University of Reading

The Clinical Development of an Affordable Orally Inhaled Combination Product for the Treatment of Respiratory Diseases

A Thesis Submitted to the University of Reading in Partial Fulfilment for the Degree of Doctor of Philosophy

by

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August 2020

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AUTHOR'S DECLARATION

Declaration: I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Richard James Allan

ACKNOWLEDGEMENTS

I would like to thank my academic supervisors, Dr Nilesh Patel and Dr Sam Bizley for their support and guidance during the write-up of my thesis and their support in preparing me for my viva.

I would like to thank Dr Jon Ward for his support and guidance during the conduct of the work that formed the basis of my thesis and in particular for his support during my PhD and the encouragement for me to apply for a PhD in the first place.

I would like to thank my co-authors and colleagues at Mylan for all the work performed during the studies that formed the basis of my thesis; this was a huge team effort and would not have been possible without everyone. In particular, I would like to acknowledge Jon Ward, Scott Haughie, Dik Ng, Nolan Wood, Rachel Bramley and Bruce Williams.

I would like to acknowledge and thank my employer, Mylan as the sponsor of the studies that formed the basis of my thesis and for sponsoring my PhD itself.

I would like to thank my family for supporting me during my PhD, acknowledging that writing up my thesis has taken up family time which is always precious.

ABSTRACT

Generic alternatives to orally inhaled products (OIPs) to treat respiratory diseases are challenging to develop clinically, due to uncertainty of what methodology could be used to demonstrate bioequivalence (BE) and the relevance of traditional methods of demonstrating BE.

OIPs assert their pharmacological effect directly in the lung and have minimal systemic absorption, thus it is necessary to test the effect of these drugs in the lung to demonstrate that a generic product is BE to the reference product; this is termed local therapeutic equivalence (LTE).

This thesis describes the identification and testing of a clinical development pathway to demonstrate BE for a generic form (Wixela™ Inhub™) of Advair® Diskus® to provide a more affordable inhaled corticosteroid/ long-acting β_2-receptor agonist (ICS/LABA) to patients with respiratory diseases in the USA.

Two methodologies (F_{eNO} and methacholine challenge) were assessed to determine whether they could be used to prove LTE; however, upon completion of these studies it was ascertained that neither were suitable. Therefore, there was no suitable pharmacodynamic method available to demonstrate LTE.

The method utilised to provide LTE was a large study in asthma patients using lung function as the endpoint, i.e., if FEV_1 was the same between the test and reference products, the LTE would be proven. The lung function measures were bioequivalent and therefore, LTE for Wixela™ Inhub™ when compared to Advair® Diskus® was demonstrated.

Additionally, pharmacokinetic (PK) BE was demonstrated, measuring fluticasone propionate (FP) and salmeterol concentrations in plasma at each available dose strength following dosing of Wixela™ Inhub™ or Advair® Diskus®.

The successful completion of the clinical development program means that the development of a generic ICS/LABA is viable clinically, because of the demonstration of BE in both plasma and the lung. Therefore, this should increase the availability of affordable, quality medicines for patients with respiratory diseases.

DETAILS OF PUBLICATIONS SUPPORTING THE PhD

F_{eNO} Study - Local Therapeutic Equivalence - ICS

The F_{eNO} study is described in the following references:

Conference Abstract (American Thoracic Society 2017)

Allan R, Haughie S, Kerwin E, Ward, J. *A Randomized, Double-blind, Placebo-controlled, Three-way Crossover Incomplete Block Study to Assess the Dose Responsiveness of Exhaled Nitric Oxide to Advair® Diskus® in Asthmatic Subjects.* American Journal of Respiratory and Critical Care Medicine 2017; 195: A3195. https://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A3195

Full Paper

Allan R, Haughie S, Kerwin E, Ward J. *A Dose-Response Study to Examine the Methodology for Demonstrating the Local Therapeutic Equivalence of the Fluticasone Propionate Component of an Orally Inhaled Combination Therapy of Fluticasone Propionate/Salmeterol Dry Powder.* Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2019 Dec;32(6):364-73.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2018.1520

Extent of Contribution to Research

Richard Allan designed the study, wrote the Clinical Protocol, led the Sponsor team conducting the study, trained external Clinical Investigators in the study procedures (study procedures involving human subjects were performed by the external Clinical Investigators), oversaw the study and, was actively involved in analysis, interpretation and reporting of the data from the study. Richard Allan was the primary author of the paper. Additionally, Richard Allan presented the data at the American Thoracic Society meeting in 2017.

Statistical analyses were performed by Scott Haughie.

Methacholine Challenge Study – Local Therapeutic Equivalence - LABA

The methacholine challenge study is described in the following references:

Conference Abstract (American Thoracic Society 2017)

Allan R, Haughie S, Ahrens RC, Ward, J. *A Randomized Double-blind Placebo- and Active-controlled Five-way Crossover Study to Assess the Dose Responsiveness of Methacholine-induced Bronchial Hyperreactivity to Single Inhaled Doses of Advair® Diskus® in Adult Asthmatics.* American Journal of Respiratory and Critical Care Medicine 2017; 195: A3196. https://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A3196

Full Paper

Allan R, Haughie S, Ahrens R, Singh S, Ward J. *A Dose-Response Study Examining the Use of Methacholine Challenge to Demonstrate Local Therapeutic Equivalence of the Salmeterol Component of Generic Inhaled Fluticasone Propionate/Salmeterol Combination Products.* Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2019 Dec;32(6):352-63.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2018.1519

Extent of Contribution to Research

Richard Allan designed the study, wrote the Clinical Protocol, led the Sponsor team conducting the study, trained external Clinical Investigators in the study procedures (study procedures involving human subjects were performed by the external Clinical Investigators), oversaw the study and, was actively involved in analysis, interpretation and reporting of the data from the study. Richard Allan was the primary author of the paper. Additionally, Richard Allan presented the data at the American Thoracic Society meeting in 2017.

Statistical analyses were performed by Scott Haughie.

Local Therapeutic Equivalence Study - Asthma

The local therapeutic equivalence (LTE) study is described in the following references:

Conference Abstract (ATS 2019)

Allan R, Kerwin EM, White MV, Miller SD, Haughie S, Ward JK, Ng D. *Pulmonary Therapeutic Bioequivalence of Wixela™ Inhub™ and Advair® Diskus® in Adults With Asthma.* American Journal of Respiratory and Critical Care Medicine 2019; 199: A2205. https://doi.org/10.1164/ajrccm-conference.2019.199.1_meetingabstracts.a2205

Full Paper

Ng D, Kerwin EM, White MV, Miller SD, Haughie S, Ward JK, Allan R. *Clinical Bioequivalence of Wixela Inhub and Advair Diskus in Adults With Asthma*. Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2020 Apr;33(2):99-107.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2019.1547

Extent of Contribution to Research

Richard Allan designed the study, wrote the Clinical Protocol, led the Sponsor team prior to clinical conduct of the study, developed training materials to train external Clinical Investigators in the study procedures (study procedures involving human subjects were performed by the external Clinical Investigators) and, was actively involved in analysis, interpretation and reporting of the data from the study. Additionally, Richard Allan presented the data at the American Thoracic Society meeting in 2019.

Statistical analyses were performed by Scott Haughie.

PK Bioequivalence Studies

The PK bioequivalence studies are described in the following publications:

Conference Abstract (ATS 2019)

Ward JK, Wood N, Allan R, Haughie S. *Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses of Advair Diskus® and Wixela™ Inhub™: Results of 3 Pharmacokinetic Equivalence Studies.* American Journal of Respiratory and Critical Care Medicine 2019; 199: A2208. https://doi.org/10.1164/ajrccm-conference.2019.199.1_meetingabstracts.a2208

Full Paper

Haughie S, Allan R, Wood N, Ward J. *Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses from Advair Diskus and Wixela Inhub: Results of Three Pharmacokinetic Bioequivalence Studies.* Journal of Aerosol Medicine and Pulmonary Drug Delivery. 2020 Feb;33(1):34-42.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2019.1537

Extent of Contribution to Research

Richard Allan designed the studies, wrote the Clinical Protocols, led the Sponsor team conducting the studies, trained external Clinical Investigators in the study procedures (study procedures involving human subjects were performed by the external Clinical Investigators), oversaw the studies and, was actively involved in analysis, interpretation and reporting of the data from the studies. Richard Allan was the co-primary author of the paper with Scott Haughie.

Statistical analyses were performed by Scott Haughie

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ABBREVIATIONS

AUC	Area Under The Curve
BE	Bioequivalence
BID	Twice Daily
BMI	Body Mass Index
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
C_{max}	Maximal Concentration
CV	Coefficient of Variation
DPI	Dry Powder Inhaler
E_0	Basal (placebo) Effect
ED_{50}	Dose that produces half E_{max}
E_{max}	Maximal Effect above E_0
FDA	Food and Drug Administration
F_{eNO}	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume In One Second
FP	Fluticasone Propionate
GMR	Geometric Mean Ratio
GINA	Global Initiative For Asthma
GOLD	Global Initiative For Chronic Obstructive Lung Disease
GSK	GlaxoSmithKline
ICS	Inhaled Corticosteroid
LABA	Long-Acting β_2-Receptor Agonist
Log_e	Natural Log
LS	Least Square
LTE	Local Therapeutic Equivalence
pMDI	Pressurised Metered Dose Inhaler
µg	Microgram
mL	Millilitre
N/A	Not Applicable
OIP	Orally Inhaled Product
PC_{20}	Provocative Concentration Causing a 20% Decrease in FEV_1
pg	Picogram
PK	Pharmacokinetic
PPB	Parts Per Billion
QD	Once Daily
RP	Reference Product
SABA	Short Acting β_2-Receptor Agonist
SD	Standard Deviation
SE	Standard Error
TP	Test Product
WS-SD	Within Subject Standard Deviation
WSV	Within Subject Variance
USA	United States of America

1 CONTEXTUAL CHAPTER

1.1 Project Aim

The clinical development of generic alternatives to currently available orally inhaled medicines, is challenging due to the need to demonstrate treatment equivalent effects in the lung as well as demonstrating systemic pharmacokinetic (PK) bioequivalence (BE).

This is particularly the case for inhaled corticosteroids/ long-acting β_2-receptor agonist (ICS/LABA) combination products meaning that despite the loss of patent exclusivity of medicines such as Advair® Diskus®, limited generic alternatives are currently available, particularly in the USA. Therefore, the price paid by patients and payors is still high. Wixela™ Inhub™ was the first generic orally inhaled dry powder product to achieve approval in the USA; however, a suitable regulatory pathway needed to be defined and tested for this medicine, which is described here.

1.2 Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are common respiratory diseases, with a significant morbidity and mortality and are prevalent throughout the World. The global prevalence of asthma is estimated at approximately 358 million people and COPD is estimated at approximately 174.5 million people with estimated deaths of 0.4 million and 3.2 million people related to asthma and COPD respectively [1].

Asthma and COPD are routinely treated with orally inhaled bronchodilators such as salbutamol (a short-acting β_2-receptor agonist), salmeterol or formoterol (LABA) or ipratropium (a short-acting muscarinic-receptor antagonist – SAMA), tiotropium (a long-acting muscarinic-receptor antagonist – LAMA) and ICS such as fluticasone propionate (FP) or budesonide. Herein, these will also be referred to as orally inhaled products (OIPs). These drugs are used alone or in combination as per the Global Initiative for Asthma (GINA) [2] and Global Initiative for Chronic Obstructive Lung Disease (GOLD) [3], which are also consistent with UK NICE Guidance for asthma and COPD [4, 5]. The use of bronchodilators leads to a relaxation of the bronchial smooth muscle, resulting in reduced symptoms such as dyspnoea in patients. Corticosteroids have a different effect, acting as an anti-inflammatory agent, reducing the abnormal inflammation associated with asthma and COPD, which in turn reduces the risk of acute exacerbations and progression of these diseases.

Bronchodilators and corticosteroids are mainly delivered to the lung using inhalers, which are most often used by patients as single inhalers. However, this can sometimes lead to problems with medicines adherence because patients are using multiple devices and may have poor inhaler technique [6]. The combination of bronchodilators and corticosteroids in a single inhaler is convenient to patients as this only requires them to use one inhaler to deliver drugs that work in a complimentary manner in the lungs and are therefore a major component of therapy in respiratory diseases. The use of a single combination inhaler is also associated with lower healthcare resource utilisation and improved cost-effectiveness compared with multiple inhalers [7].

Orally inhaled products are specifically used for the treatment of respiratory diseases because they target the site of the disease activity, i.e., the lung. They can directly deposit the drug in the lung using small doses leading to greater efficacy due to this direct effect. There is also minimal subsequent systemic exposure, reducing the potential for significant adverse effects such as those to the cardiovascular system and the adrenal cortex if they are administered systemically.

Whilst it is clear that orally inhaled products offer an advantage to patients by targeting the lung and minimising systemic adverse events, the use of the correct drug and inhaler is important. OIPs are typically administered using a pressurised metered dose inhaler (pMDI), breath actuated inhaler, or a dry powder inhaler (DPI). A review of inhaler types demonstrated that there was no systematic difference in treatment effects of different inhaler devices [8]. However, the prescribing of a specific inhalation device is often determined by a combination of availability, cost, patient preference and importantly a patient's likely ability to use the inhalation device. As an example, pMDIs, which primarily consist of an aerosol containing the drug and a mouthpiece to direct the drug towards the airway, whilst regularly prescribed to patients, require coordination of inhaling and actuation of the device, or the use of a spacer which is bulky. This potentially leads to poor inhaler technique and thus reducing the patient's ability to take a prescribed medicine appropriately, leading to reduced efficacy of the drug. In contrast, most DPIs do not require such coordination as the device is activated e.g., by pressing a lever and simply inhaling, but without the need to perform these steps simultaneously, and thus may be preferred by patients [9]. If the patient can generate sufficient inspiratory effort to aerosolise the powder in the inhaler, they will likely receive a consistent dose of drugs. Modern dry powder inhalers such as the Diskus®,

Ellipta® or Turbohaler® can be used by most patients [10-12] and drugs delivered using DPIs have become widely prescribed to treat asthma and COPD.

Combination products of ICS and LABA such as Advair® and Symbicort® are routinely used as maintenance therapy to treat both asthma (Step 3 per GINA [2], i.e., for patients that do not achieve control with a low dose ICS) and COPD (Group D per GOLD [3], i.e., for patients with a prior history of exacerbations and symptoms, particularly if they have high eosinophils). Formoterol containing ICS/LABA combinations are also used in asthma as a combined maintenance and rescue medication (Single Maintenance and Reliever Therapy - SMART) in some countries [4].

Advair® Diskus®, the combination of FP and salmeterol administered using a DPI (known as Seretide® Accuhaler® in UK) [13] was launched in 1999 and is one of the most frequently prescribed medications for treating asthma and COPD.
Advair® Diskus® had Global sales of £2.44 billion in 2018 [14] and £1.73 billion in 2019 [15], which was after the introduction of generic competition in the USA in early 2019.

Advair® Diskus® is available in the USA in three strengths (100/50, 250/50 and 500/50 µg), described according to the variable nominal FP dose and acknowledging the 50 µg dose of salmeterol in each product.

In countries such as the UK where the NHS negotiates the price of drugs with manufacturers, patients do not pay the full cost of medications or large co-pays (a payment made by a patient towards the cost of the medication, even if they have insurance coverage). As a result it is relatively easy for prescribers to follow treatment guidelines such as those set by GINA [2], GOLD [3] or NICE [4, 5] based on clinical need rather than having to consider a patient's ability to pay for their medication. It is noteworthy that Advair® Diskus® has a reported list price of up to $550.40 in the USA for a month's supply [16]. In contrast, the cost of Seretide® in the UK is £32.74 for a month's supply, demonstrating the impact that price negotiations by a single payor can have on the cost of drugs.

Asthma management per guidelines is poor in the USA. Nearly 90% of people with asthma have disease that is considered mild persistent asthma or worse [17]. If all patients with asthma were receiving guideline-directed treatment, 90% would be using daily controller medication, such as an ICS; however, only 22% of surveyed patients

with asthma were using daily long-term controller medication [18]. It is a similar case for prescribing in COPD with low levels of prescribing of maintenance therapy to patients [19]. Non-adherence to medication is an issue due to multiple factors, including a perception from the patient that they do not need the treatment or concerns about adverse events [20]. In some countries such as the USA where patients pay a large proportion of the cost of medication, an additional, significant factor in the non-adherence to respiratory treatments is the cost of treatment to a patient [21-24]. The high cost of respiratory treatments is problematic to individual patients who may experience a greater symptom burden as a result of treatment non-adherence. It is also an issue for the wider society with regards to increased healthcare utilisation and the costs associated with this. Specifically, non-adherence to medications to treat asthma and COPD are linked with increased adverse outcomes for patients and increased overall costs to health care systems [25].

With the high prevalence of, and high morbidity/mortality of asthma and COPD and a significant cost to both individual patients and society associated with these diseases, effective and affordable medication are clearly required by patients and providers. One way to address this is the development of generic alternatives. The clinical development of generic alternatives to currently available orally inhaled medicines is challenging due to the need to demonstrate equivalent treatment effects in the lung as well as demonstrating systemic PK BE. This complexity of development is particularly the case for ICS/LABA combination products as BE has to be demonstrated for both drugs in the combination. This means that despite the loss of patent exclusivity of medicines such as Advair® Diskus®, limited generic alternatives are currently available, particularly in the USA, therefore, the price paid by patients and payors is still high.

The pricing of the first US generic of Advair® Diskus® (Wixela™ Inhub™) is significantly cheaper than the originator, with a list price of $182.88 in the USA for a month's supply [26], demonstrating the impact that a generic equivalent of an inhaled product can have on access to medicines in a market that is not managed by a single payor. However, as Wixela™ Inhub™ was the first generic orally inhaled dry powder product to achieve approval in the USA, a suitable regulatory pathway needed to be defined and tested and is described here.

