feasible dose of glycopyrrolate + formoterol fumarate combination metered dose inhaler in Sprague Dawley rats." Rats (n=3/sex) were exposed to GFF pMDI for 45, 60, or 90 min. Two rats (n=1/sex) were sacrificed immediately post-dose, and 3 and 24 hrs post-dose. An additional 6 animals were used as controls and were sacrificed the day following the final exposure. Clinical observations were performed and clinical chemistry and hematology samples were collected at necropsy. Average total aerosol exposure concentrations were 0.050, 0.059, and 0.067 mg/L for the 45, 60, and 90 min exposures. Average GP exposure concentrations were 4.14, 5.1, and 5.75 µg/L for the 45, 60, and 90 min exposures, respectively. Average FF exposure concentrations were 0.83, 1.01, and 1.17 µg/L for the 45, 60, and 90 min exposures, respectively. The average MMAD (GSD) for the study drug was (b) (4) µm. Estimated inhaled GP doses for the 45, 60, and 90 min exposures were 120/132, 195, 214, and 327/354 µg/kg for males/females, respectively. Estimated inhaled FF doses for the 45, 60, and 90 min exposures were 24/26, 39/42, and 66/72 µg/kg for males/females, respectively.

One male animal in the high-dose group displayed labored breathing at 3 hrs post-dose and was euthanized. No correlating gross findings were observed in this animal. At necropsy, mild (red) discoloration was noted the lungs in 1 male and 1 female in the high-dose group at 24 hrs post-dose. BUN and GLU were increased in all exposure groups immediately post-dose (BUN: 63-95%; GLU: 78-169%) and returned to control levels at 3 hrs post-dose. CRE was increased in low- and mid-dose exposure groups immediately post-dose (73%) and returned to control levels at 3 hrs post-dose. Highest plasma levels of GP and FF occurred immediately post-dose in all dose groups (GP: 838-3578 pg/mL; FF: 362-1403 pg/mL). GP remained elevated (up to 130 pg/mL) at 24 hrs post-dose. Based on congestion in the lungs, the MTD (as achieved dose) of GP/FF was determined to be 120/24 µg/kg and 132/26 µg/kg in males and females, respectively. The pulmonary deposited dose at the MTD was 12.0/2.4 and 13.2/13.0 µg/kg in males and females, respectively (assuming a 10% deposition factor in rats).

Study no. FY08-041D, "Determining the maximum tolerated or maximum feasible dose of glycopyrrolate and formoterol fumarate combination metered dose inhaler in beagle dogs." Dogs (n=1/sex) were exposed to GFF pMDI for 15 or 45 min. The 45 min exposure occurred after a 3-day washout period following the 15 min exposure. The female dog was re-exposed to GFF pMDI for 45 min after a 4-day washout period (As per the sponsor, the male dog was not re-exposed due to a non-test article-related

illness). Blood was collected from each animal prior to each exposure session, immediately post-dose, and 0.5, 1, 2, 4, 8, and 24 hrs post-dose. Clinical observations were performed throughout the study and clinical chemistry and hematology samples were collected prior to exposure and 24 hrs post-dose. Average total aerosol exposure concentrations were 0.040, 0.067, and 0.073 mg/L for the 15, 45, and confirmatory 45 min exposures. Average GP exposure concentrations were 2.92, 5.34, and 8.67 µg/L for the 15, 45, and confirmatory 45 min exposures, respectively. Average FF exposure concentrations were 0.54, 1.04, and 1.11 µg/L for the 15, 45, and confirmatory 45 min exposures, respectively. The average MMAD(GSD) for the study drug was (b) (4) µm for the 15, 45, and confirmatory 45 min exposures, respectively. Estimated inhaled GP doses for the 15, 45, and confirmatory 45 min exposures were 14.4/13.9, 79.2/78.0, and 128.5 (females only) µg/kg for females/males, respectively. Estimated inhaled FF doses for the 15, 45, and confirmatory 45 min exposures were 2.7/2.6, 15.4/15.2, and 16.5 (females only) µg/kg for females/males, respectively.

Test article-related clinical observations noted after the 15 min exposure included erythema in the male and female. Erythema, increased respiration (2-fold versus baseline), and increased HR (2-fold versus baseline) were observed in the male and female after the 45 min exposure. Erythema and increased HR were noted in the female after the confirmatory 45 min re-exposure. There were no test article-related effects on clinical chemistry or hematology. Highest plasma levels of GP and FF occurred immediately post-dose in all dose groups (GP: 619-31069 pg/mL; FF: 2.6-16.5 pg/mL). GP remained elevated (14-272 pg/mL) at 24 hrs post-dose, while FF was close to or below the LLOQ of 0.010 ng/mL. GP AUC_{0-t} for the male and female ranged from 1490-21413 pg*hr/mL. FF AUC_{0-t} for the male and female ranged from 551-5554 pg*hr/mL. The GP and FF $T_{1/2}$ ranged from 5-11 and 5-17 hrs respectively. The MTD (as achieved dose) of GP/FF was determined to be 129/78 µg/kg for the 45 min exposure for both the male and female dog, based on clinical signs (erythema and increased respiration and HR). The pulmonary deposited dose at the MTD was 32.3/19.5 µg/kg (assuming a 25% deposition factor in dog).

6.2 Repeat-Dose Toxicity

Study title: Glycopyrrolate and formoterol fumarate pMDI: 14-day nose-only inhalation exposure study with recovery in rats

Study no.:	FY09-086
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 10, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	30 µg glycopyrrolate and 4.5 µg formoterol fumarate pMDI, ex valve; Lot #'s N-89-22-A and N-89-18-A

Key Study Findings

- No dose-limiting toxicity or target organs of toxicity were identified. The high-dose was identified as the NOAEL. For the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 36.8/6.9 and 39.4/7.3 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 7.3/1.2 and 6.3/1.2 µg/kg lung weight/day for males and females, respectively.

Methods

Doses:	See below
Frequency of dosing:	Once daily for 14 days
Route of administration:	Inhalation (nose-only)
Dose volume:	See below
Formulation/Vehicle:	See below
Species/Strain:	Sprague Dawley Rats ((b) (4))
Number/Sex/Group:	10/sex/group in main study
Age:	5 weeks of age on arrival
Weight:	Females: 176.51, 176.25, 176.43, 176.29, and 176.37 g in air control, placebo, low-, mid-, and high-dose groups Males: 250.40, 250.59, 250.69, 250.57, and 250.71 g in air control, placebo, low-, mid-, and high-dose groups
Satellite groups:	5/sex/group for 14-day recovery analysis; 2/sex from test article-treated groups were sacrificed at 30 min, 3 hrs, and 24 hrs following the first inhalation exposure and 2/sex from the air control and placebo groups were sacrificed 24 hrs following the first inhalation exposure for TK

	analysis (TK1); 2/sex from test article-treated groups were sacrificed at 30 min, 3 hrs, and 24 hrs following the last inhalation exposure and 2/sex from the air control and placebo groups were sacrificed 24 hrs following the last inhalation exposure for TK analysis (TK2)
Unique study design:	See below
Deviation from study protocol:	Only minor deviations occurred

Doses: Rats received the GFF formulation at aerosol concentrations of 4.49-6.33 µg/L GP and 0.89-1.18 µg/L FF for 25, 60, or 90 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 90 min of exposure. An air control group that received filtered room air for 90 min was also included. The resultant average daily glycopyrrolate doses were 72, 226, and 368 µg/kg/day for low-, mid-, and high-dose males, respectively, and 77, 241, and 394 µg/kg/day for low-, mid-, and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 14, 42, and 69 µg/kg/day for low-, mid-, and high-dose males, respectively, and 15, 45, and 73 µg/kg/day for low-, mid-, and high-dose females, respectively.

Filter samples were sampled directly from the reference port of each of the exposure systems. All filter samples collected throughout the study were analyzed gravimetrically to determine the total aerosol (i.e. excipients plus drug) concentration. Filters were then transferred and analyzed by HPLC-UV to determine the GP and FF aerosol concentrations.