Originator products are developed over many years, requiring the demonstration of efficacy and safety in hundreds or thousands of patients. Generic products are

developed to have the same effects as the originator product and normally for drugs formulated as oral solid dosage forms this can usually be demonstrated clinically in a small number of healthy volunteers [27]. Unlike most oral solid dosage forms of drugs that have a clear clinical pathway to approval for a generic form, based on well-established regulatory guidelines from agencies such as the US FDA [27], for OIPs it is more difficult to prove equivalence. Typically, generic versions of oral solid dosage forms of drugs can be clinically tested and subsequently approved by regulatory authorities based on PK BE. These drugs are systemically absorbed, and the target organ is usually within the systemic compartment, hence systemic exposure (usually measured in plasma) of most oral drugs is a meaningful surrogate for both safety and efficacy. However, OIPs require a different clinical development strategy due to the low systemic exposure associated with their dosing and the target organ being the lungs. Therefore, systemic exposure is not considered a suitable surrogate for efficacy and safety alone by some regulatory authorities, such as the US FDA, Health Canada and the Japanese PMDA. These particular regulatory authorities require a measure of local therapeutic equivalence (LTE), i.e., the treatment effect in the lung.

Prior to completion of the studies described in Section 1.4.1 and Section 1.4.2 below, and subsequent discussions with the FDA, no written regulatory guidance was available from the FDA to provide a pathway to develop a generic ICS/LABA product.

Despite the clear need for affordable OIPs, the standards required to demonstrate that a generic alternative is bioequivalent to the originator product need to remain high in order to assure patients and prescribers that a generic product will provide the same level of efficacy and safety as the originator's product. Therefore, a suitable and robust strategy to develop generic alternatives of orally inhaled ICS/LABA products was necessary and is described herein.

The aim of the project, and the basis of this thesis was to identify and test a clinical development pathway to demonstrate bioequivalence for a generic form (Wixela™ Inhub™) of Advair® Diskus® to provide a more affordable form of ICS/LABA to patients with asthma and COPD in the USA.

The following sections summarise the rationale and findings from the studies included in the thesis, specifically Section 1.3 and Section 1.4 for PK BE and LTE, respectively.

1.3 Pharmacokinetic Bioequivalence

The aim of the PK BE studies was to demonstrate systemic equivalence between Wixela™ Inhub™ (the generic product) and Advair® Diskus® (the originator product) by measuring plasma concentrations of FP and salmeterol in healthy subjects.

PK BE is the cornerstone of demonstrating clinically that a generic alternative to the originator's product is appropriate and that a generic product will deliver the same efficacy and safety profile. However, it is acknowledged that for an OIP this is not a fully adequate demonstration of BE, as the systemic exposure for most OIPs is primarily via lung absorption and the systemic exposure does not drive the efficacy of the product.

Nonetheless, PK BE does provide at least some assurance that a generic OIP will likely have the same risk profile as the originator's product. As the adverse effects of many inhaled products are associated with the extent of systemic exposure, PK BE does act as a suitable surrogate for the safety of the generic product in addition to the direct safety data generated during clinical studies. By demonstrating equivalent exposure, it is an appropriate method of indirectly demonstrating that a generic ICS/LABA would have a similar safety profile to an originator's product. This is particularly true of OIPs that have poor systemic bioavailability such as FP [13] as the measured systemic exposure would be almost entirely related to the lung dose of the drug.

Given the challenges of studying the systemic exposure of drugs administered from OIPs, there are a number of key considerations to make when designing PK BE studies for these products, including the choice of population, the PK sampling times and assay sensitivity.

For most oral products, e.g., tablets, healthy volunteers can be used unless there is a specific difference in absorption, distribution and metabolism in patients vs., healthy subjects. Patients with respiratory diseases have differences in their lungs vs., healthy subjects [28], therefore this needs to be properly considered. Patients with asthma are a heterogeneous population with, by definition, variable airway obstruction whose airway calibre, potentially influencing the degree of inhaled drug deposition and distribution in the airways, vary broadly among individuals and within an individual from week to week. This would be of concern because the clinical phase of these studies would run over 2-7 weeks to enable the study of both test and reference products and ensure a

washout between study periods. To maintain consistent lung function, patients with asthma would need to continue their medication (e.g., ICS/LABA and SABA) during the study which may directly impact the measurement of systemic drug concentrations, if the subject is taking the same medication as the test and reference products. Any asthmatic episode or use of a rescue medication could also significantly affect a subject's PK data.

Prior publication has shown that plasma concentrations of the inhaled corticosteroids budesonide and FP are significantly lower (up to a 60% decrease) when a patient has reduced lung function caused by methacholine-induced bronchospasm [29]. Therefore, any changes in lung function between study visits would confound the data and make any bioequivalence assessment invalid. Furthermore, an increase in symptoms could lead to discontinuation from the study and loss of the subject from the study before completion of all periods, therefore meaning their data cannot be fully utilised, increasing the variability of the overall data from the study. To have the best chance of maintaining constant lung function over 2-7 weeks and minimising the chance of the need for maintenance or rescue medication, volunteer selection would favour the mildest and most stable patients with asthma. However, patients with mild asthma that are clinically stable would have lung function ≥80% of predicted, which is comparable to using healthy subjects [30], and would not therefore be the population in whom the test or reference products would be used per guidelines. A similar argument can also be made for patients with COPD for whom a prolonged period of not taking regular medications to treat their COPD would likely have detrimental effects on their disease status unless they have particularly mild symptoms with near normal lung function.

Based on the above considerations, healthy subjects are considered a more sensitive population to detect product differences and therefore, healthy subjects were selected for the PK BE studies [31]. In summary:

- Healthy subjects are a more homogenous population and are naïve to drugs administered in these studies.
- Healthy subjects are better able to inhale the medication than patients with asthma and the resulting systemic drug levels are higher [32]. As healthy subjects deposit / absorb the drugs more effectively in / from the lungs than do patients with asthma, plasma exposure is therefore higher with any given

dose, increasing study sensitivity and allowing PK to be compared at a dose that is not a large excess of the clinical dose.

- Since bioequivalence is relative and depends on the same baseline subject characteristics from week to week, a better and more reliable comparison could be obtained with healthy subjects.
- Comparing PK following inhalation from different FP DPIs, bioavailability has been shown to be independent of study population (i.e., patient or healthy subjects), as the relative bioavailability between the two devices in healthy subjects and patients was the same [33].

As it is important to fully describe the PK profile of both the generic and originator's products in the BE studies to demonstrate that they are the same, it is necessary to ensure that sampling times are sufficient for the anticipated measurable systemic exposure and ensuring that this will cover the duration of action of the drug. For a twice daily administration such as FP/salmeterol combinations, sampling over a 48 hour period post dose is likely sufficient. However, other drugs such as once daily bronchodilators may require a longer sampling time, up to 72 or 96 hours and the design of the study should be adjusted accordingly.

A robust, discriminatory and sensitive analytical method needs to be in place to measure the systemic concentrations in plasma of an OIP, this is particularly important for combination products such as FP and salmeterol. The lower limit of quantification (LLOQ) of the assay needs to be appropriate to detect the drug for the duration of sampling during the study. For a product such as FP and salmeterol this should be ≤1 pg/mL for each analyte, reflecting the low systemic exposure of these drugs. As OIPs are designed such that they have limited systemic exposure it may be necessary to administer more than one dose of study drug at a time to ensure that systemic exposure is sufficient to be measured. In the PK BE studies described in this thesis, three inhalations of each of the study drugs were administered and this ensured that the concentrations were sufficiently high that they could be measured throughout the study periods. This enabled a full description of the PK profile (describing both maximal concentration – C_{max} and overall exposure – area under the curve [AUC]) of both components of the drug and ensured that the variability of the data was sufficiently low (CV <30%), such that the studies were practical to conduct. In addition, for a generic alternative to an orally inhaled ICS/LABA combination product, systemic PK BE must be demonstrated at all the approved dose strengths, i.e., 100/50, 250/50 and 500/50 µg

for Advair® Diskus® to provide assurance to patients and prescribers that the systemic exposure to both FP and salmeterol are equivalent at each approved dose.

1.3.1 Results and Discussion

Each of the three PK studies demonstrated that the systemic exposure following dosing from the generic product Wixela™ Inhub™ was similar to Advair® Diskus® for both FP and salmeterol at each dose strength and for both the maximum concentration (C_{max}) and overall concentration (AUC).

This demonstrated that Wixela™ Inhub™ was bioequivalent to Advair® Diskus®, as summarized in Table 1.1.

Table 1.1: PK Bioequivalence Studies - Summary of Bioequivalence and FP and Salmeterol Plasma PK Parameters

Treatment	$AUC_{(0-t)}$ (pg·h/mL)	$AUC_{(0-t)}$ TP/RP ratio (90% CI)	C_{max} (pg/mL)	C_{max} TP/RP ratio (90% CI)
100/50 Study FP/salmeterol (Total Dose 300/150 µg) (N=64)				
FP				
Wixela™ Inhub™	600.3	1.04 (1.00, 1.08)	103.7	0.92 (0.87, 0.96)
Advair® Diskus®	576.4	N/A	112.9	N/A
Salmeterol				
Wixela™ Inhub™	696.4	1.08 (1.04, 1.11)	347.7	1.00 (0.94, 1.04)
Advair® Diskus®	644.9	N/A	348.3	N/A
250/50 Study FP/salmeterol (Total Dose 750/150 µg) (N=61)				
FP				
Wixela™ Inhub™	1251	1.07 (1.02, 1.13)	164.2	1.01 (0.95, 1.07)
Advair® Diskus®	1164	N/A	162.7	N/A
Salmeterol				
Wixela™ Inhub™	641.2	1.03 (0.99, 1.07)	296.2	0.93 (0.87, 1.00)
Advair® Diskus®	623.3	N/A	317.4	N/A
500/50 Study FP/salmeterol (Total Dose 1500/150 µg) (N=65)				
FP				
Wixela™ Inhub™	2689	0.97 (0.92, 1.00)	252.8	0.90 (0.86, 0.93)
Advair® Diskus®	2783	N/A	281.8	N/A
Salmeterol				
Wixela™ Inhub™	672.3	1.00 (0.96, 1.04)	334.9	0.86 (0.81, 0.91)
Advair® Diskus®	670.8	N/A	388.6	N/A

Data presented as natural-log transformed geometric mean (based on least squares mean).
Abbreviations: $AUC_{(0-t)}$ = area under the concentration-time curve from time zero to the last quantifiable concentration; CI = confidence interval; C_{max} = maximum plasma concentration; N/A = not applicable; RP=reference product (Advair® Diskus®); TP = test product (Wixela™ Inhub™).

The design of the PK BE studies reflected a traditional BE design, i.e., 2-way crossover of one batch of test vs., one batch of reference product; this was possible due to a clear understanding of the *in vitro* characteristics of both the test and reference FP/salmeterol formulations. Other groups have postulated that it is not possible to demonstrate PK

bioequivalence of one batch vs., one batch of test and reference products due to the inherent variability of Advair® Diskus® [34]. However, the PK BE studies demonstrated that it is possible if both the test and reference products are well characterised and appropriate batches of the two products are selected prior to conduct of the clinical study. The clear understanding of these characteristics was also key to achieving *in vitro* equivalence for the product [35].

The demonstration of PK BE for both FP and salmeterol at all of the approved dose strengths indicated that Wixela™ Inhub™ was bioequivalent to Advair® Diskus®. Patients will receive the same systemic exposure from the generic product as the originator and therefore the same safety profile can be assumed.

1.4 Local Therapeutic Equivalence

As the PK BE data are not considered by all regulatory authorities to fully demonstrate the equivalent efficacy of generic and originator OIPs, there is a need to demonstrate that the constituent parts of the generic product exert the same effect as the originator product (e.g., Advair® Diskus® in this case) in the lungs.

One potential methodology would be to utilise imaging technologies to compare the generic product to the originator. It is possible to label an OIP with technetium to explore the lung deposition of a drug using scintigraphy [36]. With labelled drugs it is possible to ascertain where in the lung the drug is deposited [37] and a comparison of two drugs could theoretically be made based on their relative deposition. It is, however, very challenging/impossible to label in a manner that guarantees that the aerodynamic properties of the products are entirely unaffected, therefore a labelled drug may not be representative of deposition of a non-labelled drug. This would also mean that the absorption and or efficacy of a drug might be different to a non-labelled drug. Additionally, as the process to label the originator's reference drug would require the commercial inhaler to be taken apart and the powder removed to be labelled before reassembly, this further increases the likelihood of altering the properties of the product. As the *in vitro* properties, and hence the manner in which a drug performs when aerosolised is critical to the deposition (and subsequent absorption) in the lung, scintigraphy studies are unlikely to be a sensitive, reliable and appropriate manner of demonstrating lung bioequivalence.

For most OIPs, the US FDA typically requires a test of LTE to demonstrate comparable efficacy in addition to systemic PK BE to act as a surrogate for safety. LTE is an assessment of the pharmacodynamic properties or efficacy of the drug in the lungs to demonstrate that the generic product has the same efficacy as the originator reference product. The LTE should ideally include an assessment of dose-response [38, 39], whilst remaining within the approved daily dose range of the product. The inclusion of more than one dose of reference product is preferred, because a dose-scale analysis of relative potency (RP) (the ability to demonstrate that the test product of unknown potency can produce the same effect as the reference product under the same conditions) can be more sensitive than a more simple response-scale analysis which only compares one dose of the generic product with one dose of the reference product. However, the ability to demonstrate LTE using a dose-scale analysis poses significant difficulties, such as the need to establish clinical methods that can show a dose-response for the reference product while staying within the clinically approved dose range.

The assessment of treatment effects in the lung for a combination product such as Advair® is further complicated by the differing pharmacological actions of the constituent parts, i.e., an ICS and a LABA so methods that discriminate between the two drugs that form the combination product would be required to test the independent effects of the drugs.

1.4.1 Local Therapeutic Equivalence – ICS

1.4.1.1 Background

The aim of the ICS study was to identify a methodology that could be utilized to demonstrate LTE for the ICS component of a generic version of Advair® vs., the originator. To meet the requirements of the FDA it would be necessary to identify a methodology that would discriminate between the effect of the ICS and the LABA and demonstrate a dose-response. The dose-response would need to be large and the variability of the study would need to be low for the methodology to be suitable for a future LTE study with a feasible sample size.

The anti-inflammatory effect of the ICS component is chronic in nature, requiring regular dosing over a period of time, thus determination of LTE of this component requires multiple-dose studies. Crossover designs when requiring multiple doses can be lengthy for individual subjects. This is particularly challenging in a disease such as

asthma that is characterised by variation in disease status unless well-controlled by pharmacological treatment. In addition, demonstration of a dose-response for ICS is extremely challenging. The dose-response relationship of most ICS products at therapeutic doses is relatively flat, especially when measured outside of an individual subject, e.g., with different doses being compared between two or more populations of asthmatics within a parallel group setting [40, 41].

The literature regarding the ICS dose-response assessment includes clinical efficacy studies as well as pharmacodynamic studies utilising challenge models, such as allergen or adenosine monophosphate [42-44], inflammatory markers, including sputum eosinophilia [43] and fractional exhaled nitric oxide (F_{eNO}) [45]. These different methods all largely include the study of doses of ICS that are lower than recommended doses for treating asthma and even then, the dose-response between these low doses and high doses is not clear in most of the studies.

Most methods described in the literature are unlikely to deliver an appropriate dose-response for an ICS, especially using clinically approved doses. One methodology of interest was F_{eNO}, which is a measure of allergic/eosinophilic inflammation, measured by chemiluminescence in exhaled breath. F_{eNO} is typically raised in asthmatic patients, reflecting the patient's state of allergic inflammation. A previous publication demonstrated that F_{eNO} could distinguish between 100 and 800 µg/day beclometasone dipropionate (BDP) [46]. This was therefore considered the most likely methodology to be successful. These data are consistent with a number of other publications [43, 45] which also demonstrate an ICS dose-response using F_{eNO} as the study endpoint. The dose-response is further pronounced in patients with marked lung inflammation as determined by elevated F_{eNO} (>100 ppb). As F_{eNO} is unaffected by bronchodilators such as LABA [47, 48] this provided further promise for this methodology as it would be possible to assess the pharmacodynamic effect of the ICS component without interference from the LABA component. However, the doses of ICS utilised in the prior literature that demonstrated a dose-response include doses of ICS that are lower than those equivalent to FP 100 µg taken BID (e.g., 50-100 µg/day BDP [45, 46], or 100 µg/day budesonide [43]) as per the asthma indication for Advair® Diskus®. Prior to the study conducted [49] no one had rigorously examined the dose-response relationship of the FP component of a FP/salmeterol combination product in asthmatic patients using F_{eNO}; hence there was uncertainty as to whether this would be a suitable method of demonstrating LTE.

1.4.1.2 Results and Discussion

The study demonstrated that all Advair® Diskus® treatments decreased F_{eNO} compared with placebo (Table 1.2). The largest treatment effects were noted after 14 days of treatment, reflecting that the maximal effect required multiple doses of the ICS to reduce inflammation in the lung. However, as the treatment effects were similar for all the BID treatment arms, a dose-response was only clearly identified between QD Advair® and the BID treatment arms. The subsequent sample size estimates for an LTE study using F_{eNO} were very large.

Table 1.2: Local Therapeutic Equivalence ICS Study – Change in F_{eNO50} From Day 1 to Day 14

	Advair® (Total Daily FP dose)				
	Placebo BID (0 µg)	**Advair® 100/50 µg QD (100 µg)**	**Advair® 100/50 µg BID (200 µg)**	**Advair® 250/50 µg BID (500 µg)**	**Advair® 500/50 µg BID (1000 µg)**
Day 1					
Mean	61.8	73.8	72.0	80.4	64.2
Geometric mean	54.4	69.0	64.9	71.5	59.8
Day 14					
Mean	59.0	42.4	27.9	30.6	32.4
Geometric mean	49.4	36.8	24.0	25.5	25.7
Change from day 1 to day 14					
Mean change	-2.7	-31.5	-46.3	-49.8	-31.9
GMR	0.91	0.53	0.36	0.36	0.43
(% change)	(-9%)	(-47%)	(-64%)	(-64%)	(-57.0%)

Data are shown as ppb unless otherwise specified.
F_{eNO50}, fractional exhaled nitric oxide measured at a flow rate of 50 mL/second.

Linear regression analyses revealed a significant dose-response slope, with the steepest part of the slope between the 100 and 200 µg FP/day (i.e., Advair® Diskus® 100/50 µg QD vs., Advair® Diskus® 100/50 µg BID) dose levels (slope, -0.0039, p=0.020).

A three-parameter E_{max} model analysis (Table 1.3) indicated that the F_{eNO50} response plateaued at approximately 200 µg FP/day (Advair® Diskus® 100/50 µg BID), with an estimated ED_{50} of 69.04 µg FP/day.

Table 1.3: Local Therapeutic Equivalence ICS Study - F_{eNO50} Dose-Response Analysis Using an E_{max} Model

	Estimate F_{eNO50} (ppb)*	Estimate Ln Change from Day 1 F_{eNO50} (ppb)	Approximate Standard Error	95% CI
Change from Day 1 at Day 14				
E_0	-7.01	-0.12	0.12	-0.36, 0.13
E_{max}	-33.46	-0.89	0.13	-1.16, -0.62
ED_{50} (Total Daily Dose µg)		69.04	39.53	-11.48, 149.56
	Mean			
Placebo	-7.01	0.89	1.13	0.70, 1.13
Advair® 100/50 µg QD	-30.25	0.53	1.12	0.42, 0.66
Advair® 100/50 µg BID	-34.49	0.46	1.10	0.38, 0.56
Advair® 250/50 µg BID	-37.10	0.41	1.10	0.34, 0.49
Advair® 500/50 µg BID	-39.10	0.39	1.11	0.32, 0.48
Maximum (E_0+E_{max})	-40.47			

*Assuming a Day 1 (Baseline) mean of 63.8 ppb
E_0 - Basal (placebo) Effect, E_{max} - Maximal Effect above E_0, ED_{50} - Dose that produces half E_{max}.

As many factors as were feasible to increase the treatment effect and reduce variability were incorporated into the design of the F_{eNO} study [49]. These included restricting the study to subjects with raised F_{eNO} (≥45 ppb) at baseline, ensuring that the F_{eNO} variability was minimised (CV of within ±23% of the screening value at each treatment period) and ensuring that subjects were clinically stable in the absence of asthma-controller medications such as ICS or leukotriene receptor antagonists.

Only a shallow dose response was observed between BID doses of Advair® Diskus® in the study. If a lower dose of Advair® Diskus®, incorporating a 50 µg BID dose was available (c.f., the approved dose of FP as monotherapy, e.g., Flovent®/Flixotide®) then F_{eNO} might be a feasible methodology, as the ED_{50} (the dose that produces 50% of the maximum effect) estimated is 69 µg of FP (total daily dose). This is consistent with a study that demonstrated a difference between 50 µg BID and 250 µg BID Flixotide®, using F_{eNO} [50]. This is in contrast to the findings from a study sponsored by the FDA [51] which did not demonstrate a clear dose-response; however, the FDA study was complicated by the small number of subjects studied and difference in baseline F_{eNO} observed at different study periods. The lack of a dose-response for FP at doses approved in the clinical label is also consistent with that observed for other methods of assessing FP efficacy [40].

When a QD dose of Advair® Diskus® was included in the statistical model, a large dose-response for FP/salmeterol was demonstrated. However, this is a total daily dose that is lower than the approved dosing regimen. This finding is consistent with

published data for both FP and other ICS products, [43, 45, 46, 50] where a clear dose-response can only be identified when low daily doses of ICS are included in the models described. The data are also consistent with a small pilot study that reported differences in the effect of FP on F_{eNO} when different doses of Advair® were dosed once daily for a week [52]. Advair® Diskus® is approved as a BID drug for the treatment of asthma and COPD with a relatively short plasma half-life of 7.8 hours [13] and a study of Advair® Diskus® BID vs., QD regime in asthma subjects [53] confirmed that BID Advair® Diskus® was more efficacious than a QD regime. Therefore, both the PK properties and the clinical data in asthma are strongly indicative that FP is most efficacious within the approved BID dosing regimen. It is therefore unacceptable to the FDA that a QD dose of FP/salmeterol could be used in an LTE study to demonstrate a dose-response.

Despite the inability to develop a method which could demonstrate a dose-response within an approved dose regimen, the study clearly demonstrated that FP/salmeterol led to reductions from baseline F_{eNO} compared with placebo. This indicated that FP was working as anticipated and the study was robust and interpretable. The F_{eNO} data were used to estimate the likely sample size required for an LTE study to compare a generic FP/salmeterol to Advair® Diskus® and it was estimated that 540 patients would be required to demonstrate BE for the F_{eNO} methodology using a BID dosing regimen if a dose-response element was required. This is clearly unfeasible for a F_{eNO} study as it would require a very large number of skilled Investigator centres to identify as many patients as this to run a successful study.

1.4.2 Local Therapeutic Equivalence - LABA

The aim of the LABA study was to identify a methodology that could be utilized to demonstrate LTE for the LABA component of a generic version of Advair® vs., the originator. To meet the requirements of the FDA it would be necessary to identify a methodology that would discriminate between the effect of the LABA and the ICS and demonstrate a dose-response. The dose-response would need to be large and the variability of the study would need to be low for the methodology to be suitable for a future LTE study with a feasible sample size.

By focusing on the acute effect of a LABA (i.e., effects following a single dose) it is possible to distinguish between the treatment effect of the ICS and the LABA in the combination product as the ICS has limited acute effects on the lung compared with a

bronchodilator [54]. However, the bronchodilator effect of salmeterol is at or very near maximal following a single 50 µg orally inhaled dose [55], suggesting that use of acute bronchodilation is unlikely to yield a useful dose-response relationship.

An alternative method of assessing the efficacy of bronchodilators is to investigate their effect on induced bronchospasm. Spasmogens such as methacholine and histamine can be used to induce an acute reduction in lung function in asthmatic subjects; bronchodilators can prevent the occurrence of this bronchospasm. Additionally, the bronchoprotective effect against directly active spasmogens such as methacholine or histamine, is not acutely affected by the ICS component [56, 57], and is therefore more likely to be related to the bronchodilator, i.e., LABA component. The data for induced bronchospasm caused by inhalation of either allergens or histamine would suggest a lack of a dose-response between 50 and 100 µg of salmeterol [58], therefore these would not be suitable to study. The literature relating to the dose-response for the protective effect of single inhaled doses of salmeterol on bronchospasm induced by methacholine is however unclear. Specifically, the literature data are unclear whether 50 µg salmeterol (the approved dose of salmeterol in Advair®) produces maximal effects: with one study suggesting a dose-response does not exist for salmeterol at doses >50 µg [59] and another study suggests that a dose-response may exist [60]. Other literature existed, suggesting that methacholine challenge could be used to demonstrate a dose-response for another LABA, formoterol [61] and for the short acting β_2-receptor agonist salbutamol/albuterol [62-64]. As the literature for some of the β_2-receptor agonist products were able to demonstrate a dose-response and the literature for salmeterol was unclear, the methacholine challenge methodology was selected for further study.

1.4.2.1 Results and Discussion

The study demonstrated that all treatment groups led to bronchoprotection vs., placebo, as would be anticipated for a β_2-receptor agonist. The dose-response for Advair® was not statistically significant and subsequent sample size estimates for an LTE study using methacholine challenge were very large.

The Advair® Diskus® doses demonstrated bronchoprotection as compared to placebo at the first assessment, 1 hour post-dose and maintained the bronchoprotective effect at the 10 hour post-dose evaluation.

To explore whether there was a statistically significant dose-response between low and high doses of Advair®, analyses were performed just including the active treatments (Table 1.4).

Table 1.4: Local Therapeutic Equivalence LABA Study - Dose Response Analysis Using a Linear Mixed Model Method

Time Point (post dose)	Advair® 100/50 µg one inhalation* (50 µg salmeterol)	Advair® 100/50 µg two inhalations* (100 µg salmeterol)	Treatment Difference (95% CI)	Dose-Ratio ** (95% CI)	Slope*** (95% CI)	Model Mean Squared Error
1 hour	1.101	1.316	0.215 (-0.094, 0.523)	1.239 (0.911, 1.687)	0.310 (-0.135, 0.754	0.4976
6 hours	1.276	1.382	0.106 (-0.205, 0.417)	1.112 (0.815, 1.518)	0.153 (-0.296, 0.602)	0.5054
10 hours	1.289	1.242	-0.047 (-0.520, 0.427)	0.954 (0.594, 1.532)	-0.068 (-0.750, 0.615)	1.1018

* REML estimates of Mean Log_e (PC_{20}); ** Ratio of geometric mean PC_{20}s = Antilog of difference in PC_{20}; *** $\Delta Log_e(PC_{20}) / \Delta Log_e(Dose)$.

The steepest dose-response between the high and low dose was seen at 1 hour post-dose for Advair® (Table 1.4). The dose-response was not statistically significant, with the lower ends of the 95% confidence intervals overlapping zero and the upper end of the confidence interval exceeding twice the slope estimate.

Consistent with the linear mixed model, an E_{max} model suggested that the lowest administered dose of the salmeterol component of Advair® administered in the study considerably exceeded the respective ED_{50}. (28.26 µg).

Based on the relatively shallow dose-response slopes observed, along with the observed variability, a sample size of approximately 240 patients (90% power, 2x2 crossover) would be required to achieve statistical significance in the slope at 1 hour post-dose between Advair® Diskus® 100/50 µg one inhalation and Advair® Diskus® 100/50 µg two inhalations, i.e., 50 vs., 100 µg of salmeterol.

As the variability of a study can influence the sample size required for an LTE study it is important to try to control factors that may influence the variability of the study. As many factors as possible were incorporated into the design of the study [65], including those recommended in other literature [61]. When compared with the range of literature studies reporting β_2-receptor agonists and methacholine challenge studies, the study did not exceed the variability of the majority of other studies (Table 1.5). The dose-response slope also has a significant impact on the feasibility of methacholine challenge and is likely primarily influenced by the pharmacology and approved doses of the particular drug studied, salmeterol in this study. Results from the study suggest that the dose-response is substantially lower than has been observed for studies of approved doses of salbutamol/albuterol and formoterol. It is possible that the lack of dose-response observed in the study is due to 50 µg salmeterol being at or near the top of the plateau for the dose-response curve.

A comparison of both variability (WS-SD (s) – Within Subject Standard Deviation) and dose-response (dose-response slope (b)) from the study with a variety of other clinical studies reporting β_2-receptor agonists and methacholine challenge studies was made. This allows for an assessment of both these key factors in the interpretation of the data (Table 1.5) and a direct comparison between studies to assess whether it was representative of other literature. The variability of the data (as measured by WS-SD) from the study was consistent with or lower than most reported studies. A low s/b ratio would lead to a lower sample size than one with a high s/b ratio. The s/b ratio (2.29) for

the study is much greater than observed in a formoterol study [61] and some salbutamol/albuterol studies [62-64, 66]; however it is consistent with or even lower than the other literature for salmeterol [59, 66].

Table 1.5: Local Therapeutic Equivalence LABA Study – Methacholine Challenge Variability and Dose-Response Slope Comparisons of Studies in the Literature

Study	Treatment	Dose (µg)	n	SLOPE (b)	Ln		s/b	Estimated Sample Size	
					WSV	WS-SD (s)		0.8-1.25	0.67-1.50
Allan 2019 [65]	Salmeterol	50-100	46	0.310	0.50	0.71	2.29	1240	460
Higham 1997 [66]	Salmeterol	25-100	16	0.350	0.53	0.73	2.07	1041	382
Palmqvist 1999 [59]	Salmeterol	50-250	15	0.276	0.92	0.96	3.48	2900	1069
Langley 2005 [67]	Salmeterol	50	33	NA	0.93	0.96	NA	NA	NA
Derom 1992 [60]	Salmeterol	50-100	12	0.749	NA	NA	NA	NA	NA
Prabhakaran 2011 [61]	Formoterol	12-24	10	1.121	0.09	0.30	0.27	18	7
Palmqvist 1999 [59]	Formoterol	12-60	15	0.623	1.86	1.37	2.19	1162	425
Allan 2019 [65]	Albuterol	90-180	46	0.374	0.50	0.71	1.90	858	316
Ahrens 1999 [62]	Albuterol	90-270	24	0.722	0.54	0.74	1.02	249	92
Creticos 2002 [63]	Albuterol	90-180	13	0.976	0.27	0.51	0.53	68	26
Parameswaran 1999 [64]	Salbutamol	100-200	18	0.871	0.06	0.24	0.27	20	8
Higham 1997 [66]	Salbutamol	100-400	16	0.800	0.76	0.87	1.09	285	103
Giannini 2000 [68]	Salbutamol	100	18	NA	0.96	0.98	NA	NA	NA
Inman 1998 [69]	Salbutamol	200	20	NA	0.28	0.53	NA	NA	NA

WSV – Within Subject Variance, WS-SD – Within Subject Standard Deviation.

An assessment of what the sample size would be for an LTE study for a generic FP/salmeterol compared to Advair® Diskus® indicated that 1240 patients would be required if the geometric mean ratio and 90% CI was within 0.80-1.25 and 460 patients and 90% CI to be within 0.67-1.50 (both assuming 90% power). This is clearly unfeasible to use a methacholine challenge study as an LTE study for the comparison of a generic OIP with the originator product as it would require a very large number of skilled Investigator centres to identify as many patients as this to run a successful study.

1.4.3 Local Therapeutic Equivalence - Asthma

The aim of the asthma LTE study was to demonstrate local (lung) BE between Wixela™ Inhub™ (the generic product) and Advair® Diskus® (the originator product) using clinical endpoints in asthmatic patients.

Neither the F_{eNO}, nor the methacholine methodologies were suitable to be used as an LTE design due to the size of studies that would be required to demonstrate a dose-response with either methodology. Following completion of those studies and discussions with the agency, the FDA published a draft guidance (2013) for the development of generic FP/salmeterol products [70]. This guidance recognised the inability to identify a suitable LTE that incorporated dose-response using the approved dose regime for FP/salmeterol despite best efforts. This suggested that LTE, based upon a large asthma patient study (without the need for dose-response) should be conducted. This study design assesses pulmonary function (FEV_1):

1. After the first dose of drug, reflecting the effect of salmeterol

and,

2. After four weeks of treatment, primarily reflecting the effect of FP.

The study design incorporated a comparison of the test (generic product), with the reference product (original brand product) and with placebo. The inclusion of placebo ensures assay sensitivity, as both the test and reference products need to demonstrate statistically significant differences from placebo as well as demonstrating bioequivalence.

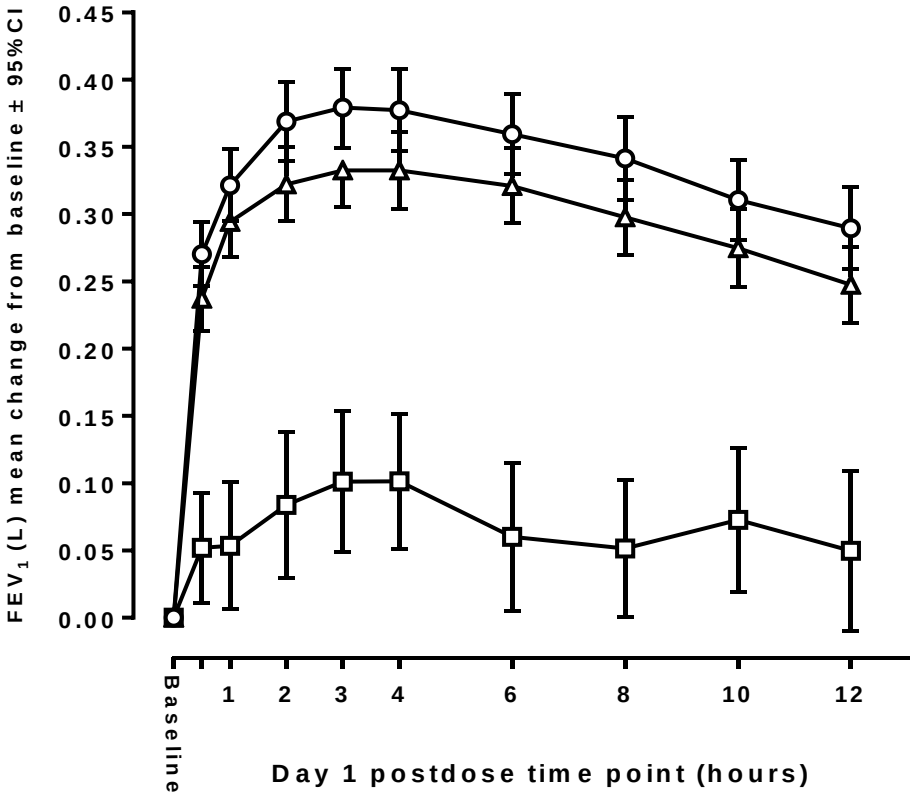
The LTE study [71] was conducted using asthma patients, studying the treatment effect after 4 weeks of dosing on trough FEV_1 and post-dose FEV_1 (0-12 hours) after the first dose of study drugs.