Table 6: Estimated glycopyrrolate dose in rats

Exposure Group	Exposure Duration (minutes)	Aerosol Concentration (µg/L)	Average Daily Dose (µg/kg/day)
	Males		
Low Exposure	25	4.49	72
Mid Exposure	60	5.86	226
High Exposure	90	6.33	368
	Females		
Low Exposure	25	4.49	77
Mid Exposure	60	5.86	241
High Exposure	90	6.33	394

Table 7: Estimated formoterol fumarate dose in rats

Exposure Group	Exposure Duration (minutes)	Aerosol Concentration (µg/L)	Average Daily Dose (µg/kg/day)
	Males		
Low Exposure	25	0.89	14
Mid Exposure	60	1.09	42
High Exposure	90	1.18	69
	Females		
Low Exposure	25	0.89	15
Mid Exposure	60	1.09	45
High Exposure	90	1.18	73

Particle size was determined with an impactor. Representative samples were collected directly from the chamber exhaust three times throughout the study for both the GFF pMDI and the placebo pMDI systems; samples were collected on exposure days 2, 8, and 15 for the GFF pMDI system and on days 3, 9, and 15 for the pMDI placebo system. Mass median aerodynamic diameter (MMAD) and geometric standard deviations (GSD) for GFF pMDI and placebo pMDI are presented in the following table:

Table 8: MMADs and GSDs for GFF pMDI and placebo pMDI systems: 14-day rat inhalation toxicity

Test Atmosphere	Date Taken	MMAD µm (GSD)
Combination pMDI	Day 2	(b) (4)
Combination pMDI	Day 8	
Combination pMDI	Day 15	
Placebo pMDI	Day 3	
Placebo pMDI	Day 9	
Placebo pMDI	Day 15	

GP and FF pulmonary deposited doses expressed as both µg/kg/day and µg/g lung weight/day are presented in the following tables:

Table 9: GP Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day in rats

Sex	Dose (µg/kg/day)	Deposited Dose[1] (µg/kg/day)	Body Weight[2] (g)	Total Dose (µg/day)	Lung Weight[3] (g)	Deposited Dose (µg/g LW/day)
Males	72	7.2	292.17	2.10	1.5	1.40
	226	22.6	289.72	6.55	1.5	4.37
	368	36.8	277.64	10.22	1.4	7.30
Females	77	7.7	199.38	1.54	1.3	1.18
	241	24.1	199.26	4.80	1.2	4.00
	394	39.4	192.33	7.58	1.2	6.31

[1] A deposition factor of 0.10 was used in calculations.
[2] Mean terminal body weights at the end of the treatment period.
[3] Absolute lung weights from the sponsor's data.

Table 10: FF Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day in rats

Sex	Dose (µg/kg/day)	Deposited Dose[1] (µg/kg/day)	Body Weight[2] (g)	Total Dose (µg/day)	Lung Weight[3] (g)	Deposited Dose (µg/g LW/day)
Males	14	1.4	292.17	0.41	1.5	0.27
	42	4.2	289.72	1.22	1.5	0.81
	69	6.9	277.64	1.92	1.4	1.28
Females	15	1.5	199.38	0.30	1.3	0.23
	45	4.5	199.26	0.90	1.2	0.75
	73	7.3	192.33	1.40	1.2	1.17

[1] A deposition factor of 0.10 was used in calculations.
[2] Mean terminal body weights at the end of the treatment period.
[3] Absolute lung weights from the sponsor's data.

Route, Dose Volume, Formulation/Vehicle: Animals were exposed to GFF using a nose-only inhalation exposure system for up to 90 min per day for 14 consecutive days at target concentrations of 0.08 mg/L. The placebo target aerosol concentration was 0.06 mg/L. The aerosols were generated with a dedicated (b) (4) pMDI aerosol generation system in separate, dedicated exposure chambers. The (b) (4) pMDI aerosol generation system coupled six pMDIs to an expansion chamber that allowed the propellant to expand and evaporate prior to transitioning into the exposure plenum. The pMDI vials were actuated at a rate of 90 shots every minute (six vials on the system at a time) which equated to 15 shots per vial per minute. The pMDI vials were changed on a continuous basis during the exposures at 15 min ± 1 min. Air control animals received filtered room air for 90 minutes per day using a similar nose-only inhalation exposure system with the exception of the (b) (4) pMDI aerosol generation system attached.

Observations and Results

Mortality

Animals were observed for mortality/morbidity twice daily. All animals survived until scheduled sacrifice.

Clinical Signs

Animals were observed for clinical signs twice daily. No test article-related clinical signs were observed.

Body Weights

Body weights were recorded prior to randomization, and once weekly during the treatment and recovery phases, as well as at scheduled necropsy days. Body weight was statistically significantly decreased in high-dose males and females during Week 1 versus air controls (5 and 3%, respectively) and placebo controls (4 and 5%, respectively).

Feed Consumption

Food consumption was not recorded.

Ophthalmoscopy

Ophthalmic exams were performed by a board-certified veterinary ophthalmologist in the pre-dose phase and prior to the treatment and recovery phase necropsies. All animals were examined following pupil dilation with 1% tropicamide using slit lamp biomicroscopy and indirect ophthalmoscopy. No test article-related changes were observed.

ECG

ECG was not performed.

Hematology

Blood (~2 mL) was collected via cardiac puncture from all animals at the treatment and recovery phase necropsies for analysis of hematology parameters. A complete battery of hematology parameters was assessed. At the end of the treatment phase, RBC and HGB were significantly increased in high-dose males and females (RBC: 5 and 4%, respectively; HGB: 4.4 and 5%, respectively) versus air controls. Reticulocytes (%) were significantly increased in mid- and high-dose males (63 and 32%, respectively) and low-, mid-, and high-dose females (40, 60, and 75%, respectively) versus air controls. Reticulocytes (%) were also significantly increased in mid-dose males (58%) and mid- and high-dose females (33 and 45%, respectively) versus placebo controls. At the end of the recovery phase, platelets were significantly decreased in high-dose females versus air and placebo controls (19 and 24%, respectively).

Clinical Chemistry

Blood (~4 mL) was collected via cardiac puncture from all animals at the treatment and recovery phase necropsies for analysis of clinical chemistry parameters. A complete battery of clinical chemistry parameters was assessed. At the end of the treatment phase, calcium was significantly increased in mid- and high-dose males versus air controls (5 and 4%, respectively). Phosphorus was significantly increased in high-dose males and females versus air (13.3 and 10.3%, respectively) and placebo (17.2 and 12.9%, respectively). ALP was significantly increased in mid- and high-dose females

versus air (25.0 and 27.8%, respectively) and placebo (18.6 and 21.6%, respectively) controls. Globulin was significantly decreased and A/G ratio was increased in mid- and high-dose males versus air controls (GLOBN: 11.8 and 12.4%, respectively; A/G ratio: 16 and 16%, respectively). Cholesterol and triglycerides were significantly increased in low-, mid-, and high-dose males (CHOL: 28, 35, and 28%, respectively; TRIG: 46.3, 39.0, and 43.9%, respectively) and high-dose females (CHOL: 33.8%; TRIG: 30.6%) versus placebo controls. At the end of the recovery phase, CRE-S was significantly increased in high-dose females (~45.5%) versus air and placebo controls.

Urinalysis

Urinalysis was not performed.

Gross Pathology

All treatment and recovery phase animals were euthanized with an overdose of Euthasol and weighed. Body surfaces, orifices, cranial, thoracic, and abdominal cavities were examined for abnormalities and lesions. Protocol-specified tissues and organs were examined, collected, weighed, and fixed. All tissues were fixed win 10% neutral buffered formalin (with the exception of epididymis, testes, and eyes/optic nerves which were fixed in Bouin's). The right testis and epididymis of high-dose male E001 were described as small, diffuse (severity). These gross findings correlated with microscopic findings in both the testis and epididymis (see Histopathology below). The testis of high-dose male E006 was described as discolored and dark red. This gross finding also correlated with a microscopic finding (see Histopathology).