The study treatments were Wixela™ Inhub™ 100/50 µg BID (test product), Advair® Diskus® 100/50 µg BID (reference product) and placebo BID. Subjects were randomised to treatments in a 5:1 ratio of active to placebo, with 512 subjects randomised to receive Wixela™, 512 subjects randomised to receive Advair® and 103 subjects randomised to receive placebo.

1.4.3.1 Results and Discussion

The study demonstrated that both Wixela™ Inhub™ and Advair® Diskus® were efficacious, with substantial improvements in FEV_1 on both Day 1 and Day 29 vs., placebo. The treatment effects of both Wixela™ and Advair® were similar at both Day 1 and Day 29 and therefore bioequivalence between the generic product and the original branded product was demonstrated.

The improvement vs., placebo for day 1 FEV_1 (mean 237-270 mL) was evident by the earliest time point measured (30 minutes post-dose; Figure 1.1). Wixela™ Inhub™ and Advair® Diskus® demonstrated similar FEV_1 responses, with overlapping, superimposed 95% CIs over the 12 hours of assessments and a clear separation from placebo for both treatments.



Placebo (□), Wixela™ (○) and Advair® (Δ)

Figure 1.1: Local Therapeutic Equivalence Study - Change from Baseline FEV_1 On Day 1 of Asthma Study

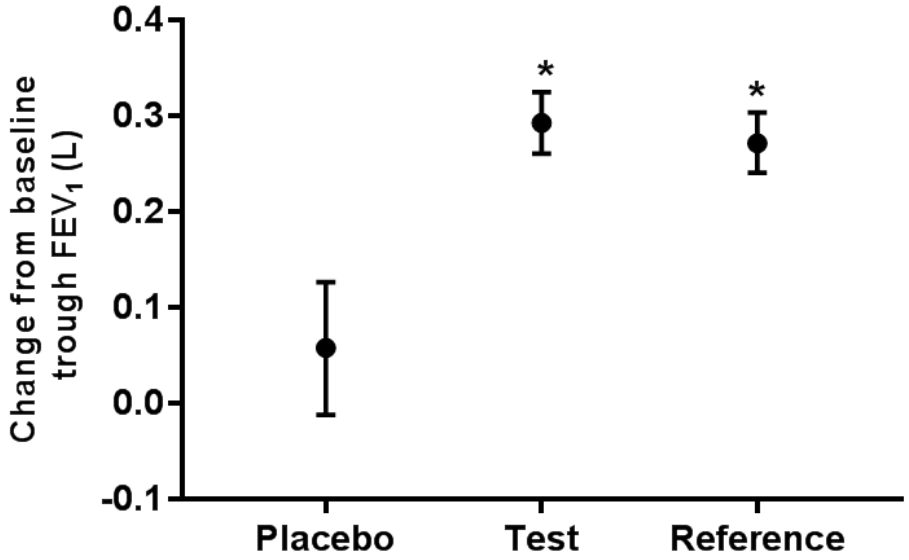


Figure 1.2: Local Therapeutic Equivalence Study – Change From Baseline in Trough FEV_1 Day 29 of Asthma Study

Both treatments also significantly increased trough FEV_1 over placebo following twice-daily dosing for 28 days (235 mL [Wixela™ Inhub™], 215 mL [Advair® Diskus®], each $p < 0.0001$) (Figure 1.2).

A comparison of the treatment effects of Wixela™ Inhub™ (Test) and Advair® Diskus® (Reference), using the LS mean Test/Reference ratios (90% CIs) for Day 1 FEV_1 $AUC_{(0\text{-}12)}$ and Day 29 trough FEV_1 were 1.120 (1.016, 1.237) and 1.069 (0.938, 1.220), respectively. As the 90% CIs for both endpoints were between 0.80 and 1.25 (Figure 1.3), this indicates that Wixela™ Inhub™ and Advair® Diskus® were bioequivalent.

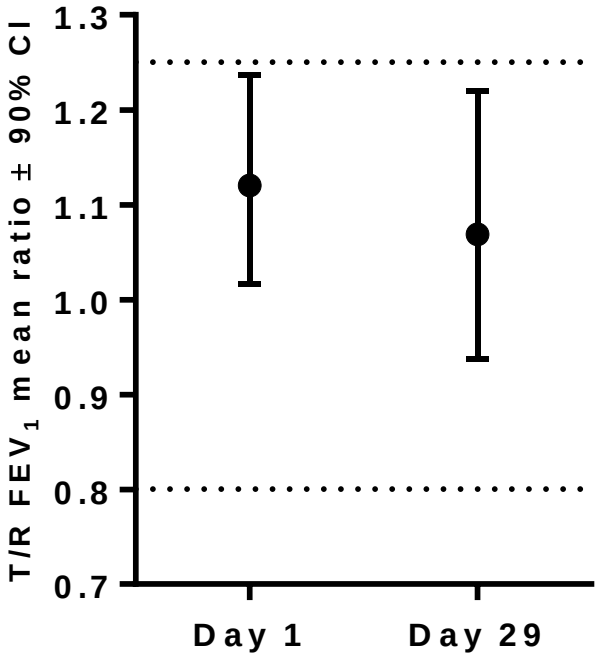


Figure 1.3: Local Therapeutic Equivalence Study - Day 1 and Day 29 Bioequivalence Test of Asthma Study

The LTE study demonstrated a clear treatment effect of Wixela™ Inhub™ and Advair® Diskus® vs., placebo and that Wixela™ Inhub™ was equivalent to Advair® Diskus® for both endpoints.

The absolute treatment effects observed for both Advair® and Wixela™ in the LTE study, were numerically smaller than those observed for prior clinical studies conducted using Advair® Diskus® vs., placebo [72, 73]. However, the use of ICS/LABA has become more routine in recent years (as recommended in guidance such as GINA [2]) and indeed approximately 54% of patients participating in the study were taking ICS or ICS/LABA containing products prior to the study. The population of asthma patients naïve to ICS/LABA or those willing/able to refrain from ICS/LABA during placebo-controlled studies has therefore likely changed to represent a milder phenotype of asthma patient with a higher baseline lung function. With a milder asthmatic patient population participating in the study, the absolute improvements that an ICS/LABA will make are therefore potentially smaller than those participating in prior studies associated with the original approval of Advair® Diskus®.

Nonetheless, as the effect of both test and reference products in the study was consistent, it can be assumed that the treatment effect across the population of asthmatic

patients that use FP/salmeterol would be the same and that Wixela™ Inhub™ will have the same efficacy as Advair® Diskus®.

1.5 Regulatory Considerations

The development of Wixela™ Inhub™ commenced prior to the issue of guidance from the FDA in 2013 [70] and discussions and data provided during the development period allowed the guidance documents to be developed using actual clinical data. The guidance detail the requirements to demonstrate LTE using a large asthma study (reflecting that pharmacodynamic methods are unfeasible) and PK BE. It also states that *in vitro* equivalence must be demonstrated, assessing single actuation content and aerodynamic particle size distribution; this was demonstrated for Wixela™ Inhub™ [35]. Additionally, as Inhub™ is a new inhaler, it is also necessary to demonstrate that patients can use the inhaler and that the inhaler is robust, such that it maintains pharmaceutical performance after patient use [74]. As the studies ultimately conducted were successful and the product was subsequently approved, this endorsed the guidance as being feasible and a pathway that can be followed for the development of other generic OIPs.

The totality of evidence approach is mandated by regulatory authorities such as the US FDA, Health Canada and Japan's PMDA to approve a generic alternative to an OIP, therefore, BE must be demonstrated across the range of methodologies (LTE and PK BE) and for each of the selected endpoints in those studies. This assures patients and prescribers that a generic OIP is bioequivalent to the originator's product and therefore, this high standard of evidence provides assurance of equivalent efficacy and safety when used by patients.

It is worthy of note that international regulatory authorities may have differing requirements for study designs and doses to be studied for the demonstration of LTE and PK BE [75]. For example, the European Medicines Agency (EMA) advocates a stepwise approach to approval. The stepwise approach means that an LTE study may not be required if PK BE has been demonstrated for a product. The EMA also recommends the use of patients in PK BE studies, unless an appropriate justification for using healthy subjects can be made; the discussion in Section 1.3 should however suffice as to why healthy subjects were chosen for the work presented in this thesis. The EMA also requires a differentiation between systemic exposure of drug due to lung absorption and GI absorption, e.g., using charcoal blockade or assessing $AUC_{0\text{-}30min}$ to

demonstrate exposure due solely to lung absorption. The use of charcoal has previously demonstrated the blockade of GI absorption of drugs such as salmeterol [76] and subsequently decrease the systemic pharmacological effects such as changes in heart rate [77].

With adaptations to individual clinical studies the principles of this development program could satisfy most, if not all regulatory authorities.

1.6 Conclusion

The original clinical development strategy was to combine PK BE with an LTE method that could differentiate between the effect of FP and salmeterol and demonstrate a dose-response for both of these drugs, consistent with guidance for other OIPs. The completion of the F_{eNO} and the methacholine challenge methodology development studies demonstrated that this LTE strategy would not be possible to undertake in studies of a feasible size. Therefore, the alternative LTE study in asthmatic subjects was necessary in addition to PK BE.

As BE was achieved in all the pivotal studies (LTE at 100/50 µg strength in asthmatic patients and PK BE at all dose strengths in healthy subjects) it can be determined that from a clinical perspective, Wixela™ Inhub™ is a suitable generic alternative to Advair® Diskus®. The additional requirement to demonstrate *in vitro* equivalence was also demonstrated for Wixela™ Inhub™ [35]. Wixela™ Inhub™ can therefore be prescribed to patients with confidence that they will attain the same level of safety and efficacy as the originator product but at a considerably lower cost than for Advair® Diskus®.

Following this pathway led to the approval of Wixela™ Inhub™ in the USA and subsequently also in Australia, New Zealand and Canada. Additionally, this clinical development program for the generic product, Wixela™ Inhub™, provides specific details regarding clinical study design, anticipated variability of endpoints in the studies and the magnitude of treatment effects. This can therefore be used as a blueprint for the development of further generic alternatives to orally inhaled ICS/LABA combinations. The principles of the development program can also be applied to the development of alternatives to products with other pharmacological activity such as orally inhaled long-acting muscarinic receptor antagonists.

The information gathered, during these studies, therefore increases the likelihood of success for future high quality, accessible OIPs to treat respiratory diseases.

1.7 References

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2 PUBLICATIONS

2.1 FeNO

2.1.1 ATS 2017

Allan R, Haughie S, Kerwin E, Ward, J. A Randomized, Double-blind, Placebo-controlled, Three-way Crossover Incomplete Block Study to Assess the Dose Responsiveness of Exhaled Nitric Oxide to Advair® Diskus® in Asthmatic Subjects. American Journal of Respiratory and Critical Care Medicine 2017; 195: A3195. https://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A3195

A3195

A Randomized, Double-blind, Placebo-controlled, Three-way Crossover Incomplete Block Study to Assess the Dose Responsiveness of Exhaled Nitric Oxide to Advair® Diskus® in Asthmatic Subjects

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INTRODUCTION

- Asthma is widely treated using inhaled corticosteroid (ICS)/long-acting beta agonist combinations, such as fluticasone propionate/salmeterol dry powder inhaler (FPS), marketed as Advair® Diskus®
- Given that global estimates suggest that asthma may affect 334 million people,[1] a range of economical and effective therapies is essential, and generic treatments that satisfy the appropriate regulatory hurdles could provide a significant advantage in cost without sacrificing efficacy[2]
- Some regulators require generics to demonstrate local (lung) therapeutic equivalence (LTE) for each component of the FPS reference, ideally with a dose-response within the approved FPS dose range
- The ability to demonstrate therapeutic equivalence poses difficulties, such as the need to establish clinical methods that measure local delivery of the proposed generic drug[3,4]
- Demonstration of LTE for a fixed-dose combination generic version of FPS will require measurement of each therapeutic component (ICS and long-acting beta agonist)
- This study evaluated potential LTE endpoints for the ICS component of FPS in patients with asthma

OBJECTIVE

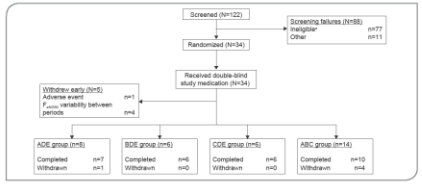
- To assess whether a fractional exhaled nitric oxide measured at a flow rate of 50 mL/sec (F_{ENO50}) methodology can demonstrate a dose-response for the fluticasone propionate (FP) component of FPS in patients with persistent asthma

METHODS

Study design, patients, and treatments

- This was a randomized, double-blind, placebo-controlled, five-period, three-way crossover study (incomplete block design) of the dose-response of 14 days of FPS (Advair Diskus) on F_{ENO50}
- Patients were not using ICS for the last 4 weeks, were non- or exsmokers, aged 18 to 65 years with asthma diagnosis for ≥6 months with a prebronchodilator forced expiratory volume in 1 second (FEV_1) of ≥60% of predicted values, F_{ENO50} ≥45 parts per billion (ppb), and either:
  - Albuterol (360 µg) reversible (≥12% and ≥200 mL) OR
  - A provocative concentration of methacholine producing a 20% fall in mean FEV_1 ≤4 mg/mL

Figure 1: Randomization and patient flow



*Sixty-three patients had low F_{ENO50} (<45 ppb).
Group and treatment sequence abbreviations: A = FPS 100/50 µg QD; B = FPS 100/50 µg BID; C = FPS 250/50 µg BID; D = FPS 500/50 µg BID; E = placebo BID.
BID = twice daily; F_{ENO50} = fractional exhaled nitric oxide measured at a flow rate of 50 mL/sec; FPS = fluticasone propionate/salmeterol dry powder inhaler; ppb = parts per billion; QD = once daily.

- Patients were randomized to one of 24 sequences, receiving three of five treatments in an incomplete block design (**Figure 1**; each separated by 14±2 days of washout):
  - A = FPS 100/50 µg once daily (QD)
  - B = FPS 100/50 µg twice daily (BID)
  - C = FPS 250/50 µg BID
  - D = FPS 500/50 µg BID
  - E = matching placebo BID
- To maintain the blind, patients received two Advair Diskus inhalers for each treatment period (one for use in the morning and one for the evening containing active or placebo treatment)

Primary endpoint

- F_{ENO50} on treatment days 1, 2, 3, 5, 7, and 14 of each period

Safety endpoints

- Safety assessments included adverse event (AE) reporting, laboratory tests, vital signs and electrocardiogram measurements, physical examinations, and daily home asthma monitoring

Statistical analyses

- Analyses of F_{ENO} were performed using the full analysis set (all randomized patients who received study drug and provided any F_{ENO} data)
- The F_{ENO50} post day 1 response was analyzed using linear regression and a three-parameter E_{max} model (E_0, the zero dose-response [placebo]; E_{max}, the maximum attributable drug effect [above placebo]; and ED_{50}, the dose producing 50% of the E_{max} effect)
- The sample size for a future LTE study designed to demonstrate local bioequivalence for a generic FPS versus Advair Diskus was calculated

RESULTS

- The study encountered a high screening failure rate (88/122, 72%); 63 of the 88 screen failure patients failed to meet the F_{ENO50} ≥45 ppb criterion (**Figure 1**)

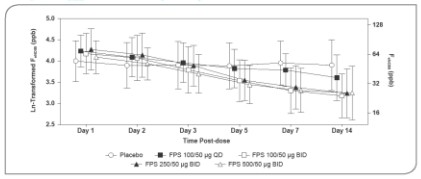
Table 1. Baseline demographics and clinical characteristics

	Study 1002 (N = 34)
Age, mean (range) years	33.1 (18–61)
Males, n (%)	21 (61.8)
Race, n (%)	
White	28 (82.4)
Black/African American	5 (14.7)
Other	1 (2.9)
BMI, mean (SD) kg/m^2	26.97 (5.45)
Smoking history, n	
Never smoked (%)	29 (85.3)
Exsmokers (%)	5 (14.7)
Spirometry, mean (SD)	
FEV_1, L	3.27 (0.86)
FEV_1 percent predicted normal	84.5 (16.6)
F_{ENO50}, mean (SD) ppb	70.16 (30.19)

BMI = body mass index; F_{ENO50} = fractional exhaled nitric oxide measured at flow rate of 50 mL/sec; FEV_1 = forced expiratory volume in 1 second; L = liters; ppb = parts per billion; SD = standard deviation.

- Thirty-four patients randomized to treatment (**Table 1**)
- Mean age was 33.1 years, 61.8% of patients were male with 82.4% white, and mean (SD) FEV_1 percent predicted was 84.5 (16.6)

Figure 2: F_{ENO50} flow rate from day 1 to day 14



Data are mean F_{ENO50} (ppb) ± SD.
BID = twice daily; FPS = fluticasone propionate/salmeterol dry powder inhaler; F_{ENO50} = fractional concentration of exhaled nitric oxide at a flow rate of 50 mL/sec; Ln = natural logarithm; ppb = parts per billion; QD = once daily; SD = standard deviation.

- All FPS treatments decreased the F_{ENO50} compared with placebo, with the largest decreases and potential differentiation between FP doses noted on day 14 (**Figure 2**)
- The three BID treatments decreased F_{ENO50} over days 1 to 7 to a similar extent, with minimal further change during days 7 to 14
- Decreases in F_{ENO50} for all treatments, including 100/50 µg QD compared with placebo, were apparent by day 5

Table 2: Impact of FP dose on F_{ENO50} flow rate from day 1 (baseline) to day 14

	FP dose (Treatment group)				
	0 µg (Placebo BID)	100 µg (FPS 100/ 50 µg QD)	200 µg (FPS 100/ 50 µg BID)	500 µg (FPS 250/ 50 µg BID)	1000 µg (FPS 500/ 50 µg BID)
n	20	20	18[a]	17	19
Day 1					
Mean	61.765	73.823	71.969	80.350	64.232
Geometric mean	54.396	69.035	64.868	71.503	59.752
Day 14					
Mean	59.028	42.360	27.935	30.550	32.387
Geometric mean	49.441	36.838	23.968	25.481	25.678
Change from day 1 to day 14					
Mean change	−2.737	−31.46	−46.33	−49.800	−31.845
GMR (% change)	0.909 (−9.1)	0.534 (−46.6)	0.355[b] (−64.5)	0.356 (−64.4)	0.430 (−57.0)

Data are shown as ppb unless otherwise specified.
[a]n = 17 at days 5, 7, and 14.
BID = twice daily; FP = fluticasone propionate; FPS = fluticasone propionate/salmeterol dry powder inhaler; F_{ENO50} = fractional exhaled nitric oxide measured at a flow rate of 50 mL/sec; GMR = geometric mean ratio; ppb = parts per billion; QD = once daily.

- The geometric mean ratio (percent change) from days 1 to 14 ranged from −46.6% to −64.5% with FPS versus −9.1% with placebo (**Table 2**)
- The E_{max} model analysis at day 14 indicated that the F_{ENO50} response plateaued at 200 µg FP/day, ie, 100/50 µg BID, with an ED_{50} of 69.04 µg FP/day (95% confidence interval −11.48, 149.56)

Table 3: F_{ENO50} dose-response slope by analysis

Linear regression model	Dose-response slope, mean (95% CI)	*P* value
3 doses (total daily dose FPS 100/50, 200/100, and 500/100 µg)	−0.0011 (−0.0017, −0.0006)	<0.001
2 doses (total daily dose 100/50 and 200/100 µg FPS)	−0.0039 (−0.0070, −0.0008)	0.020
2 doses (total daily dose 200/100 and 500/100 µg FPS)	−0.0008 (−0.0016, −0.0001)	0.131

CI, confidence interval; F_{ENO50} = fractional concentration of exhaled nitric oxide at a flow rate of 50 mL/sec; FPS = fluticasone propionate/salmeterol.

- Linear regression analyses at day 14 revealed a significant dose-response slope, with the steepest part of the slope between the 100 and 200 µg FP/day (ie, 100 µg QD versus 100 µg BID) dose levels (slope, −0.0039, *P*=0.020) (**Table 3**). If the QD dose was not included in the model, the dose response was nonsignificant
- The shallow dose response is consistent with other reported F_{ENO50} studies in the literature[2,3]

Sample sizes for FPS LTE clinical trials

- Sample size estimates for evaluation of the LTE of generic FPS products were calculated from F_{ENO50} dose-response data
  - Assuming approved BID dosing of FPS, the estimated sample size would be 540 patients
  - Due to the high screen failure rate (including ≈52% for F_{ENO50} <45 ppb), it may be necessary to screen almost 2000 patients to achieve a randomization population of 540
  - If a QD dose (ie, a nonapproved dose) of FPS was included, the sample size would be 124 patients

Safety evaluation

- Seventeen of 34 patients (50%) experienced a total of 23 treatment-emergent AEs (placebo, n = 6; FPS 100/50 µg QD, n = 4; FPS 100/50 µg BID, n = 6; FPS 250/50 µg BID, n = 3; and FPS 500/50 µg BID, n = 4)
- AEs reported by ≥1 patient were nasopharyngitis and headache
- One patient was withdrawn due to a serious AE (severe asthma on day 1; resolved 19 days after onset)
- One clinically significant laboratory finding (mild neutropenia) was observed and resolved 9 days later

CONCLUSIONS

- This study indicates that F_{ENO50} assessment is not a feasible methodology with which to demonstrate LTE for the FP component of FPS as the methodology failed to show a steep enough dose-response in the range of doses approved for clinical use
- F_{ENO50} could be a valid and feasible endpoint if QD dosing is included; however, QD dosing is not within the approved label for FPS, thus it is unlikely to be acceptable to regulators
- A large clinical endpoint bioequivalence study is therefore necessary, as suggested in draft US Food and Drug Administration guidelines for the development of generic FPS formulations, where responses of the lowest approved dose of test and reference products can be compared using conventional clinical endpoints

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ACKNOWLEDGEMENTS

Mylan Inc. (Canonsburg, PA) provided funding for medical writing and editorial support in the development of this poster. Tina Rideout, MS, and Roger J. Hill, PhD, of Ashfield Healthcare Communications (Middletown, CT) drafted and revised the poster based on input from the authors, and Paula Stuckart of Ashfield Healthcare Communications copyedited and styled the poster per congress requirements.

The authors acknowledge Rachel Bramley (Mylan) for clinical operations related to the study.

The authors acknowledge the other investigators involved in the study; Dr Chandar Abbey, Dr Richard Ahrens, Dr Stephen Maddock, Dr Mark Moss, Dr Sheldon Spector, Dr Tom Casale, Dr Gregory Gottschlich, and Dr Anthony Montanaro.

DISCLOSURES

RA: employee of Mylan Pharma UK Limited

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EK: consultant for Amphastar, Astra Zeneca, Boehringer-Ingelheim, Forest, Mylan, Novartis, Pearl, Sunovion, Teva, and Theravance Biopharma

JW: employee of Mylan Pharma UK Limited

Presented at the Annual Meeting of the American Thoracic Society • May 19-24, 2017 • Washington, DC, US

2.1.2 Journal of Aerosol Medicine and Pulmonary Drug Delivery 2019

Allan R, Haughie S, Kerwin E, Ward J. A Dose-Response Study to Examine the Methodology for Demonstrating the Local Therapeutic Equivalence of the Fluticasone Propionate Component of an Orally Inhaled Combination Therapy of Fluticasone Propionate/Salmeterol Dry Powder. Journal of aerosol medicine and pulmonary drug delivery. 2019 Dec;32(6):364-73. PubMed PMID: 31259655. Epub 2019/07/02. eng.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2018.1520

JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 32, Number 6, 2019

Pp. 364–373
DOI: 10.1089/jamp.2018.1520

A Dose–Response Study to Examine the Methodology for Demonstrating the Local Therapeutic Equivalence of the Fluticasone Propionate Component of an Orally Inhaled Combination Therapy of Fluticasone Propionate/Salmeterol Dry Powder

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Abstract

Background: Asthma is widely treated using inhaled corticosteroid/long-acting beta-agonist combinations, such as fluticasone propionate/salmeterol (FPS) dry powder inhaler. Some regulators require generic medications to demonstrate local therapeutic equivalence (LTE) for each component of the FPS reference product. Fractional exhaled nitric oxide (F_{eNO}) was developed as a possible LTE endpoint for the fluticasone propionate (FP) component of FPS in a randomized, double-blind, crossover study in steroid-naive asthma patients with elevated F_{eNO} (≥45 parts per billion).
Methods: Thirty-four patients received three of five treatments: FPS 100/50 μg once daily (QD), FPS 100/50 μg twice daily (BID), FPS 250/50 μg BID, FPS 500/50 μg BID, or placebo, each for 2 weeks separated by 14-day washout. F_{eNO} was measured on days 1, 2, 3, 5, 7, and 14 of each period, according to American Thoracic Society standards.
Results: FPS treatments decreased F_{eNO} compared with placebo, with the largest differentiation between doses noted on day 14; the mean decreases from days 1 to 14 ranged from −46.6% to −64.5% with FPS versus −9.1% with placebo. The dose–response plateaued at 200 μg/day (FPS 100/50 μg BID). Linear regression analysis revealed significant slopes between FPS doses, with the steepest between 100/50 μg QD and 100/50 μg BID (−0.0039, $p = 0.020$). An estimated sample size (SS) of 160 or 48 patients would be required to demonstrate LTE of generic and FPS reference products (0.80–1.25 and 0.67–1.50 bioequivalence limits, respectively). However, as the slope between BID FPS doses was shallow, a larger SS may be needed if only an approved dose regimen was used.
Conclusion: F_{eNO} could be a valid endpoint to determine LTE between the FP component of generic and reference FPS products, but only if QD dosing and wide equivalence limits are included. As QD dosing is not an approved regimen, this approach is unlikely to be acceptable.

Keywords: Advair, asthma, fluticasone propionate, fractional exhaled nitric oxide, ICS/LABA, local therapeutic equivalence

Introduction

THE CONTROL OF ASTHMA SYMPTOMS with orally inhaled corticosteroids (ICS) and long-acting β2-adrenergic agonists (LABA) forms a key therapeutic strategy recommended by disease management guidelines such as the Global Initiative for Asthma and the National Asthma Education and Prevention Program (NAEPP).[1,2] Given that global estimates suggest that asthma may affect 334 million people,[3] a range of economical and effective therapies is essential.

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Generic treatments (that satisfy the appropriate regulatory hurdles) could provide a significant advantage in cost without sacrificing efficacy.[4]

Fluticasone propionate/salmeterol (FPS) dry powder inhaler is a widely prescribed ICS/LABA fixed-dose combination drug, marketed as Advair® Diskus® in the United States (GlaxoSmithKline, Brentford, UK). As U.S. patent protection for Advair Diskus expired in 2016,[5] several generic versions are progressing toward regulatory approval by the U.S. Food and Drug Administration (FDA).[6,7]

Guidelines for the approval of generic orally inhaled drugs in the United States include the demonstration of local therapeutic equivalence (LTE) at the site of action (i.e., the lung) as part of a weight of evidence approach (together with *in vitro* pharmaceutical equivalence and systemic pharmacokinetic bioequivalence [BE]). The LTE method should be an accurate, sensitive, and reproducible approach that measures local delivery of the products.[8–10] LTE can be established using a response–scale analysis, which compares the responses for two different drug products at the same dose, or a dose–scale analysis, which compares the doses required for two different drug products to give an equivalent response. In ideal circumstances, a dose–scale analysis of relative potency (RP) can be more sensitive than a response–scale analysis. However, the ability to demonstrate LTE using a dose–scale analysis poses significant difficulties, such as the need to establish clinical methods that can show a dose–response for the reference product while staying within the clinically approved dose range.[11,12] Moreover, demonstration of LTE for a generic version of an FPS dry powder inhaler–fixed-dose combination will require measurements specific for each therapeutic component (ICS and LABA).

There are inherent challenges associated with the identification of a suitable LTE method for an ICS. The anti-inflammatory effect of the fluticasone propionate (FP) component is chronic in nature; thus, determination of LTE of this component requires multiple dose studies, which, if crossover designs are required, can be lengthy for individual patients. This is particularly challenging in a disease such as asthma that is characterized by variation in disease status. In addition, the demonstration of dose–response for ICS is challenging because the dose–response relationship of most ICS products at therapeutic doses is relatively flat, especially when dose–response is measured outside of an individual subject (e.g., with different doses being compared between two or more populations of asthmatics within a parallel-group setting). The literature regarding the ICS dose–response assessment includes clinical efficacy studies, as well as pharmacodynamic studies utilizing challenge models[13–16] and inflammatory markers, including fractional exhaled nitric oxide (F_{eNO})[17] and sputum eosinophilia.[14]

F_{eNO} is the methodology considered most likely to deliver an appropriate dose–response relationship using an ICS-responsive pharmacodynamic endpoint,[18] where F_{eNO} could distinguish between 100 and 800 μg/day beclomethasone dipropionate. The dose–response would be more pronounced in patients with marked lung inflammation as determined by elevated F_{eNO} (>100 parts per billion [ppb]). These data were consistent with a number of other publications,[14,17] which also demonstrated an ICS dose–response using F_{eNO} as the study endpoint. As F_{eNO} is unaffected by bronchodilators such as LABA,[19,20] this methodology provides further promise because it would be possible to assess the pharmacodynamic effect of the FP component without interference from the salmeterol component. However, the doses of ICS utilized in the prior literature that demonstrated a dose–response included doses of ICS that are lower than those equivalent to FP 100 μg taken twice daily (BID) as per the asthma indication for Advair Diskus.

No one has rigorously examined the dose–response relationship of the FP component of an FPS combination product in asthmatic patients using F_{eNO} and it is important to determine whether it can be utilized as part of the development of a generic orally inhaled FPS product.

The purpose of this study was to identify whether F_{eNO} methodology could be developed to support the LTE assessment of a generic equivalent to Advair Diskus (FPS).

As Advair Diskus would form the basis of a reference product in the United States, this study assessed the effect of multiple doses of Advair Diskus on F_{eNO} in asthmatic patients. The doses of Advair Diskus were 100/50 μg once daily (QD), 100/50 μg BID, 250/50 μg BID, and 500/50 μg BID, in addition to a matching placebo (lactose).

Materials and Methods

Study design and conduct

This was a randomized, double-blind, placebo-controlled, five-treatment, three-way crossover study (incomplete block design) of the dose–response impact of 14 days of FPS (Advair Diskus) administration (five dose levels) on F_{eNO}. This study was conducted at nine centers in the United States between July 2012 and April 2013.

The study was carried out in accordance with Good Clinical Practice guidelines contained within the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH-E6)[21] and the U.S. Code of Federal Regulations. All patients provided written informed consent. The study was approved by the New England Independent Review Board, University of Iowa Institutional Review Board (IRB), Western IRB, and Creighton University IRB.

Patients and treatments

The study included patients (male and female) 18–65 years of age with an asthma diagnosis for ≥6 months (per NAEPP criteria [2]) with a prebronchodilator forced expiratory volume in 1 second (FEV_1) of ≥60% of predicted values. Patients had F_{eNO} measured at a flow rate of 50 mL/s (F_{eNO50}) of ≥45 ppb at screening. Patients were required to demonstrate either a postbronchodilator FEV_1 reversibility of ≥12% and ≥200 mL (15–45 minutes after 360 μg albuterol inhalation) or a methacholine histamine provocative concentration causing a 20% drop in FEV_1 of ≤4 mg/mL. Patients were also required to be nonsmokers (or quit smoking ≥6 months before the study) and not currently taking ICS.

Patients were excluded from the study if they had any of the following: a respiratory condition other than asthma and allergic rhinitis, unstable asthma (exacerbation in the 3

months before the study or hospitalization in the 12 months before the study), history of life-threatening asthma episodes, contraindication to FPS, presence or recent history of serious conditions that could interfere with study outcomes, or suspected hypersensitivity to any of the study agents. Patients were also excluded if they had received an investigational drug within 1 month, an anti-immunoglobulin E antibody within 6 months, oral corticosteroids within 3 months, ICS within 4 weeks, medications contraindicated with FPS or methacholine within 4 weeks, LABAs within 2 weeks, or any of the following agents within 2 weeks: nedocromil or cromolyn sodium, long- or short-acting antimuscarinics, leukotriene inhibitors, methylxanthines, oral β2-adrenergic agonists, or over-the-counter bronchodilators.

Patients were randomized to 1 of 24 sequences, receiving three of the following five treatments (A–E) in an incomplete block design: (A) Advair Diskus 100/50 μg QD; (B) Advair Diskus 100/50 μg BID; (C) Advair Diskus 250/50 μg BID; (D) Advair Diskus 500/50 μg BID; or (E) matching placebo. The incomplete block design was selected to allow for comparisons between multiple treatments, but to minimize the duration of the study for an individual patient. The three 14-day treatment periods were separated by washout periods of 14 $\pm$ 2 days.

Patients and study personnel were blinded to the study treatment throughout, with randomization and study medication managed by automated interactive response technology. Patients were required to inhale from two inhalers at each dosing time on each treatment day (two Advair Diskus inhalers containing active or placebo treatment, that is, a double-dummy system was utilized to maintain the blind).

Assessments

Study design. This double-blind, double-dummy study consisted of 20 clinic visits and screening, including a 1- to 2-week run-in, three 2-week treatment periods (each followed by a 2-week washout) and a 1- to 2-week follow-up. Screening to follow-up was $\sim$14 weeks for each patient. The visit structure was

- **Visit 1:** Screening visit 1 was conducted 1 to 2 weeks before the first dosing day (eligible patients entered a 1- to 2-week single-blind placebo run-in period).
- **Visit 2:** Period 1, day 1 randomization visit and start of period 1.
- **Visits 3–7:** Period 1 clinic visits on days 2, 3, 5, 7 ($\pm$1 day), and 14 ($\pm$2 days).
- **Visit 8:** Period 2, day 1 start of period 2.
- **Visits 9–13:** Period 2 clinic visits on days 2, 3, 5, 7 ($\pm$1 day), and 14 ($\pm$2 days).
- **Visit 14:** Period 3, day 1 start of period 3.
- **Visits 15–19:** Period 3 clinic visits on days 2, 3, 5, 7 ($\pm$1 day), and 14 ($\pm$2 days).
- **Visit 20:** Follow-up visit (7–14 days after visit 19).

Patients underwent a 1- to 2-week single-blind placebo run-in period (BID inhalation of placebo from Diskus) to ensure that all had a consistent baseline assessment of F_{eNO} measured at randomization and that they remained clinically stable while treated with as-needed albuterol (i.e., no concomitant ICS, LABA, etc.)

To minimize variability in data and demonstrate that patients did not have a significant increase in inflammation, before randomization patients had to demonstrate baseline F_{eNO} measures at randomization (visit 2) with a coefficient of variation (CV) within $\pm$23% compared with screening: CV = [A – B]/[A + B] $\times$ 100, in which A = mean F_{eNO} at screening and B = mean F_{eNO} at randomization.

In addition, their FEV_1 at the randomization visit (visit 2) also had to fulfill the inclusion criteria (i.e., $\geq$60% predicted), and demonstrate that their asthma symptoms were controlled as determined by the investigator during the run-in period.

All clinic visits were conducted with dosing between 06:00 and 12:00 to reduce the variability of measurements due to diurnal variation.