Organ Weights

The following protocol-specified organ weights were recorded: adrenals (paired), brain, heart, kidney (paired), liver, thymus, spleen, lungs, ovary (paired), epididymis (paired), testis (paired), and ovary (paired). Organ-to-body weight and organ-to-brain weight ratios were also determined. No apparent test article-related changes in absolute organ weights or organ-to-body or –brain weights were observed.

Histopathology

Adequate Battery

Protocol-specified tissues/organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. An adequate battery of tissues was examined microscopically, including the following: Abnormal tissue, adrenals (paired), aorta, brain, epididymis (paired), eye/optic nerve (paired), gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum, colon, rectum), harderian gland (paired), heart, kidneys, lacrimal glands (paired) liver, mammary gland, mesenteric lymph nodes, esophagus, ovary (paired), pancreas, parathyroid (paired), pituitary, prostate, sciatic nerve, skin, seminal vesicles + coagulating gland, spinal cord, spleen, sternum with bone marrow, mandibular lymph node, submandibular salivary gland (paired), testes (paired), thigh muscle, thymus, thyroid (paired), tongue, urinary bladder, uterus (body and horns), vagina, tracheobronchial lymph node, larynx, lungs, nasal cavity, trachea. Femur with

bone marrow was collected (fixed) but not processed or read. Tissues from air, placebo, and high-dose groups were processed and examined histologically, as well as tissues from low- or mid-dose groups with abnormal observations at necropsy. Sections of lungs and respiratory tract were prepared and read from all animals.

Peer Review

A peer review was not performed.

Histological Findings

Rat microscopic findings are summarized in the following table:

Table 11: Microscopic findings in rats following a 14-day nose-only inhalation toxicity study with GFF pMDI with 14-day recovery

Observation	Sex	GFF MDI				
		Air (n=10/sex)	Placebo (n=10/sex)	LD (n=10/sex)	MD (n=10/sex)	HD (n=10/sex)
Lung						
-Histiocytosis	M	**2(1)**	**1(1)**	**4(1)**	**4(1)**	**4(1)**
	F	**1(1)**	**3(1)**	**2(1)**	**1(1)**	**2(1)**
-Recovery (n=5)	*M*	*2(1)*	*2(1)*	*2(1)*	*2(1)*	*2(1)*
	F	*2(1)*	*1(1)*	*0*	*1(1)*	*1(1)*
-Mineralization	M	**4(1)**	**3(1)**	**4(1)**	**5(1)**	**6(1)**
	F	**2(1)**	**2(1)**	**3(1)**	**5(1)**	**3(1)**
-Recovery (n=5)	*M*	*3(1)*	*3(1)*	*3(1)*	*4(1)*	*3(1)*
	F	*3(1)*	*3(1)*	*2(1)*	*3(1)*	*1(1)*
Nasal turbinate IV						
-Inflam., submucosa, oral	M	0	0	0	**1(3)**	**1(1)**
	F	0	0	0	0	0
-Recovery (n=5)	*M*	*0*	*0*	*0*	*0*	*0*
	F	*1(1)*	*1(1)*	*0*	*0*	*0*
-Inflam., subacute, oral	M	0	0	0	0	0
	F	0	0	0	**1(2)**	0
Lymph node, tracheobronchial						
-Erythrocytosis	M	0	0	0	0	0
	F	0	0	0	**1(1)**	**1(2)**
-Recovery (n=5)	*M*	*0*	*0*	*0*	*0*	*1(1)*
	F	*0*	*1(2)*	*1(1)*	*0*	*0*
Lymph node, mesenteric						
-Erythrocytosis	M	0	0	NE	NE	**1(1)**
	F	0	0			0
Epididymis						
-Hypospermia, unilateral	M	0	0	NE	NE	**1(4)***
-Sperm granuloma, unilateral	M	0	0			**1(2)***
Testis						
-Degeneration, tubular, unilat	M	0	0	NE	NE	**1(5)***
-Recovery (n=5)	*M*	*1(1)*	*0*			*1(1)*

( ) Indicates average severity of findings: 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, 5 = severe/high
* Male E001

No dose-limiting target organs of toxicity were identified in this study. Findings in the lung (histiocytosis and mineralization) showed no significant increase in severity with increased dose. Although histiocytosis incidence was slightly increased compared to controls, this finding was not associated with signs of inflammation or necrosis. Nasal inflammation was observed in high-dose rats in low incidence. This finding is attributed to the route of administration in the rat (nose-only) which does not correlate to human inhalation administration and therefore is not deemed toxicologically significant to humans. Low incidence, low-severity findings of erythrocytosis were observed in the lymph nodes of mid- and high-dose animals at the end of the treatment phase and in placebo and low-dose animals at the end of the recovery phase (Erythrocytosis was also observed across all dose groups in the 14-week dog toxicity study, see below). The toxicological significance of this finding in rats is considered low, as erythrocytosis of lymph nodes can also be an artifact that results from euthanasia or tissue dissection during necropsy[1]. Moderately severe to severe microscopic findings were also observed in the epididymis (unilateral hypospermia) and testis (unilateral tubular degeneration); however, these findings were not considered to be test article-related, as severe unilateral findings in the male reproductive organs are likely a congenital condition or the result of a pre-existing injury. The high-dose was identified as the NOAEL. For the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 36.8/6.9 and 39.4/7.3 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 7.3/1.2 and 6.3/1.2 µg/kg lung weight/day for males and females, respectively.

Special Evaluation

N/A

Toxicokinetics

Blood samples were collected from main study animals and recovery animals at necropsy for analysis of plasma levels of GP and FF. Two/sex from test article-treated groups were sacrificed at 30 min, 3 hrs, and 24 hrs following the first inhalation exposure and 2/sex from the air control and placebo groups were sacrificed 24 hrs following the first inhalation exposure for TK analysis (TK1); 2/sex from test article-treated groups were sacrificed at 30 min, 3 hrs, and 24 hrs following the last inhalation exposure and 2/sex from the air control and placebo groups were sacrificed 24 hrs following the last inhalation exposure for TK analysis (TK2). Plasma levels of GP and FF were determined using a validated LC-MS/MS protocol with a LLOQ of 10.0 pg/mL. No formal TK analyses were performed. C_{max} and T_{max} were noted from raw bioanalytical data. Data were also reviewed for gender differences and drug accumulation. T_{max} occurred at 30 min following both the first (TK1) and last (TK2) inhalation exposures. TK1 and TK2 GP and FF C_{max} observational values are presented in the following table:

[1] Elmore, Susan A. (2006) Histopathology of the lymph nodes. Toxicol. Pathol. 34:425-454.

Table 12: GP and FF C_{max} values in rats on Day 1 (TK1) and Day 14 (TK2)

Group	Drug	Sex	Cmax (pg/mL)		
			LD	MD	HD
TK1	GP	M	660	4393	4525
		F	1390	5163	3768
	FF	M	503	3849	2062
		F	531	3324	2698
TK2	GP	M	2025	3557	7687
		F	1437	5137	7098
	FF	M	445	1625	2437
		F	375	2433	4507

On Day 1 (TK1), a clear dose-response in plasma GP or FF was not observed. In addition, the mid-dose groups had similar or higher levels of GP and FF than high-dose groups. On Day 2 (TK2), plasma levels of GP and FF showed a dose-response in both genders. In addition, an accumulation of GP was observed in the low- and high-dose groups from Day 1 to Day 14. Finally, there were no clear gender differences in plasma levels of GP or FF at either time point.

Stability and Homogeneity

Results of formulation analyses for dose concentration and stability of GFF pMDI were within the accepted criteria.

Study title: Gycopyrrolate and formoterol fumarate pMDI: 14-day face mask inhalation exposure study with recovery in beagle dogs

Study no.:	FY09-087
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 21, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	30 µg glycopyrrolate and 4.5 µg formoterol fumarate pMDI, ex valve; Lot #'s N-89-22-A and N-89-18-A

Key Study Findings

- Test article-related clinical signs included erythema in 5/12 low-dose dogs (3 M, 2 F), 11/12 mid-dose dogs (5 M, 6 F), and 12/12 high-dose dogs (6 M, 6 F).
- Mean heart rates were increased for males and females in all dose groups after the first and last exposures. Following the first exposure, HR was increased by 44, 84, and 115% in low-, mid-, and high-dose animals (male and female average) versus placebo control. Following the second exposure, HR was

increased by 31, 58, and 87% in low-, mid-, and high-dose animals (male and female average) versus placebo control. No rhythm abnormalities were observed.

- The target organs of toxicity were the heart and liver, which are characteristic for β_2 adrenergic agonists.
- In the heart, minimal to moderate fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 and 2/8 mid- and high-dose animals, respectively, at the end of the treatment period. These findings in dogs are generally attributed to increased heart rate that is monitorable in a clinical setting. At the end of the recovery period, minimal to mild fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 high-dose animals
- In the liver, minimal to mild hepatocellular alteration was present in 8/8, 7/8, and 8/8 dogs in the low-, mid-, and high-dose groups. Affected hepatocytes were associated with the portal triad and periacinar regions. This finding resolved at the end of the recovery period. This finding (glycogen deposition), was attributed to the pharmacological action of formoterol and not judged as adverse.
- A NOAEL was not identified based upon histopathological findings in the liver at all doses; however, findings in the liver were not considered adverse. In addition, findings in the heart at the mid- and high-dose were judged to be monitorable in a clinical setting; therefore, the high-dose was used to determine safety factors. At the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 18.5/3.1 and 18.9/3.2 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 2.0/0.4 and 2.1/0.4 µg/kg lung weight/day for males and females, respectively. GP systemic exposures for males and females at the high-dose were 17046 and 13437 pg*hr/mL, respectively. FF systemic exposures for males and females at the high-dose were 3633 and 4155 pg*hr/mL, respectively.

Methods

Doses:	See below
Frequency of dosing:	Daily for 14 days
Route of administration:	Inhalation, see below
Dose volume:	See below
Formulation/Vehicle:	See below
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group in main study
Age:	Approximately 6 months of age on arrival
Weight:	Females: 7.86, 7.74, 7.70, 7.88, and 7.85 kg in the air control, placebo, low-, mid-, and high-dose groups, respectively. Males: 8.84, 8.79, 8.71, 8.79, and 8.90 kg in the air control, placebo, low-, mid-, and high-dose groups, respectively.
Satellite groups:	2/sex/group for a 14-day recovery analysis
Unique study design:	See below
Deviation from study protocol:	Only minor deviations occurred

Doses: Dogs received the GFF formulation at aerosol concentrations of 5.15-7.79 µg/L GP and 1.00-1.34 µg/L FF for 10, 20, or 30 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 30 min of exposure. An air control group that received filtered room air for 45 min was also included. The resultant average daily glycopyrrolate doses were 17.0, 51.3, and 73.8 µg/kg/day for low-, mid-, and high-dose males, respectively, and 17.3, 52.3, and 75.4 µg/kg/day for low-, mid-, and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 3.3, 8.8, and 12.5 µg/kg/day for low-, mid-, and high-dose males, respectively, and 3.4, 9.0, and 12.8 µg/kg/day for low-, mid-, and high-dose females, respectively.

Filter samples were sampled directly from the reference port of each of the exposure systems. All filter samples collected throughout the study were analyzed gravimetrically to determine the total aerosol (i.e. excipients plus drug) concentration. Filters were then transferred and analyzed by LC-MS/MS to determine the GP and FF aerosol concentrations.

Table 13: Estimated glycopyrrolate dose in dogs

Exposure Group	Exposure Duration (minutes)	Aerosol Concentration (µg/L)	Average Daily Deposited Dose (µg/kg/day)
Males			
Low Exposure	10	5.15	17.0
Mid Exposure	20	7.79	51.3
High Exposure	30	7.50	73.8
Females			
Low Exposure	10	5.15	17.3
Mid Exposure	20	7.79	52.3
High Exposure	30	7.50	75.4

Table 14: Estimated formoterol fumarate dose in dogs

Exposure Group	Exposure Duration (minutes)	Aerosol Concentration (µg/L)	Average Daily Deposited Dose (µg/kg/day)
Males			
Low Exposure	10	1.00	3.3
Mid Exposure	20	1.34	8.8
High Exposure	30	1.27	12.5
Females			
Low Exposure	10	1.00	3.4
Mid Exposure	20	1.34	9.0
High Exposure	30	1.27	12.8

Particle size was determined with an impactor. Representative samples were collected directly from the chamber exhaust three times throughout the study for both the GFF pMDI and the placebo pMDI systems. Based on the results below, the particle sizes were within the respirable range (< 5 um). Mass median aerodynamic diameter (MMAD) and geometric standard deviations (GSD) for GFF pMDI and placebo pMDI are presented in the following table:

Table 15: MMADs and GSDs for GFF pMDI and placebo pMDI systems: 14-day dog inhalation toxicity

Test Atmosphere	Date Taken	MMAD (GSD) µm (b) (4)
Combination pMDI	Day 4	
Combination pMDI	Day 11	
Combination pMDI	Day 14	
Placebo pMDI	Day 4	
Placebo pMDI	Day 11	
Placebo pMDI	Day 14	

GP and FF pulmonary deposited doses expressed as both µg/kg/day and µg/g lung weight/day are presented in the following tables:

Table 16: GP Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day in dogs

Sex	Dose (µg/kg/day)	Deposited Dose[1] (µg/kg/day)	Body Weight[2] (kg)	Total Dose (µg/day)	Lung Weight[3] (g)	Deposited Dose (µg/g LW/day)
Males	17.0	4.25	8.78	37.32	72.7	0.51
	51.3	12.83	9.05	116.11	78.0	1.49
	73.8	18.45	9.09	167.71	82.4	2.04
Females	17.3	4.33	7.68	33.25	71.4	0.47
	52.3	13.08	7.93	103.72	68.5	1.51
	75.4	18.85	7.92	149.29	70.7	2.11

[1] A deposition factor of 0.25 was used in calculations.
[2] Mean terminal body weights at the end of the treatment period.
[3] Absolute lung weights from the sponsor's data.

Table 17: FF Pulmonary deposited dose expressed as µg/kg/day and µg/g LW/day in dogs

Sex	Dose (µg/kg/day)	Deposited Dose[1] (µg/kg/day)	Body Weight[2] (kg)	Total Dose (µg/day)	Lung Weight[3] (g)	Deposited Dose (µg/g LW/day)
Males	3.3	0.83	8.78	7.29	72.7	0.10
	8.8	2.20	9.05	19.91	78.0	0.26
	12.5	3.13	9.09	28.45	82.4	0.35

Females	3.4	0.85	7.68	6.53	71.4	0.09
	9.0	2.25	7.93	17.84	68.5	0.26
	12.8	3.20	7.92	25.34	70.7	0.36

[1] A deposition factor of 0.25 was used in calculations.
[2] Mean terminal body weights at the end of the treatment period.
[3] Absolute lung weights from the sponsor's data.

Route, Dose volume, Formulation/Vehicle: Animals were exposed to GFF using a face mask inhalation exposure system for up to 30 min per day for 14 consecutive days at target concentrations of 0.08 mg/L. The placebo aerosol maximum achievable concentration was 0.07 mg/L and this was the target concentration for the placebo group for 30 min. The aerosols were generated with a dedicated (b) (4) pMDI aerosol generation system in separate, dedicated exposure chambers. The (b) (4) pMDI aerosol generation system coupled six pMDIs to an expansion chamber that allowed the propellant to expand and evaporate prior to transitioning into the exposure plenum. The pMDI vials were actuated at a rate of 90 shots every minute (six vials on the system at a time) which equated to 15 shots per vial per minute. The pMDI vials were changed on a continuous basis during the exposures at 15 min ± 1 min. Air control animals received filtered room air for 30 minutes per day using a similar face mask inhalation exposure system with the exception of the (b) (4) pMDI aerosol generation system attached.