The first visit in the study was a screening visit to assess eligibility for the study, primarily regarding asthma diagnosis and likely eligibility regarding F_{eNO}, and to commence a single-blind placebo run-in. At the second visit, patients had to demonstrate a F_{eNO} of $\geq$45 ppb and stability versus the visit 1 assessment. If eligible, patients were randomized to receive 2 weeks of study treatment with subsequent visits occurring at days 2, 3, 5, 7, and 14. Patients then underwent a washout of $\geq$2 weeks before commencing the subsequent study periods. At the first day of each study period, patients had to demonstrate a F_{eNO} of $\geq$45 ppb and stability versus the visit 1 assessment.

Patients were discontinued from the study if they demonstrated uncontrolled asthma, as determined by the investigator (to ensure that patients were adequately treated with as-needed albuterol and the study treatments).

Study drug administration. The study drugs were administered through the Diskus inhaler, using a double-dummy approach to maintain the blind. Patients inhaled through two Diskus inhalers at each time of dosing (BID) to receive the study drugs. Patients were instructed about the method of inhalation to ensure that study drug delivery was appropriate.

Patients received one of the following five treatments at study periods in an incomplete block design. There were four blocks in total, each consisting of three treatments (ADE, BDE, CDE, and ABC), in which:

- A = Advair Diskus 100/50 μg QD (Advair Diskus 100/50 μg 1 dose in morning + Advair Diskus placebo 1 dose in evening).
- B = Advair Diskus 100/50 μg BID (Advair Diskus 100/50 μg 1 dose in morning + Advair Diskus 100/50 μg 1 dose in evening).
- C = Advair Diskus 250/50 μg BID (Advair Diskus 250/50 μg 1 dose in morning + Advair Diskus 250/50 μg 1 dose in evening).
- D = Advair Diskus 500/50 μg BID (Advair Diskus 500/50 μg 1 dose in morning + Advair Diskus 500/50 μg 1 dose in evening).
- E = Advair Diskus placebo BID (Advair Diskus placebo 1 dose in morning + Advair Diskus placebo 1 dose in evening).

Each block contained all six possible sequences.

Assessment of F_{eNO}. F_{eNO} was measured by chemiluminescence, using a commercially available system (NIOX® Flex) produced by Aerocrine (Solna, Sweden; now part of Circassia). The NIOX Flex device has a range of detection of 2–200 ppb, with an analytical precision of <2.5 ppb of the measured value at <50 ppb and <5% of the measured value at >50 ppb.

Procedures followed manufacturer's recommendation and American Thoracic Society guidelines[22] after the patient rested for a minimum of 5 minutes. The flow rate of primary interest was F_{eNO} at an expiratory flow rate of 50 (45–55) mL/s (F_{eNO50}; ppb). The site personnel were trained and certified in the use of the F_{eNO} analyzer device.

F_{eNO50} was always measured first at each visit. A minimum of two measurements were obtained, and the F_{eNO50} measures had to be reproducible within 10% of each other (or 2.5 ppb if the measured F_{eNO} was <25 ppb). The mean value of the two was subsequently recorded.

F_{eNO} was measured at visits 1 through 19. At visits 2, 8, and 14, mean F_{eNO50} had to demonstrate that the CV was within ±23% compared with screening ($CV = [A - B]/[A + B] \times 100$, in which A = mean F_{eNO} at screening and B = mean F_{eNO} at visits 2, 8, or 14) for the subject to continue with dosing. If F_{eNO50} fell outside these limits, visits 2, 8, or 14 were rescheduled after ~1 week (up to two repeat assessments could be made) with visits occurring until a CV within ±23% was achieved. If a subject failed to reach F_{eNO50} consistency at these rescheduled visits, then the subject was not randomized (if at visit 2, i.e., day 1 of period 1) or was withdrawn from the study (if at visits 8 or 14, i.e., day 1 of periods 2 or 3).

Statistical analyses

The sample size (SS) was based on simulating by-subject data and fitting a linear mixed model between placebo (E) and Advair Diskus 500/50 BID (D), assuming that the primary endpoint (F_{eNO50}) was distributed log normally (ln) and the highest dose (Advair Diskus 500/50 BID) would halve the baseline F_{eNO50}. These simulations showed that 30 patients receiving both Advair Diskus 500/50 BID (D) and placebo (E) would give at least 90% power for the slope to be statistically significant (using a 5% significance level). Since there were three blocks containing both D and E, each with six sequences, the number of patients receiving these blocks had to be a multiple of 18. Therefore, it was necessary to increase the number of patients receiving D and E to 36. Twenty-four additional patients (four patients per sequence) were allocated to the remaining block (ABC), giving a total of 60 patients. Approximately 72 patients were to be randomized to achieve this.

It should be noted that, following a meeting with the FDA and discussion of results for an FDA-sponsored F_{eNO} study[23] that failed to show a dose–response for a BID FP pressurized metered dose inhaler (pMDI), the FDA advised the cessation of the study; the decision was made by the sponsor to stop further enrollment into the study and only 34 patients were randomized.

The primary analyses were performed using the full analysis set (all randomized patients who received study medication and provided F_{eNO} data for at least one treatment period). F_{eNO50} measured at days 1, 2, 3, 5, 7, and 14 of each treatment period and the change from day 1 at postday 1 visits were summarized using descriptive statistics. The F_{eNO50} values were ln-transformed for analysis; the raw exhaled nitrous oxide values were ln-transformed before calculating the change from day 1.

The ln-transformed change in F_{eNO50} from days 1 to 14 was analyzed using a three-parameter maximum attributable drug effect (E_{max}) model with FP total daily dose (0, 100, 200, 500, and 1000 μg), treatment period, and baseline (ln-transformed F_{eNO50} measured on day 1) as explanatory variables. A subject-specific random intercept term was included in the model. Final estimates and standard errors for the parameters in the model were calculated, along with the 95% confidence interval (CI) of the parameter estimates. Model-based estimates of the mean change from day 1 were derived for each dose, along with corresponding 95% CIs. These quantities were exponentiated to provide geometric mean ratios for the change from day 1 and the corresponding 95% CIs.

Additionally, the ln-transformed change in F_{eNO50} from days 1 to 14 was analyzed using a linear mixed model that included the FP total daily dose (continuous variable) and the treatment period as fixed effects and the subject as a random effect. Sequence was not fitted as a term in the model because of the small number of patients ($n = 34$) relative to the number of possible sequences.[24] When three doses were included, the dose was fitted as an additional random effect (random coefficients model). Contrast statements were used to perform a linear dose–response trend analyses for piecewise segments of the dose–response curve (treatments ABC, AB, and BC). Model-based estimates of the mean change from day 1 were derived for each dose (and differences between doses), along with the corresponding 95% CIs and p-values (for dose differences). These quantities were exponentiated to provide geometric mean ratios for the change from day 1 for each dose, comparisons between doses, and corresponding 95% CIs. The piecewise linear regression analyses did not include placebo and were repeated for ln-transformed dose to provide slope parameters on the appropriate scale for sample sizing future LTE studies.

Results

Patients

A total of 122 patients were screened for this study and the 34 patients determined to be eligible were randomized and treated. Thirty-three patients were included in the full analysis set.

The overall screen failure rate was 72%. Of the 88 patients who were not randomized, 63 failed because their F_{eNO50} was <45 ppb.

Of the 34 patients randomized, 29 (85.3%) completed the study, and 5 (14.7%) were withdrawn. Reasons for withdrawal from the study included one patient who had an adverse event (AE) and four patients who were withdrawn because of baseline variability in F_{eNO50} between study periods (Fig. 1).

The data and analyses presented used the full analysis set.

The patients randomized were predominantly men (61.8%) with an average age of 33 years (Table 1). Mean F_{eNO50} at baseline was 65.0 ppb. The incomplete block

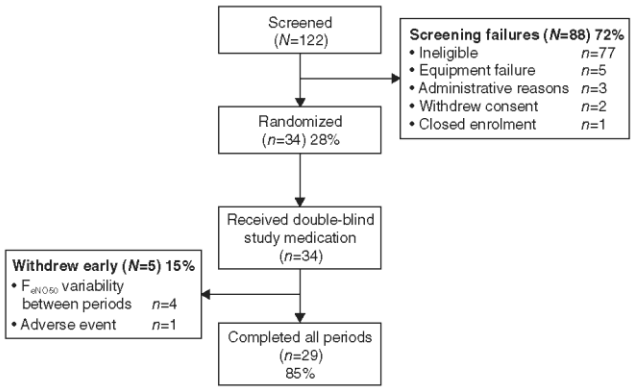

FIG. 1. Patient flow. F_{eNO50}, fractional exhaled nitric oxide measured at a flow rate of 50 mL/s.

crossover design led to similar patients receiving each dose of treatment (as shown in Table 2). However, the patients who received low- to medium-dose FPS (daily FP doses of 100, 200, and 500 μg) had higher baseline F_{eNO50} levels ranging from 72 to 80 ppb, while the placebo and FP 1000 μg daily dosing groups had lower baseline F_{eNO50} of 62–64 ppb.

Study results

F_{eNO} measured at a flow rate of 50 mL/s. The effect of Advair Diskus was evident with reductions in F_{eNO50} apparent at all doses of study drug versus placebo, with the most pronounced treatment effects observed at day 14 of the study. All Advair Diskus treatments demonstrated a steady decrease in F_{eNO50} from days 1 to 14, with the placebo treatment demonstrating a relatively flat profile over the course of the treatment period (Fig. 2).

The differences between active-treatment arms and placebo were apparent (and statistically significant) for all treatment arms by day 5, including the lowest dose (Advair Diskus 100/50 μg QD).

The geometric mean ratio (percent change) from days 1 to 14 ranged from –47% to –64% with Advair Diskus versus –9% with placebo (Table 2). All active doses showed marked improvements in F_{eNO50} from baseline, beginning by day 3 through day 5. Percent reductions in F_{eNO50} were considerable by day 5, but showed continuing improvements out to days 7 and 14. For instance, the percent change from baseline in F_{eNO50} for the 100 μg FP and 200 μg FP doses was –34% and –49% on day 5, and –36 and –60% on day 7, increasing to –47% and –64% on day 14, respectively (Table 2). Three to 5 days of treatment appeared adequate to produce a definitive treatment response and apparent numerical dose separation, with later ongoing benefits of FPS to suppress F_{eNO50} continuing to at least 14 days.

A three-parameter E_{max} model analysis (Table 3) indicated that the F_{eNO50} response plateaued at 200 μg FP/day (Advair Diskus 100/50 μg BID), with an estimated median effective dose (ED_{50}) of 69.04 μg FP/day (95% CI –11.48 to 149.56). The relatively wide CIs around the ED_{50} likely reflect the small SS of the study.

Linear regression analyses revealed a significant dose–response slope, with the steepest part of the slope between 100 and 200 μg FP/day dose levels (i.e., Advair Diskus 100 μg QD vs. 100 μg BID [slope, –0.0039; $p=0.020$]) (Table 4). If the QD dose was not included in the model (i.e., comparing 200 and 500 μg FP/day [Advair Diskus 100 μg BID vs. 250 μg BID]), although the slope was shallower, the dose–response was also significant.

Sample sizing for future studies

The linear regression analyses were repeated using ln-transformed dose, and the estimates obtained for slope and

TABLE 1. DEMOGRAPHICS AND BASELINE CHARACTERISTICS, RANDOMIZED PATIENTS

Characteristic	N = *34*
Age, mean (range) years	33.1 (18–61)
Males, *n* (%)	21 (61.8)
Race, *n* (%)	
White	28 (82.4)
Black/African American	5 (14.7)
Other	1 (2.9)
BMI, mean (SD) kg/m^2	26.97 (5.45)
Smoking history	
Never smoked, *n* (%)	
Exsmokers, *n* (%)	20 (58.8)
Consumption by exsmokers, mean (range) pack-years	5 (14.7) 0.62 (0.0–7.5)
Spirometry, mean (SD)	
FEV_1, L	3.27 (0.86)
FEV_1% predicted normal	84.5 (16.6)
F_{eNO50}, geometric mean (GSD) ppb	65.0 (1.48)

BMI, body mass index; F_{eNO50}, fractional exhaled nitric oxide measured at a flow rate of 50 mL/s; FEV_1, forced expiratory volume in 1 second; GSD, geometric standard deviation; SD, standard deviation; ppb, parts per billion.

TABLE 2. IMPACT OF FLUTICASONE PROPIONATE DOSE ON FRACTIONAL EXHALED NITRIC OXIDE MEASURED AT A FLOW RATE OF 50 mL/s FROM DAY 1 (BASELINE) TO DAY 14

	FP dose (treatment group)				
N	*0 μg (placebo BID)* 20	*100 μg (FPS 100/50 μg QD)* 20	*200 μg (FPS 100/50 μg BID)* 18[a]	*500 μg (FPS 250/50 μg BID)* 17	*1000 μg (FPS 500/50 μg BID)* 19
Day 1					
Mean	61.8	73.8	72.0	80.4	64.2
Geometric mean	54.4	69.0	64.9	71.5	59.8
Day 5					
Mean	57.6	50.2	38.7	39.0	34.3
Geometric mean	48.7	45.7	34.3	34.6	31.3
Change from days 1 to 5					
Mean change	−4.2	−23.6	−35.6	−41.4	−29.9
GMR (% change)	0.90 (−10)	0.66 (−34)	0.51 (−49)	0.48 (−52)	0.52 (−48)
Day 7					
Mean	60.0	47.2	30.7	33.4	31.0
Geometric mean	52.0	44.0	27.1	29.3	27.2
Change from days 1 to 7					
Mean change	−1.8	−26.6	−43.6	−47.0	−33.2
GMR (% change)	0.96 (−4)	0.64 (−36)	0.40 (−60)	0.41 (−59)	0.46 (−54)
Day 14					
Mean	59.0	42.4	27.9	30.6	32.4
Geometric mean	49.4	36.8	24.0	25.5	25.7
Change from days 1 to 14					
Mean change	−2.7	−31.5	−46.3	−49.8	−31.9
GMR (% change)	0.91 (−9)	0.53 (−47)	0.36[a] (−64)	0.36 (−64)	0.43 (−57)

Data are shown as ppb unless otherwise specified.
[a]$N = 17$ at days 5, 7, and 14.
BID, twice daily; FP, fluticasone propionate; FPS, fluticasone propionate/salmeterol dry powder inhaler; GMR, geometric mean ratio; QD, once daily.

within-subject variance from the linear mixed model were used to calculate the SS for a future LTE study (Fig. 3). Simulations were performed using the same primary endpoint (the change from baseline in F_{eNO50} measured at day 14) in a three-period crossover study with four treatments in an incomplete block design (formed by deleting the fourth period of a four-period Williams square design with four sequences and four treatments). The simulated studies ($N = 1000$) assumed a true RP of 0.95 and the four-point parallel line assay analysis method (Finney) was applied to the simulated data. The number of patients required for 90% of the simulated studies to have a positive outcome was

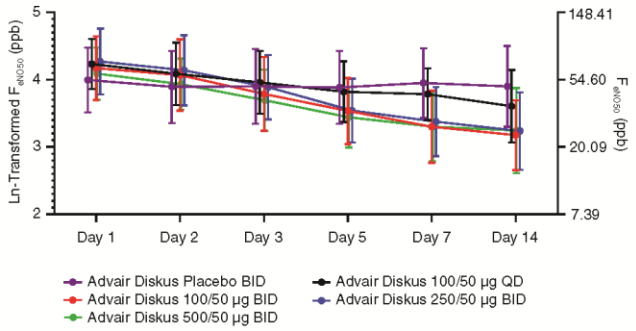

FIG. 2. F_{eNO50} summary by visit. BID, twice daily; ppb, parts per billion; QD, once daily.

Table 3. Fractional Exhaled Nitric Oxide Measured at a Flow Rate of 50 mL/s Dose–Response Maximum Response Analysis

	Estimate F_{eNO50} (ppb)[a]	*Estimate*	*Approximate standard error*	*95% CI*
Change from day 1 at day 14				
E_0	−7.01	−0.12	0.12	−0.36 to 0.13
E_{max}	−33.46	−0.89	0.13	−1.16 to −0.62
ED_{50} (total daily dose μg)		69.04	39.53	−11.48 to 149.56
	Mean			
Placebo	−7.01	0.89	1.13	0.70 to 1.13
100/50 μg QD	−30.25	0.53	1.12	0.42 to 0.66
100/50 μg BID	−34.49	0.46	1.10	0.38 to 0.56
250/50 μg BID	−37.10	0.41	1.10	0.34 to 0.49
500/50 μg BID	−39.10	0.39	1.11	0.32 to 0.48
Maximum ($E_0 + E_{max}$)	−40.47			

[a]Assuming a day 1 (baseline) mean of 63.8 ppb.
CI, confidence interval; E_0, response at baseline; ED_{50}, median effective dose; E_{max}, maximum response

calculated (corresponding to 90% power). For a positive outcome in a study, the two-sided 90% CI for the observed RP had to be within 0.80–1.25 limits. Simulations were repeated for 0.67–1.50 limits and for 80% power.

Based on the dose–response slope estimate for Advair Diskus 100/50 BID versus 250/50 BID (approximately −0.28), along with a variance estimate of ∼0.1, a SS of ∼540 patients randomized (and ∼2000 patients screened at current screen fail rates of 72%) would be required to complete a study to demonstrate BE for a generic FPS compared with Advair Diskus. If the two-sided 90% CI for observed RP was within wider limits of 0.67–1.50, the SS would be smaller at ∼180 patients randomized. For 80% power, corresponding SS would be ∼440 patients (0.80–1.25) or 132 patients (0.67–1.50).