Observations and Results

Mortality

Animals were observed for mortality/morbidity twice daily. All animals survived until scheduled sacrifice.

Clinical Signs

Animals were observed for clinical signs twice daily. Erythema was observed in 5/12 low-dose dogs (3 M, 2 F), 11/12 mid-dose dogs (5 M, 6 F), and 12/12 high-dose dogs (6 M, 6 F).

Body Weights

Body weights were recorded prior to randomization, and once weekly during the treatment and recovery phases, as well as at scheduled necropsy days. There were no test article-related effects on body weight.

Feed Consumption

Food consumption was not recorded.

Ophthalmoscopy

Ophthalmic exams were performed by a board-certified veterinary ophthalmologist in the pre-dose phase and prior to the treatment phase necropsies. All animals were examined following pupil dilation with 1% tropicamide using slit lamp biomicroscopy and indirect ophthalmoscopy. No test article-related changes were observed.

ECG

ECGs (using leads I, II, III, aVR, aVL, and aVF) were performed during the pre-dose phase, immediately following the first and last exposures, and on recovery phase animals prior to necropsy. Heart rate and waveform morphology (P, QRS, and T waves) were evaluated. Following the first exposure, HR was increased by 44, 84, and 115% in low-, mid-, and high-dose animals (male and female average) versus placebo control. Following the second exposure, HR was increased by 31, 58, and 87% in low-, mid-, and high-dose animals (male and female average) versus placebo control. HR returned to control values at the end of the recovery period. There were no test article-related changes in waveform.

Hematology

Blood was collected from all animals at the treatment and recovery phase necropsies for analysis of hematology parameters. A complete battery of hematology parameters was assessed. At the end of the treatment phase, monocytes were increased by 45 and 43% in mid- and high-dose males versus placebo. Eosinophils were decreased by 48 and 35% in mid- and high-dose males and by 47% in high-dose females versus air controls. Eosinophils remained decreased by 66% in high-dose males at the end of the recovery phase. Large unstained cells were decreased by 11 and 33% in mid- and high-dose males versus air controls.

Clinical Chemistry

Blood was collected from all animals at the treatment and recovery phase necropsies for analysis of clinical chemistry parameters. A complete battery of clinical chemistry parameters was assessed. There were no test article-related changes in clinical chemistry parameters.

Urinalysis

Urinalysis was performed on urine collected from all animals at necropsy. The following parameters were evaluated (unless quantity collected was not sufficient): occult blood, pH, glucose, protein, urobilinogen, ketones, bilirubin, color, appearance, and specific gravity. pH was significantly increased in mid- and high-dose males at the end of the treatment phase versus air controls (5.8 in air controls versus 7.4 and 7.6 in mid-, and high-dose males, respectively). pH remained increased in low-, mid-, and high-dose males at the end of the recovery phase versus air controls (5.0 in air controls versus 6.3, 6.0, and 6.3 in low-, mid-, and high-dose males, respectively). Specific gravity was increased in high-dose males versus air and placebo controls at the end of the recovery

phase (1.037 and 1.038 in air and placebo controls, respectively, versus 1.057 in high-dose males).

Gross Pathology

All treatment and recovery phase animals were euthanized with an overdose of Euthasol and weighed. Body surfaces, orifices, cranial, thoracic, and abdominal cavities were examined for abnormalities and lesions. Protocol-specified tissues and organs were examined, collected, weighed, and fixed. All tissues were fixed win 10% neutral buffered formalin (with the exception of epididymis, testes, and eyes/optic nerves which were fixed in Bouin's). No test article-related macroscopic findings were observed.

Organ Weights

The following protocol-specified organ weights were recorded at necropsy: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, spleen, testis, thymus, and uterus. Organ-to-body weight and organ-to-brain weight ratios were also determined. Absolute thymus weight and thymus-to-body and thymus-to-brain weights were decreased in placebo (46, 46, and 45%, respectively), low- (43, 40, and 44%, respectively), mid- (41, 40, and 39%, respectively), and high-dose (43, 44, and 49%, respectively) males versus air controls at the treatment phase sacrifice. Similarly, absolute thymus weight and thymus-to-body and thymus-to-brain weights were decreased in placebo (15, 16, and 22%, respectively), low- (32, 26, and 37%, respectively), mid- (48, 50, and 51%, respectively), and high-dose (33, 34, and 36%, respectively) females versus air controls at the treatment phase sacrifice. Absolute adrenal weight and adrenal-to-brain weight ratio were significantly decreased in high-dose males versus air controls (26 and 29%, respectively) at the recovery sacrifice. Absolute heart weight and heart-to-body and heart-to-brain weights were decreased in high-dose males versus air controls (25, 18, and 26%, respectively) at the recovery sacrifice. Absolute liver weight was significantly decreased in high-dose males versus air and placebo controls (19 and 14%, respectively) at the recovery sacrifice.

Histopathology

Adequate Battery

Protocol-specified tissues/organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. An adequate battery of tissues was examined microscopically, including the following: abnormal tissue, adrenals, aorta-thoracic, brain, cervix, epididymides, esophagus, eyes/optic nerves, gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum, colon, rectum), heart, kidneys, larynx, liver with gallbladder, lungs, lymph nodes (mandibular, bronchial, mesenteric, cervical, retropharyngeal), mammary gland (inguinal), nasal cavity/nasal turbinates, ovaries, pancreas, parathyroids, pharynx, pituitary, prostate, rib with bone marrow, sciatic nerve, salivary gland (mandibular), skin (thigh), spinal cord (cervical, midthoracic, lumbar), spleen, sternum with bone marrow, testes, thymus, thyroids, tongue, trachea (anterior and carina), urinary bladder, uterus (body and horns), and vagina. Tissues from air, placebo, and high-dose groups were processed and examined histologically, as well as

tissues from low- or mid-dose groups with abnormal observations at necropsy. Sections of lungs and respiratory tract were prepared and read from all animals.

Peer Review

A peer review was not conducted.

Histological Findings

Dog microscopic findings are summarized in the following table:

Table 18: Microscopic findings in dogs following a 14-day face mask inhalation toxicity study with GFF pMDI with 14-day recovery

Observation	Sex	GFF MDI				
		Air (n=4/sex)	Placebo (n=4/sex)	LD (n=4/sex)	MD (n=4/sex)	HD (n=4/sex)
Heart						
-Fibrosis, myocardium,	M	0	0	0	**3(1.3)**	**1(3)**
papillary muscle, left vent.	F	0	0	0	**0**	**1(2)**
-Recovery (n=2)	*M*	*0*	*0*	*0*	*0*	*2(1)*
	F	*0*	*0*	*0*	*0*	*1(2)*
-Mineralization, myocardium,	M	0	0	0	0	**1(1)**
papillary muscle, right vent.	F	0	0	0	0	**1(2)**
Liver						
-Alteration, hepatocellular,	M	0	0	**4(1.3)**	**3(1.3)**	**4(1.5)**
portal/periacinar	F	0	0	**4(1.3)**	**4(1.8)**	**4(1.3)**
Lymph node, bronchial						
-Erythrocytosis	M	0	0	0	0	0
	F	0	**1(1)**	**1(2)**	**1(2)**	**1(1)**
-Recovery (n=2)	*M*	**0**	*0*	*0*	*0*	*0*
	F	**0**	*0*	*1*	*0*	*1*
Lymph node, cervical						
-Erythrocytosis	M	**2(1)**	0	**1(2)**	**1(2)**	**1(2)**
	F	**1(3)**	**1(1)**	**1(3)**	**2(1.5)**	**3(1)**
-Recovery (n=2)	*M*	*0*	*1(2)*	*1(2)*	*0*	*0*
	F	*0*	*1(1)*	*1(1)*	*2(1.5)*	*1(1)*
Lymph node, mandibular						
-Erythrocytosis	M	0	0	NE	NE	0
	F	0	0			**1(1)**
Lymph node, mediastinal						
-Erythrocytosis	M	1(3)	1(3)	NE	NE	2(3)
	F	0	0	1(3)		0
Lymph node, mesenteric						
-Erythrocytosis	M	0	0	NE	NE	**1(1)**
	F	0	**1(1)**			**2(1.5)**
-Recovery (n=2)	*M*	*1(1)*	*1(1)*			*1(1)*
	F	*1(1)*	*0*			*0*
Pancreas						
-Increased individual cell	M	0	**1(1)**	NE	NE	**2(1)**
death, acinar	F	0	0			0

( ) Indicates average severity of findings: 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, 5 = severe/high

The target organs of toxicity were the heart and liver, which are characteristic for β_2 adrenergic agonists. In the heart, minimal to moderate fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 and 2/8 mid- and high-dose animals, respectively, at the end of the treatment period. These findings in dogs are generally attributed to increased heart rate that is monitorable in a clinical setting. At the end of the recovery period, minimal to mild fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 high-dose animals. In the liver, minimal to mild hepatocellular alteration was present in 8/8, 7/8, and 8/8 dogs in the low-, mid-, and high-dose groups. Affected hepatocytes were associated with the portal triad and periacinar regions. This finding resolved at the end of the recovery period. This finding (glycogen deposition), was attributed to the pharmacological action of formoterol and not judged as adverse.

Erythrocytosis was observed in several lymph nodes (bronchial, cervical, mandibular, mediastinal, and mesenteric). There was a slight dose-related increased incidence in erythrocytosis in the mandibular, mediastinal, and mesenteric lymph nodes in high-dose animals. However, erythrocytosis of the cervical and mesenteric lymph nodes was also observed in air control groups at the end of the treatment and recovery phases, respectively. As in the 14-day rat study, the toxicological significance of this finding in dogs is considered low, as erythrocytosis of lymph nodes can also be an artifact that results from euthanasia or tissue dissection during necropsy.

In the pancreas, minimal increased acinar cell death was observed in 1/4 placebo males and 2/4 high-dose males. Because microscopic evaluation of the pancreas was not performed for mid- and low-dose groups, an Information Request was sent to the sponsor via fax on June 28, 2010 to request evidence that this was an incidental finding. The sponsor replied on July 1, 2010 (SDN4). The Study Director for dog study FY09-087 consulted with an independent Board Certified Pathologist and two Board Certified Toxicologists, who agreed with the conclusions of the original study pathologist, (b) (4) , DVM, PhD, DACVP. Dr. (b) (4) provided the following perspective in an additional signed statement:

> "Cell death (apoptosis) of individual acinar cells occurs in the pancreas of animals as a means of normal, physiological cell turnover… Minimally increased individual acinar cell death in 2/4 high-dose males was not interpreted to be a test article-related effect. Of consideration was that this finding was present in a control, that the difference in the incidence between high-dose males (2/4) and placebo-treated males (1/4) was not meaningful given the nature of the change and its minimal severity, and absence of this finding in the high-dose females."

Though the sponsor did not provide historical control evidence of this finding, the reviewer considers the sponsor's response to be adequate at this time. However, the sponsor's proposed 3-month inhalation toxicity study of GFF pMDI in dogs will be monitored for findings in the pancreas.

A NOAEL was not identified based upon histopathological findings in the liver at all doses; however, findings in the liver were not considered adverse. In addition, findings in the heart at the mid- and high-dose were judged to be monitorable in a clinical setting; therefore, the high-dose was used to determine safety factors. At the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 18.5/3.1 and 18.9/3.2 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 2.0/0.4 and 2.1/0.4 µg/kg lung weight/day for males and females, respectively. GP systemic exposures for males and females at the high-dose were 17046 and 13437 pg*hr/mL, respectively. FF systemic exposures for males and females at the high-dose were 3633 and 4155 pg*hr/mL, respectively.

Special Evaluation

Respiratory Parameters: Spontaneous breathing measurements (frequency, respiratory minute volume, tidal volume), were collected for at least 15 min in duration from all dogs during the pre-dose phase, immediately following the first and last exposures, and on recovery animals prior to necropsy. Frequency was significantly increased in low-dose males on Day 1 (21%) versus placebo controls. Frequency was significantly decreased in low-, mid-, and high-dose males versus air controls on Day 14 (18, 18, and 15%, respectively). Conversely, frequency was significantly increased in low- and mid-dose females on Day 14 (26% in both groups) versus placebo controls. Minute volume was significantly increased in low-, mid-, and high-dose males (47, 85, and 104%, respectively) versus placebo controls and in low-, mid-, and high-dose females (~50, 76, and 50%, respectively) versus air and placebo controls on Day 1. Minute volume was increased in low- and mid-dose males (27 and 23%, respectively) versus placebo controls and in low- and mid-dose females (68 and 73%, respectively) versus air and placebo controls on Day 14. Minute volume was also increased in high-dose males (34%) versus placebo controls and in high-dose females (39%) versus air and placebo controls on Day 14, though the increase was not statistically significant. Tidal volume was significantly increased in low-, mid-, and high-dose males on Day 1 (~20, 50, and 41%, respectively) and in low- and mid-dose males on Day 14 (~36 and 38%) versus air and placebo controls. Tidal volume was also increased in high-dose males versus air and placebo controls on Day 14 (31%), though the increase was not statistically significant. Tidal volume was increased in low-, mid-, and high-dose females on Day 1 (22, 44, and, 37%, respectively; increases in low- and mid-dose females were statistically significant) and Day 14 (32, 37, and 41%, respectively; increases in low- and high-dose females were statistically significant) versus air controls. Respiratory parameters returned to control values at the end of the recovery phase.

Toxicokinetics

Blood was collected from main study animals for analysis of plasma GP and FF levels after the first (TK1) and last (TK2) exposure at the following time points: immediately post-exposure (0.016 hr) and 0.5, 1, 3, 6, and 24 hrs post-dose. Plasma levels of GP and FF were determined by HPLC-MS/MS with a LLOQ of 10 pg/mL for both GP and FF. C_{max}, T_{max}, AUC_{last}, and $t_{1/2}$ were determined.

On Day 1 (TK1), T_{max} occurred immediately post-exposure (0.016 hr) for both GP and FF. Systemic exposure (C_{max} and AUC) was dose-dependent in males, but not in females. $T_{1/2}$ for GP and FF ranged from 7.09-14.53 hrs and 5.02-10.07 hrs, respectively. On Day 14 (TK2), T_{max} was variable for GP (0.016-0.629 hrs) and FF (0.016-0.754 hrs). Systemic exposure (C_{max} and AUC) was dose-dependent in males and females. $T_{1/2}$ for GP and FF ranged from 7.38-12.34 hrs and 5.54-8.16 hrs, respectively. TK results for Day 1 and Day 14 are presented in the following tables:

Table 19: Day 1 (TK1) toxicokinetic results in dogs

Exposure Group	C_{max} (pg/mL)	T_{max} (hr)	AUClast (hr*pg/mL)	$t_{1/2}$ (hr)
		Glycopyrrolate		
Males				
Low Exposure	787.85	0.016	1398.34	8.46
Mid Exposure	1500.38	0.016	4344.34	14.53
High Exposure	5990.82	0.016	5808.42	7.61
Females				
Low Exposure	533.09	0.016	939.71	10.78
Mid Exposure	4687.74	0.016	6435.02	8.97
High Exposure	3893.58	0.016	5845.45	7.09
		Formoterol Fumarate		
Males				
Low Exposure	190.66	0.262*	768.33	8.06
Mid Exposure	373.64	0.016	1599.94	5.97
High Exposure	915.41	0.016	2308.59	5.32
Females				
Low Exposure	95.04	0.016	495.61	10.07
Mid Exposure	746.02	0.016	2119.98	7.27
High Exposure	677.20	0.137[$]	2406.76	5.02

Table 20: Day 14 (TK2) toxicokinetic results in dogs

Exposure Group	C_{max} (pg/mL)	T_{max} (hr)*	AUClast (hr*pg/mL)	$t_{1/2}$ (hr)
		Glycopyrrolate		
Males				
Low Exposure	1120.88	0.137	3377.44	11.21
Mid Exposure	4034.71	0.629	9025.38	12.34
High Exposure	11552.75	0.016	17046.45	8.27
Females				
Low Exposure	634.21	0.137	1723.34	9.54
Mid Exposure	5851.91	0.016	8664.14	7.38
High Exposure	11082.58	0.137	13437.15	10.22
		Formoterol Fumarate		
Males				
Low Exposure	214.84	0.754	1434.36	8.16
Mid Exposure	645.58	0.258	2211.71	5.64
High Exposure	1037.46	0.016	3633.39	5.68
Females				
Low Exposure	143.73	0.271	781.77	8.04
Mid Exposure	708.21	0.137	2476.50	5.86
High Exposure	1141.04	0.137	4155.01	5.54

Stability and Homogeneity

Results of formulation analyses for dose concentration and stability of GFF pMDI were within the accepted criteria.