If the design of the LTE study instead utilized FPS 100/50 QD and 100/50 BID (i.e., using a nonapproved dose) to assess the dose–response of the ICS component, the SS would be smaller. Based on the dose–response slope estimate for Advair Diskus 100/50 QD versus 100/50 BID (approximately −0.56) along with the variance estimate (∼0.1), a SS of ∼160 patients would be required for 90% power and ∼124 patients would be required for 80% power. If the wider limits of 0.67–1.50 were utilized, the SS would be further decreased to ∼48 patients (90% power) or 40 patients (80% power).

Safety evaluation

During the study, 17 of 34 (50.0%) patients experienced 16 AEs (placebo, $n=0$; Advair Diskus 100/50 μg QD, $n=4$; Advair Diskus 100/50 μg BID, $n=6$; Advair Diskus 250/50 μg BID, $n=2$; and Advair Diskus 500/50 μg BID, $n=4$).

AEs reported by one or more patients were nasopharyngitis and headache. One patient was withdrawn due to a

Table 4. Fractional Exhaled Nitric Oxide Measured at a Flow Rate of 50 mL/s Dose–Response Linear Regression Analysis

Dose–response group	*Estimate*	*95% CI*	p
Change from days 1 to 14			
100/50 μg QD, 100/50 μg BID, 250/50 μg BID			
Intercept	−0.0318	−1.2840 to 1.2203	0.959
Slope	−0.0011	−0.0017 to −0.0006	<0.001
100/50 μg QD (GMR [% change])	0.5249 (−47.5)	0.4421 to 0.6233	<0.001
100/50 μg BID (GMR [% change])	0.4694 (−53.1)	0.3885 to 0.5673	<0.001
250/50 μg BID (GMR [% change])	0.3358 (−66.4)	0.2470 to 0.4565	<0.001
250/50 μg BID vs. 100/50 μg QD (GMR [% change])	0.6397 (−36.0)	0.5154 to 0.7939	<0.001
100/50 μg QD, 100/50 μg BID			
Intercept	0.8727	−0.7687 to 2.5141	0.284
Slope	−0.0039	−0.0070 to −0.0008	0.020
100/50 μg QD (GMR [% change])	0.5491 (−45.1)	0.4369 to 0.6902	<0.001
100/50 μg BID (GMR [% change])	0.3715 (−62.9)	0.2880 to 0.4792	<0.001
100/50 μg BID vs. 100/50 μg QD (GMR [% change])	0.6765 (−32.4)	0.4982 to 0.9188	0.020
100/50 μg BID, 250/50 μg BID			
Intercept	0.9173	−0.4773 to 2.3119	0.187
Slope	−0.0008	−0.0016 to −0.0001	0.031
100/50 μg BID (GMR [% change])	0.4312 (−56.9)	0.3252 to 0.5716	<0.001
250/50 μg BID (GMR [% change])	0.3343 (−66.6)	0.2533 to 0.4412	<0.001
250/50 μg BID vs. 100/50 μg BID (GMR [% change])	0.7753 (−22.5)	0.6207 to 0.9684	0.031

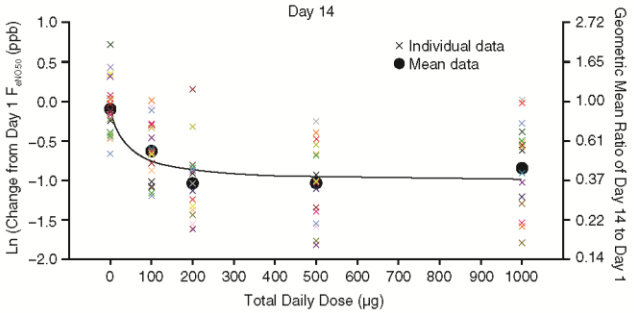

FIG. 3. F_{eNO50} E_{max} model summary. E_{max}, maximum response.

serious AE (severe asthma on day 1; resolved 19 days after onset). One clinically significant laboratory finding (mild neutropenia) was observed and resolved 9 days later.

Discussion

This study demonstrated the anti-inflammatory effect of different doses of the oral ICS FP (as part of a combination with salmeterol); as expected, the study treatments demonstrated consistent effects, and all study treatments demonstrated statistically significant differences from placebo. The percentage change in baseline F_{eNO50} may be a useful endpoint, which evens out baseline F_{eNO50} values that may vary among treatment groups.

The study confirmed that 3–5 days of dosing was adequate to discern a 30%–50% decrease from baseline in F_{eNO50} levels in ICS-naive patients, even with the lowest FPS doses of 100/50 µg QD or BID. Longer treatment times out to 14 days led to further F_{eNO} declines, but showed less dose–response discrimination. QD FPS doses began to converge in F_{eNO} suppression by 14 days, showing similar long-term efficacy. These data confirm that ICS in FPS combinations appear to exert anti-inflammatory effects gradually, and full efficacy may not plateau for 2–4 weeks or longer after treatment initiation.

The interpretation of our F_{eNO} data is potentially limited by the relatively small number of patients ($N=34$); however, the study was stopped on the advice of the FDA following completion of an FDA-sponsored study[23] that failed to show a significant dose–response between 44 µg BID and 88 µg BID FP (administered as a pMDI). Discussions with the FDA also recognized the difficulties of conducting a study of this type; it was very challenging to recruit suitable patients (the sites in our study had to screen 122 patients to randomize 34). This demonstrates the challenges of selecting patients with clinically stable asthma who could participate in a crossover study, including placebo, and who had a baseline F_{eNO50} >45 ppb at screening, indicative of lung inflammation. Despite this potential limitation, the study clearly demonstrated that FPS led to reductions from baseline F_{eNO} compared with placebo, indicating that FP was working as expected and the study was robust and interpretable.

While the study demonstrated that all of the study treatments were effective anti-inflammatory agents, the aim was to determine whether the F_{eNO} methodology could be utilized for the determination of LTE for a generic FPS product and, thus, it was important to explore the dose–response of FP because this had been considered necessary for other products that used a pharmacodynamic-based methodology.[24] The data from this study would indicate that a very large number of patients would be required to use the F_{eNO} endpoint to demonstrate local BE for the FP component of a generic FPS product.

The study demonstrated a clear dose–response relationship for FPS only when a QD dose of Advair Diskus was included in the statistical model (i.e., a total daily dose that is lower than the approved dosing regimen). This finding was consistent with published data for FP and other ICS products,[14,17,18,25,26] in which a clear dose–response could only be identified when low doses of ICS were included in the model. The recently published data from a small pilot study of Advair Diskus given QD for 7 days is consistent with our study and that of Anderson et al.[25] and Weiler et al.[26] Differences in the effectiveness of FP can be observed between doses of Advair in the Weiler study, and some comparisons are statistically significant; however, Weiler et al.[26] did not discuss the SS required to show BE based on their dose–response data. On the other hand, our study is a more robust and thorough exploration of the dose–response of Advair Diskus because it included more patients, both QD and BID treatments, and continued dosing for 14 days (consistent with the approved dosing regimen), thus, enabling a good understanding of the appropriateness of this methodology for a future BE study.

Advair Diskus is approved as a BID drug for the treatment of asthma and chronic obstructive pulmonary disease (COPD) with a relatively short half-life of 7.8 hours[27] and a study of Advair Diskus BID versus QD regimen in asthma patients[28] confirmed that BID Advair Diskus was more efficacious than a QD regimen. Considering that both the pharmacokinetic properties and the clinical data in asthma are strongly indicative that FP is most efficacious within the approved BID dosing regimen, it is unlikely to be acceptable that a QD dose of FPS could be used in an LTE study to demonstrate a dose–response. This means that all dose regimens

would be required to be BID in an LTE study despite the favorable impact on SS that inclusion of a QD dose would have. The shallow dose response between BID doses of Advair Diskus in our study greatly influences the SS required for an LTE study. If a lower dose of Advair Diskus, incorporating a 50 μg BID dose was available (c.f., the approved dose of FP as monotherapy), then F_{eNO} might be a feasible methodology, despite the findings from the FDA-sponsored study,[23] as the ED_{50} estimated from our study is 69 μg of FP (total daily dose). However, this may still require a relatively large SS depending on the BE limits selected.

The choice of BE limits for demonstrating BE is a significant factor in the feasibility of using F_{eNO} as an LTE for an ICS-containing product. If BE limits of 0.8–1.25 are selected, the SS for a comparison of FPS, regardless of the dose regimen selected, would be unlikely to be feasible; SS for an LTE study are estimated between 160 and 540 patients depending on the doses selected. A F_{eNO} study with BE limits of 0.67–1.50 could be feasible (48 patients) if a nonapproved dose regimen, that is, 100/50 QD, was also utilized as the lowest dose in the study. While the gold standard for demonstrating BE is for the two-sided 90% CI for observed RP to be within 0.80–1.25 limits, there is some precedence for 0.67–1.50 limits for variable products and/or methods of testing, such as the methacholine challenge for albuterol. However, as Advair Diskus is not considered a highly variable drug and the use of F_{eNO} would also require the use of a nonapproved dose regimen to bring the SS down to a practical number, this scenario is unlikely to be acceptable to the FDA (or other regulatory authorities).

The lack of a dose–response for FP observed in this study at doses approved for the clinical label is also consistent with that observed for other methods of assessing FP efficacy, such as FEV_1[29]; it is of note that there was no evidence of a dose–response using FEV_1 in our study.

Considering the observed variability and shallow dose–response for the FP component of Advair Diskus at approved doses in this study it can be concluded that utilizing the F_{eNO} methodology for the determination of LTE for a generic FPS would be very challenging, requiring a large number of patients, which would not be practical to conduct. An alternative approach is necessary to develop an LTE for a generic alternative to Advair Diskus as recommended by the FDA's draft FPS guidelines.[30] However, it may be possible to use this methodology for other ICS products that demonstrate a larger dose–response between approved doses and/or if BE limits of 0.67–1.50 are utilized.

Acknowledgments

Mylan, Inc. (Canonsburg, PA) provided funding for medical writing and editorial support. The authors wish to acknowledge Roger Hill, PhD, for medical writing and Paula Stuckart for editorial assistance in the preparation of the article (Ashfield Healthcare Communications, Middletown, CT). The authors also acknowledge Rachel Bramley (Mylan) for clinical operations related to the study, and acknowledge the investigators at the other clinical sites.

Author Disclosure Statement

R.A., S.H., and J.W. are employees of Mylan and have stock ownership in Mylan. E.K. has participated in consulting, advisory boards, speaker panels, or received travel reimbursement from Amphastar, AstraZeneca, Boehringer Ingelheim, Forest Laboratories, GlaxoSmithKline, Mylan, Novartis, Oriel, Pearl Pharma, Sunovion, Teva, and Theravance Biopharma. He has conducted multicenter clinical research trials for ~40 pharmaceutical companies.

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Received on December 10, 2018
in final form, May 16, 2019

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2.2 Methacholine Challenge Study

2.2.1 ATS 2017

Allan R, Haughie S, Ahrens RC, Ward, J. A Randomized Double-blind Placebo- and Active-controlled Five-way Crossover Study to Assess the Dose Responsiveness of Methacholine-induced Bronchial Hyperreactivity to Single Inhaled Doses of Advair® Diskus® in Adult Asthmatics. American Journal of Respiratory and Critical Care Medicine 2017; 195: A3196. https://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2017.195.1_MeetingAbstracts.A3196

A3196

A Randomized Double-blind Placebo- and Active-controlled Five-way Crossover Study to Assess the Dose Responsiveness of Methacholine-induced Bronchial Hyperreactivity to Single Inhaled Doses of Advair® Diskus® in Adult Asthmatics

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INTRODUCTION

- Asthma is widely treated using inhaled corticosteroid (ICS)/long-acting beta agonist (LABA) combinations, such as fluticasone propionate/salmeterol dry powder inhaler (FPS), marketed as Advair® Diskus®
- Given that global estimates suggest that asthma may affect 334 million people,[1] a range of economical and effective therapies is essential, and generic treatments that satisfy the appropriate regulatory hurdles could provide a significant advantage in cost without sacrificing efficacy[2]
- Some regulators require generics to demonstrate local (lung) therapeutic equivalence (LTE) for each component of the FPS reference, ideally with a dose response within the approved FPS dose range
- The ability to demonstrate therapeutic equivalence poses difficulties, such as the need to establish clinical methods that measure local delivery of the proposed generic drug[3,4]
- Demonstration of LTE for a fixed-dose combination generic version of FPS will require measurement of each therapeutic component (ICS and LABA)
- This study (Study 1001) evaluated potential therapeutic equivalence endpoints for the LABA component of FPS in patients with asthma

OBJECTIVE

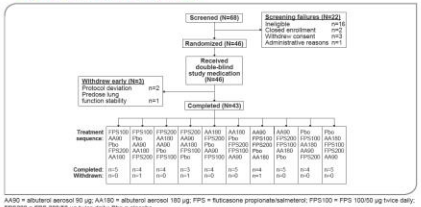
- To assess whether a methacholine challenge (MeCh) methodology can demonstrate a dose response for the LABA component (salmeterol) of FPS in patients with mild persistent asthma

METHODS

Study design, patients, and treatments

- Study 1001 was a randomized, double-blind, double-dummy, single-dose, five-way crossover study
- Patients were non- or ex-smokers aged 18–65 years, with asthma diagnosis for ≥6 months with a prebronchodilator forced expiratory volume in 1 second (FEV_1) of ≥70% of predicted values
  - MeCh was administered using a breath-actuated nebulizer (AeroEclipse® R BAN) using tidal breathing method
  - Patients demonstrated bronchial hyperresponsiveness (provocative concentration of methacholine producing a 20% fall in mean FEV_1 [PC_{20}] of ≤8 mg/mL) and a ≥4-fold increase in PC_{20} (PC_{20} ≤128 mg/mL) 30 minutes after inhalation of 180 µg albuterol aerosol (AA; Proventil®)

Figure 1: Randomization and patient flow



AA90 = albuterol aerosol 90 µg; AA180 = albuterol aerosol 180 µg; FPS = fluticasone propionate/salmeterol; FPS100 = FPS 100/50 µg twice daily; FPS200 = FPS 200/50 µg twice daily; Pbo = placebo.

- Patients were randomized equally to one of 10 sequences (**Figure 1**), receiving each of five single treatments (each separated by 3–10 days of washout):
  - Double-blind (matching) placebo
  - FPS 100/50 µg (one inhalation of 100/50 µg Advair Diskus)
  - FPS 200/100 µg (two inhalations of 100/50 µg Advair Diskus)
  - AA 90 µg (one inhalation of 90 µg Proventil)
  - AA 180 µg (two inhalations of 90 µg Proventil)
- To maintain the blind, one inhalation from each of four inhalers (two of each type) containing either active treatment or placebo was administered per subject per dosing period

Primary efficacy endpoint

- PC_{20} measured by MeCh (1, 6, and 10 hours after study treatment)

Safety endpoints

- Safety assessments included adverse event (AE) reporting, laboratory tests, vital signs, and electrocardiogram measurements and physical examinations

Statistical analyses

- Mixed effect modeling used full analysis set and comprised all randomized patients who provided any poststudy treatment PC_{20} data
- Dose-response slopes were determined using linear regression of log PC_{20} against dose (both with and without placebo) with subject within sequence, dose, period, and sequence as covariates
- Study simulation determined sample size for a future LTE study for a generic FPS versus Advair Diskus. This incorporated the following assumptions:
  - A four-period, four-sequence, four-treatment Williams design
  - Potency of generic product relative to Advair Diskus (reference product; RP) assessed on the dose scale using Finney two-by-two bioassay statistical methods
  - 80%–125% bioequivalence acceptance criteria for RP

RESULTS

Patients

Table 1. Baseline demographics and clinical characteristics

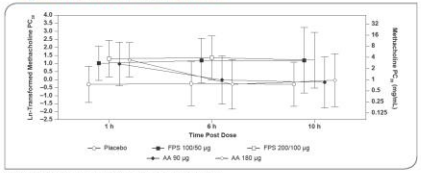
	Study 1001 (N=46)
Age, mean (range) years	37.6 (18–64)
Males, n (%)	22 (47.8)
Race, n (%)	
White	36 (78.3)
Black/African American	8 (17.4)
Other	2 (4.3)
BMI, mean (SD) kg/m²	26.79 (4.94)
Smoking history, n (%)	
Never smoked	39 (84.8)
Ex-smokers	7 (15.2)
Spirometry, mean (SD)	
FEV_1, L	3.23 (0.94)
FEV_1 percent predicted	83.1 (9.70)
PC_{20}, mean (SD) mg/mL	0.9307 (1.46512)

BMI = body mass index; FEV_1 = forced expiratory volume in 1 second; PC_{20} = provocative concentration causing a 20% change in FEV_1; SD = standard deviation

- Mean age was 37.6 years, 47.8% of patients were male with 78.3% white, and mean (standard deviation) FEV_1 percent predicted was 83.1 (9.70) (**Table 1**)

Efficacy

Figure 2. Primary efficacy measures over time



Methacholine PC_{20} versus time post dose. Data are mean PC_{20} (mg/mL) ± SD.
AA = albuterol aerosol; FPS = fluticasone propionate/salmeterol dry powder inhaler; h = hours; Ln = natural logarithm; PC_{20} = provocative concentration causing a 20% change in forced expiratory volume in 1 second; SD = standard deviation.

Table 2. Summary of methacholine PC_{20} (mg/mL)

Time Post Dose	Placebo	FPS 100/50 µg	FPS 200/100 µg	AA 90 µg	AA 180 µg
1 hour post dose					
N	43	44	44	45	44
Geometric mean	0.7444	2.7625	3.6880	2.6584	3.4157
6 hours post dose					
N	42	44	44	44	41
Geometric mean	0.7812	3.2800	3.9220	0.9781	0.7472
10 hours post dose					
N	37	42	44	41	38
Geometric mean	0.7510	3.3239	3.3507	0.8429	0.9635

AA = albuterol aerosol; FPS = fluticasone propionate/salmeterol dry powder inhaler; PC_{20} = provocative concentration causing a 20% change in forced expiratory volume in 1 second.