11 Integrated Summary and Safety Evaluation

GFF pMDI is a glycopyrrolate (quarternary ammonium antimuscarinic agent) and formoterol fumarate (β_2-adrenergic agonist) metered dose inhaler for the chronic, twice daily treatment [redacted] (b) (4) with COPD. The nonclinical safety assessment of GFF pMDI will be based upon NDA 20-831 and NDA 21-279 (Novartis, Foradil Aerolizer) as well as NDA 12-827 and NDA 17-558 (Robinul and Robinul Forte). GFF will be administered in a mixture of DSPC + $CaCl_2$ with HFA134a as the propellant.

DSPC + $CaCl_2$ are regarded as novel excipients for inhalation administration and require appropriate qualification. Over 40 drugs marketed in the US are formulated with DSPC-containing phospholipids, including 4 products approved for pulmonary routes of administration [redacted] (b) (4)

(b) (4) In addition, $CaCl_2$ is currently a component in an approved product, Pulmozyme, for administration by nebulization. (b) (4) Therefore, the sponsor is required to qualify DSPC + $CaCl_2$ as an excipient for the chronic inhalation route of administration.

GP is approved for both the oral and IV/IM routes of administration, but not for inhalation. GP is approved for oral administration up to 6 mg/day (Robinul and Robinul Forte label, NDA 12-827) and for IV/IM administration up to 0.8 mg/day for the treatment of peptic ulcers in adults (Robinul Injection label, NDA 17-558). Studies to evaluate the mutagenic and carcinogenic potential of GP have not been conducted. In reproduction studies in rats, GP decreased rates of conception in a dose-related manner. Studies in dogs suggest that this may be due to diminished seminal secretion which occurs at high doses of GP. GP also decreased pup survival in a dose-related manner. No teratogenicity was noted in rats or rabbits who received dietary GP doses of approximately 65 or 0.5 mg/kg/day, respectively. Glycopyrrolate is classified as a Pregnancy Category B drug (Robinul Injection label, NDA 17-558).

FF is approved for oral inhalation up to 24 µg/day for the long-term treatment of asthma and bronchoconstriction in patients with COPD (Foradil Aerolizer label, NDA 20-831 and NDA 21-279). FF was not mutagenic or clastogenic in the following tests: mutagenicity tests in bacterial and mammalian cells, chromosomal analyses in mammalian cells, unscheduled DNA synthesis repair tests in rat hepatocytes and human fibroblasts, transformation assay in mammalian fibroblasts, and micronucleus tests in mice and rats. The carcinogenic potential of formoterol fumarate has been evaluated in 2-year drinking water and dietary studies in both rats and mice. In rats, the incidence of ovarian leiomyomas was increased at doses of 15 mg/kg and above in the drinking water study and at 20 mg/kg in the dietary study, but not at dietary doses up to 5 mg/kg (AUC exposure approximately 450 times human exposure at the maximum recommended daily inhalation dose). In the dietary study, the incidence of benign ovarian theca-cell tumors was increased at doses of 0.5 mg/kg and above (AUC exposure at the low dose of 0.5 mg/kg was approximately 45 times human exposure at the maximum recommended daily inhalation dose). This finding was not observed in the drinking water study, nor was it seen in mice. In mice, the incidence of adrenal subcapsular adenomas and carcinomas was increased in males at doses of 69 mg/kg and above in the drinking water study, but not at doses up to 50 mg/kg (AUC exposure approximately 590 times human exposure at the maximum recommended daily inhalation dose) in the dietary study. The incidence of hepatocarcinomas was increased in the dietary study at doses of 20 and 50 mg/kg in females and 50 mg/kg in males, but not at doses up to 5 mg/kg in either males or females (AUC exposure approximately 60 times human exposure at the maximum recommended daily inhalation dose). Also in the dietary study, the incidence of uterine leiomyomas and leiomyosarcomas was increased at doses of 2 mg/kg and above (AUC exposure at the low dose of 2 mg/kg was approximately 25 times human exposure at the maximum recommended daily inhalation dose). Increases in leiomyomas of the rodent female genital tract have been similarly

demonstrated with other beta-agonist drugs. Reproduction studies in rats revealed no impairment of fertility at oral doses up to 3 mg/kg. FF has been shown to cause still birth and neonatal mortality at oral doses of 6 mg/kg and above in rats receiving the drug during the late stage of pregnancy. These effects, however, were not produced at a dose of 0.2 mg/kg. When given to rats throughout organogenesis, oral doses of 0.2 mg/kg and above delayed ossification of the fetus, and doses of 6 mg/kg and above decreased fetal weight. FF was not teratogenic in rats or rabbits following oral administration. FF is classified as a Pregnancy Category C drug (Foradil Aerolizer label, NDA 20-831 and NDA 21-279).

Toxicology studies (up to 14-days duration in rats and dogs) have been conducted with (b) (4) (GP pMDI) and (b) (4) (FF pMDI) and support clinical dosing with GP pMDI and FF pMDI up to 72 µg BID and 9.6 µg BID, respectively, for up to 14 days. Inhalation toxicology studies with GP pMDI monoproduct support GP administration by a novel route, and inhalation toxicology studies with FF pMDI FF administration by a new formulation/delivery system. Additional studies with GFF pMDI support a novel combination product. (b) (4) GFF pMDI (IND 107,739) for Agency discussion of (b) (4) combination product nonclinical development plans, including the DSPC + $CaCl_2$ qualification plan.

In the current IND 107,739 submission, the sponsor conducted two pivotal 14-day inhalation toxicity studies of GFF pMDI in rat and dog. In the rat 14-day inhalation toxicity study, rats received the GFF formulation at aerosol concentrations of 4.49-6.33 µg/L GP and 0.89-1.18 µg/L FF for 25, 60, or 90 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 90 min of exposure. An air control group that received filtered room air for 90 min was also included. The resultant average daily glycopyrrolate doses were 72, 226, and 368 µg/kg/day for low-, mid-, and high-dose males, respectively, and 77, 241, and 394 µg/kg/day for low-, mid-, and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 14, 42, and 69 µg/kg/day for low-, mid-, and high-dose males, respectively, and 15, 45, and 73 µg/kg/day for low-, mid-, and high-dose females, respectively. No dose-limiting toxicity or target organs of toxicity were identified. The high-dose was identified as the NOAEL. For the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 36.8/6.9 and 39.4/7.3 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 7.3/1.2 and 6.3/1.2 µg/kg lung weight/day for males and females, respectively.

In the dog 14-day inhalation toxicity study, dogs received the GFF formulation at aerosol concentrations of 5.15-7.79 µg/L GP and 1.00-1.34 µg/L FF for 10, 20, or 30 min of exposure for the low-, mid-, and high-dose groups, respectively. The placebo group received the vehicle (DSPC, $CaCl_2$, and HFA134a) for 30 min of exposure. An air control group that received filtered room air for 45 min was also included. The resultant average daily glycopyrrolate doses were 17.0, 51.3, and 73.8 µg/kg/day for low-, mid-, and high-dose males, respectively, and 17.3, 52.3, and 75.4 µg/kg/day for low-, mid-,

and high-dose females, respectively. The resultant average daily formoterol fumarate doses were 3.3, 8.8, and 12.5 µg/kg/day for low-, mid-, and high-dose males, respectively, and 3.4, 9.0, and 12.8 µg/kg/day for low-, mid-, and high-dose females, respectively. Test article-related clinical signs included erythema in 5/12 low-dose dogs (3 M, 2 F), 11/12 mid-dose dogs (5 M, 6 F), and 12/12 high-dose dogs (6 M, 6 F). Mean heart rates were increased for males and females in all dose groups after the first and last exposures. Following the first exposure, HR was increased by 44, 84, and 115% in low-, mid-, and high-dose animals (male and female average) versus placebo control. Following the second exposure, HR was increased by 31, 58, and 87% in low-, mid-, and high-dose animals (male and female average) versus placebo control. No rhythm abnormalities were observed. The target organs of toxicity were the heart and liver, which are characteristic for β_2 adrenergic agonists. In the heart, minimal to moderate fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 and 2/8 mid- and high-dose animals, respectively, at the end of the treatment period. These findings in dogs are generally attributed to increased heart rate that is monitorable in a clinical setting. At the end of the recovery period, minimal to mild fibrosis associated with the papillary muscle of the left ventricle was observed in 3/8 high-dose animals. In the liver, minimal to mild hepatocellular alteration was present in 8/8, 7/8, and 8/8 dogs in the low-, mid-, and high-dose groups. Affected hepatocytes were associated with the portal triad and periacinar regions. This finding resolved at the end of the recovery period. This finding (glycogen deposition), was attributed to the pharmacological action of formoterol and not judged as adverse. In the pancreas, minimal increased acinar cell death was observed in 1/4 placebo males and 2/4 high-dose males. Because microscopic evaluation of the pancreas was not performed for mid- and low-dose groups, an Information Request was sent to the sponsor via fax on June 28, 2010 to request evidence that this was an incidental finding. The sponsor replied on July 1, 2010 (SDN4). The Study Director for dog study FY09-087 consulted with an independent Board Certified Pathologist and two Board Certified Toxicologists, who agreed with the conclusions of the original study pathologist, (b) (4), DVM, PhD, DACVP. Dr. (b) (4) provided the following perspective in an additional signed statement:

> "Cell death (apoptosis) of individual acinar cells occurs in the pancreas of animals as a means of normal, physiological cell turnover… Minimally increased individual acinar cell death in 2/4 high-dose males was not interpreted to be a test article-related effect. Of consideration was that this finding was present in a control, that the difference in the incidence between high-dose males (2/4) and placebo-treated males (1/4) was not meaningful given the nature of the change and its minimal severity, and absence of this finding in the high-dose females."

Though the sponsor did not provide historical control evidence of this finding, the reviewer considers the sponsor's response to be adequate at this time. However, the sponsor's proposed 3-month inhalation toxicity study of GFF pMDI in dogs will be monitored for similar dose-related (i.e. increased incidence or severity versus air or placebo controls) pancreas findings.

A NOAEL was not identified in the 14-day dog toxicity study based upon histopathological findings in the liver at all doses; however, findings in the liver were not considered adverse. In addition, findings in the heart at the mid- and high-dose were judged to be monitorable in a clinical setting; therefore, the high-dose was used to determine safety factors. At the high-dose, pulmonary deposited doses for GP/FF calculated on a body weight basis were 18.5/3.1 and 18.9/3.2 µg/kg BW/day for males and females, respectively. Pulmonary deposited doses for GP/FF calculated on a lung weight basis were 2.0/0.4 and 2.1/0.4 µg/kg lung weight/day for males and females, respectively. GP systemic exposures for males and females at the high-dose were 17046 and 13437 pg*hr/mL, respectively. FF systemic exposures for males and females at the high-dose were 3633 and 4155 pg*hr/mL, respectively.

Safety margins for the highest proposed clinical doses of GP and FF are shown in the table below. Safety margins based upon µg/kg/day or µg/g lung weight/day were >10 and >6 for the rat and dog, respectively.

Table 21: Safety margin calculations for the highest clinical doses of glycopyrrolate and formoterol fumarate (GP/FF) pMDI at 72/9.6 µg (ex actuator) BID

Study	Sex	Safety margins for a maximum clinical dose of 144 µg/19.2 µg GP/FF			
		Pulmonary Deposited Dose (GP/FF)*		Pulmonary Deposited Dose (GP/FF)*	
		µg/kg/day	µg/g LW/day	2.9/0.4 µg/kg/day	0.144/0.0192 µg/g LW/day
14-day rat	M	36.8/6.9	7.30/1.28	12.7/17.3	50.7/66.7
	F	39.4/7.3	6.31/1.17	13.6/18.3	43.8/60.9
14-day dog	M	18.5/3.1	2.04/0.35	6.4/7.8	14.2/18.2
	F	18.9/3.2	2.11/0.36	6.5/8.0	14.7/18.8

*Pulmonary deposition factors for rats, dogs, and humans used in calculations were 10, 25, and 100%, respectively.

Safety margins for the highest proposed clinical doses of DSPC and $CaCl_2$ are shown in the table below. Safety margins, also adjusted for pulmonary deposited doses, based on µg/kg/day were >10 and >6 for the rat and dog, respectively.

Table 22: Safety margin calculations for the highest clinical dose of excipients, DSPC and $CaCl_2$

Study	Nonclinical doses of excipients		Safety margins for maximum clinical doses of excipients	
	DSPC (µg/kg/day)	$CaCl_2$ (µg/kg/day)	DSPC 17.9 µg/kg/day	$CaCl_2$ 1.3 µg/kg/day
14-day rat	262.9	18.3	14.7	14.1
14-day dog	129.0	9.0	7.2	6.9

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-107739	ORIG-1	PEARL THERAPEUTICS INC	GLYCOPYRROLATE FORMOTEROL FUMARATE INHALATION AEROSOL

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/s/

MARCIE L WOOD
07/08/2010

MOLLY E SHEA
07/08/2010

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LUQI PEI
03/10/2016

TIMOTHY W ROBISON
03/10/2016
I concur
Signing for Dr. Marcie Wood

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 208-294 **Applicant: Pearl Therapeutics** **Stamp Date: June 25, 2015**

Drug Name: Bevespi Aerosphere™ (glycopyrrolate/formoterol) **NDA/BLA Type: NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		No pharmacology, pharmacokinetics, carcinogenicity, or teratogenicity studies of glycopyrrolate (GP) were submitted per agreement at 12-APR-2010 Pre-IND meeting. The submitted studies (see section 4) are well organized for review.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		All agreed studies were submitted. They included a 6-mo inhalation toxicity study (ITS) of GP in rats, and qualification of excipient. Nonclinical requirement for a NDA submission was discussed in the pre-IND meeting held on April 12, 2010, in 107,739. A pre-NDA meeting was held on June 24, 2014, but there were no discussions on nonclinical requirements.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		The to-be-marketed formulation was tested in a 3-mo ITS in rats. The vehicle (porous particles) was tested in a 6-mo ITS in rats. Porous particles are comprised of DSPC and $CaCl_2$.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		The animals ITS and the to-be-marketed drug used the same route of administration (i.e., inhalation) to evaluate the general toxicity of the drug.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			Not applicable. No special studies were requested.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	x		The proposed labeling is in PLR format. Edits to the proposed labeling are needed.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	x		The applicant states that the impurity levels are below the ICH qualification threshold. New issues will be discussed with the CMC team upon consultation.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	x		Not applicable.
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	x		This is a 505(b)(2) application in which the applicant relies on the literature and NDA 17558 for pharmacology and reproductive toxicology data of glycopyrrolate. The application relies on the PK data from ITS to bridge the systemic exposures.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____YES____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

None

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LUQI PEI
08/13/2015

MARCIE L WOOD
08/13/2015