- Compared to placebo, bronchoprotection (increased PC_{20}) was clearly evident in all active treatment groups at 1 hour post dose (**Figure 2; Table 2**)
  - Both FPS (100/50 and 200/100 µg) and AA (90 and 180 µg) increased PC_{20} values over placebo (FPS: 3.71- and 4.96-fold, respectively, $P<0.001$; AA: 3.59- and 4.62-fold, respectively, $P<0.001$)
- The increase in PC_{20} was sustained 6 and 10 hours post dose with FPS but not AA, reflecting the long-acting effect of salmeterol

Table 3. PC_{20} dose-response slope by treatment and time point[a]

Treatment	Time post dose (hr)	Dose-response slope (Δ ln[PC_{20}] / Δ beta agonist dose) Mean (95% CI)	*P* value	Observed PC_{20} Ratio[a]
AA	1	0.0029 (−0.0005, 0.0063)	0.095	1.29
	6	−0.0033 (−0.0071, 0.0006)	0.092	0.77
	10	0.0004 (−0.0034, 0.0043)	0.815	1.17
FPS	1	0.0043 (−0.0019, 0.0105)	0.167	1.34
	6	0.0021 (−0.0041, 0.0083)	0.494	1.20
	10	−0.0009 (−0.0104, 0.0085)	0.842	1.01

[a]Ratio of geometric means (AA ratio = [PC_{20} AA 180 µg]/[PC_{20} 90 µg]; FPS ratio = [PC_{20} FPS 200/100 µg]/[PC_{20} FPS 100/50 µg]).
AA = albuterol aerosol; CI = confidence interval; FPS = fluticasone propionate/salmeterol dry powder inhaler; hr = hour; ln = natural logarithm; PC_{20} = provocative concentration causing a 20% change in forced expiratory volume in 1 second.

- Linear regression analysis with log-transformed PC_{20} values demonstrated a nonsignificant dose-response slope at all time points (**Table 3**). However, the confidence intervals for these slopes are relatively wide, encompassing both a slope of zero and relatively high slopes
  - The estimated ratio of PC_{20} values at the 1-hour time point (time at which dose response was steepest) indicated only a shallow dose response of 1.29-fold and 1.34-fold for a doubling of AA or FPS dose, respectively, with nonsignificant ($P>0.05$) increases in PC_{20} ratios
  - The shallow dose response observed for AA is not consistent with some previous reports, possibly due to the dose range (doubling versus tripling of dose) or later measurement of PC_{20} at 1 hour post dose (versus 15–30 minutes in some previous reports, **Table 4**)[5,6]
  - A shallow dose response for salmeterol is consistent with some of the literature,[7] although a steeper dose response has been reported in a single study comparing doubling doses of dry powder inhaler (50–100 µg),[8] and a single study comparing quadrupling doses of aerosol (25–100 µg)[9]

Table 4: Comparison of PC_{20} ratios between this study and similar published studies

Study	Time post dose	Observed PC_{20} Ratios (Low, high beta-agonist doses [in µg]) Albuterol	Salmeterol	Formoterol
Current Study	60 mins	1.29 (90, 180)	1.34 (50, 100)	na
Creticos et al.[5]	15 mins	1.86 (90, 180)	na	na
Parameswaran et al.[6]	15 mins	1.89 (100, 200)[a] 1.76 (100, 200)[b]	na	na
Prabhakaran et al.[10]	60 mins	na	na	2.20 (12, 24)
Derom et al.[8]	60 mins	na	1.67 (50, 100)	na
Higham et al.[9]	30 mins	3.05 (100, 400)[c]	1.77 (25, 100)[c]	na
Palmqvist et al.[7]	60 mins	na	1.26 (50, 250)[d]	1.63 (12, 60)[d]

[a]Ventolin™-CFC.
[b]Proventil-HFA.
[c]four-fold difference in beta agonist dose.
[d]five-fold difference in beta agonist dose.
na = not assessed; PC_{20} = provocative concentration causing a 20% change in forced expiratory volume in 1 second.

Sample sizes for FPS therapeutic equivalence clinical trials

- Sample size estimates for evaluation of the therapeutic equivalence of generic FPS products were calculated from PC_{20} dose-response data
  - Assuming the use of the approved dose of FPS, (50 µg salmeterol versus 100 µg salmeterol reflecting one dose versus a total daily dose), and the within-subject variance dose response observed in this study, the estimated sample size was 1240 patients

Safety evaluation

- No serious AEs, no treatment discontinuations due to an AE, and no clinically significant changes in laboratory evaluations were observed

CONCLUSIONS

- The specific study design and methodology used in this study are not suitable for LTE studies of the salmeterol component of FPS products in patients with asthma
  - The estimated dose-response slope for salmeterol was too shallow, consistent with some other literature reports, while other studies report steeper slopes
  - Study simulation using inputs from this study indicated that the required sample size for a successful LTE study would be too large to be practical (1240 subjects)
- Successful use of MeCh for assessment of LTE of products containing inhaled beta agonists may be possible if a product has a greater dose response than those observed for AA and FPS in the current study
  - The dose-response slope observed in other studies for AA and formoterol has been greater and LTE studies of these agents using this endpoint would therefore require fewer subjects
- In any LTE studies using this endpoint, it is crucial to control within-subject variability, which may be challenging if multiple study centers are necessitated by sample size

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ACKNOWLEDGEMENTS

Mylan Inc. (Canonsburg, PA) provided funding for medical writing and editorial support in the development of this poster. The authors acknowledge Bruce Williams (Mylan) for Clinical Operations related to the study. The authors acknowledge the other investigators involved in the study; Dr Craig LaForce, Dr S. David Miller, Dr Sheldon Spector, Dr Steven F. Weinstein.

Tina Rideout, MS, and Roger Hill, PhD, of Ashfield Healthcare Communications (Middletown, CT) drafted and revised the poster based on input from authors, and Paula Stuckart of Ashfield Healthcare Communications copyedited and styled the poster per congress requirements.

DISCLOSURES

RCA: no conflict of interest to declare

RA: employee of Mylan Pharma UK Limited

SH: employee of Mylan Pharma UK Limited

JW: employee of Mylan Pharma UK Limited

Presented at the Annual Meeting of the American Thoracic Society • May 19-24, 2017 • Washington, DC, US

2.2.2 Journal of Aerosol Medicine and Pulmonary Drug Delivery 2019

Allan R, Haughie S, Ahrens R, Singh S, Ward J. A Dose-Response Study Examining the Use of Methacholine Challenge to Demonstrate Local Therapeutic Equivalence of the Salmeterol Component of Generic Inhaled Fluticasone Propionate/Salmeterol Combination Products. Journal of aerosol medicine and pulmonary drug delivery. 2019 Dec;32(6):352-63. PubMed PMID: 31259673. Epub 2019/07/02. eng.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2018.1519

JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 32, Number 6, 2019

Pp. 352–363
DOI: 10.1089/jamp.2018.1519

Original Research

A Dose-Response Study Examining the Use of Methacholine Challenge to Demonstrate Local Therapeutic Equivalence of the Salmeterol Component of Generic Inhaled Fluticasone Propionate/Salmeterol Combination Products

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Abstract

Background: Asthma is widely treated using inhaled corticosteroid/long-acting beta agonist (LABA) combinations, for example, fluticasone propionate/salmeterol (FPS) dry powder inhaler, marketed as Advair® Diskus®. Some regulators require generics to demonstrate local (lung) therapeutic equivalence (LTE) for each component of the FPS reference, ideally with a dose-response within the approved FPS dose range. We sought to develop a methacholine challenge (MeCh) LTE methodology for assessing the LABA (salmeterol) component of FPS.

Methods: Forty-six patients with asthma received single doses of albuterol (active control; 90 or 180 μg), FPS (100/50 or 200/100 μg), and placebo on 5 separate study days. Spirometry and MeCh were performed 1, 6, and 10 hours after study drug inhalation. Primary endpoint was provocative concentration of methacholine producing a 20% fall in forced expiratory volume in 1 second (PC_{20}). Study entry required screening PC_{20} ≤8 mg/mL, with a greater than fourfold increase (and PC_{20} ≤128 mg/mL) after 180 μg albuterol.

Results: Both albuterol (90 and 180 μg) and FPS (100/50 and 200/100 μg) significantly increased PC_{20} compared with placebo (sustained 6 and 10 hours postdose with FPS but not albuterol). The dose-response slopes (95% confidence interval) estimated 1 hour after treatment were 0.374 (−0.068 to 0.815) and 0.310 (−0.135 to 0.754) between low and high doses of albuterol and FPS, respectively, both nonsignificant. Slopes were shallower than those available in the literature for albuterol and formoterol, but similar to those for salmeterol.

Conclusions: These data confirm that the bronchoprotective effect of FPS lasts longer than that of albuterol. The shallow dose-response slope we observed for albuterol is contrary to previous reports, probably due to the measurement of PC_{20} beginning at 1 hour postdose. The results suggest that use of MeCh to assess LTE for salmeterol formulations may be more difficult to accomplish than it is for albuterol and formoterol products.

Keywords: asthma, inhaled, methacholine challenge, salmeterol, therapeutic equivalence

Introduction

THE CONTROL OF ASTHMA SYMPTOMS with orally inhaled corticosteroids (ICS) and long-acting β2-adrenergic agonists (LABA) forms a key therapeutic strategy recommended by disease management guidelines, such as the Global Initiative for Asthma and the National Asthma Education and Prevention Program (NAEPP).[1,2] Given that global estimates suggest that asthma may affect 339 million people,[3] a range of economical and effective therapies is essential, and generic treatments that satisfy the appropriate regulatory hurdles could provide a significant advantage in cost without sacrificing efficacy.[4]

Fluticasone propionate/salmeterol (FPS) dry powder inhaler is a widely prescribed ICS/LABA fixed-dose combination drug, marketed as Advair® Diskus® in the United

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States (GlaxoSmithKline, Brentford, Middlesex, United Kingdom). As US patent protection for Advair Diskus expired in 2016,[5] several generic versions are progressing toward regulatory approval by the US Food and Drug Administration (FDA).[6,7]

Guidelines for the approval of generic orally inhaled drugs in the United States include the demonstration of local therapeutic equivalence (LTE) at the site of action (i.e., the lung) as part of a weight of evidence approach (together with *in vitro* pharmaceutical equivalence and systemic pharmacokinetic bioequivalence [BE]). The LTE method should be an accurate, sensitive, and reproducible approach that measures local delivery of the products.[8–10] LTE can be established using a dose-scale analysis, which estimates the ratio of doses of two different drug products that will produce the same level of response, known as the relative potency (RP). Alternatively, it can be established using a response-scale analysis, which compares the responses for two different drug products at the same dose of each formulation. A dose-scale analysis is considered to be more sensitive and informative about differences in dose delivered to the lung than a response-scale analysis because it communicates both the estimated difference between the two formulations (the RP), and the precision and reliability of this estimate (through the RP's 90% confidence interval [CI]). In other words, if the CI is wide, that study has low power to detect differences in the quantity of drug delivered to the lung. In contrast, a response-scale analysis provides no information about the power of the study to detect differences in the quantity delivered. Because of this, a dose-scale analysis poses greater challenges, including the need to establish clinical methods that can show a statistically significant dose response for the reference product while staying within the clinically approved dose range.[11,12] The dose-scale analysis approach for LTE is reflected in FDA Guidance for other products that have a local site of action, including albuterol, budesonide, and orlistat.[13–15] The expectation is that those developing generic versions of these products will also develop appropriate methods to demonstrate a dose-response before the conduct of pivotal LTE studies. Moreover, demonstration of LTE for a generic version of an FPS fixed-dose combination will require measurements specific for each therapeutic component (ICS and LABA).

When considering how to determine LTE for the salmeterol component, it is necessary to consider the treatment effect of a bronchodilator versus the anti-inflammatory component. By focusing on the acute effect of salmeterol (i.e., effects following a single dose) it is possible to distinguish between the treatment effect of the fluticasone propionate and salmeterol components of the FPS combination product, as fluticasone propionate has limited acute effects on lung function[16] and airway hyperreactivity.[17,18]

One possible outcome measure for assessment of LTE is the acute bronchodilator effect of salmeterol; however, it is apparent that this effect is at or very near maximal following a single 50 μg inhaled dose,[19] suggesting that this outcome is unlikely to yield a useful dose-response relationship.

The protective effect of single inhaled doses of salmeterol against bronchospasm induced by methacholine could be a promising outcome. This model has been used to support registrations of generic alternatives of inhaled short-acting beta receptor agonists (SABA).[20] It is unclear whether a sufficiently steep dose-response is present in the range of clinically used salmeterol doses. One study suggests a dose-response does not exist for salmeterol at doses >50 μg (the approved dose in Advair),[21] while it does for clinically relevant doses of formoterol.[21,22] Another study suggests that a dose-response does exist for salmeterol.[23]

While it is important to determine whether this approach can be used as part of the development of a generic inhaled FPS product, this issue has not been studied rigorously.

The purpose of this study was to identify whether a methacholine challenge (MeCh) methodology could be developed to support dose-scale LTE assessment of a generic equivalent to Advair Diskus. The doses studied were 100/50 μg and 200/100 μg (two doses of Advair 100/50 μg) to test the dose-response of Advair Diskus while staying within the approved total daily dose of this formulation. Additionally, albuterol was included at two doses (90 and 180 μg) to provide a positive control for the study.

Materials and Methods

Patients

The study included patients 18–64 years of age with an asthma diagnosis for ≥6 months (per NAEPP criteria[2]) and with a prebronchodilator forced expiratory volume in 1 second (FEV_1) of ≥70% of predicted value. Patients were required to be nonsmokers (or quit smoking ≥6 months before the study).

Patients were excluded from the study if they had any respiratory condition other than asthma and allergic rhinitis, unstable asthma (exacerbation in the 3 months before the study or hospitalization in the 12 months before the study), history of life-threatening asthma episodes, a recent (within the previous 2 weeks or during the study) respiratory tract infection, contraindication to FPS or methacholine, presence or recent history of serious conditions that could interfere with study outcomes, or suspected hypersensitivity to any of the study agents. Patients were also excluded if they had received an investigational drug within 1 month, anti-immunoglobulin E antibody within 6 months, oral corticosteroids within 3 months, medications contraindicated with FPS or methacholine within 4 weeks, LABAs within 3 weeks, or any of the following agents within 2 weeks before the study: nedocromil or cromolyn sodium, long- or short-acting antimuscarinics, leukotriene inhibitors, methylxanthines, oral β2-adrenergic agonists, or over-the-counter bronchodilators. Patients were allowed to use concomitant ICS during the study.

Before receiving study treatment at visits 3–7, patients had 12-lead electrocardiogram (ECG), vital signs, and spirometry performed. The predose FEV_1 measured at visits following screening had to be ≥70% predicted and also ±15% of the value measured at visit 1 (screening).

Study design

This was a randomized, double-blind, double-dummy, five-way crossover study of the effect of single doses of FPS (Advair Diskus, two dose levels), albuterol HFA (Proventil® [Merck & Co., Inc., Kenilworth, NJ], two-dose levels), and placebo on methacholine-induced bronchial hyperreactivity.

The study was conducted at five centers in the United States between August 2012 and December 2012.

To minimize diurnal variation, all visits were conducted in the morning, with dosing of study drugs between 06:00 and 10:00 on each treatment day, and within ±1 hour of the time of dosing at visit 2.

The first visit was a screening visit to assess eligibility for the study and the response to methacholine challenge (PC_{20}) in the absence of any bronchodilator. The second visit was also a screening visit that occurred ≥24 hours following the first. To ensure that patients were responsive to a beta agonist, PC_{20} was measured 30 minutes following administration of 180 μg of inhaled albuterol.

At the third visit, patients were randomized into the study and received their first study treatment as per their randomization schedule. The remaining four study treatments were administered at visits 4–7. Albuterol had to be withheld for at least 8 hours before the spirometry assessment, and if the patient used concomitant ICS, this was withheld on the morning of assessments. One hour following administration of study treatments, the first methacholine challenge was initiated. Additional challenges were performed at 6 and 10 hours postdose. After completion of the 10-hour methacholine challenge, safety tests (12-lead ECG, vital signs, physical examination, and spirometry) were performed. Inhaled albuterol was given if needed, and patients were discharged from the clinic when the investigator considered it safe to do so.

As the salmeterol component of FPS was the primary treatment of interest, the timing of methacholine challenge tests at visits 3–7 were based around the duration of action of this LABA. The test at 1 hour was selected to be around the time of maximal effect of salmeterol. The tests at 6 and 10 hours allowed assessment of the duration of action of salmeterol.

The study design included features found in recently conducted methacholine challenge studies, such as those described and recommended by Prabhakaran et al.[22]:

- centrally preprepared solutions of methacholine at the correct concentrations;
- tidal breathing to administer the methacholine;
- methacholine-responsive patients, that is, with a PC_{20} of <8 mg/mL at screening;
- albuterol-responsive patients at screening, that is, with at least a fourfold increase in PC_{20} following albuterol;
- maximum allowable concentration of methacholine of 128 mg/mL.

The study was conducted in accordance with Good Clinical Practice guidelines contained within the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH-E6)[24] and the US Code of Federal Regulations. All patients provided written informed consent. The study was approved by New England International Review Board (NEIRB) and University of Iowa IRB. Due to the dose of methacholine administered being higher than is approved in the United States, the study was also submitted to and approved by the FDA as an investigational new drug (Number 115116).

Study drug administration

The study drug was administered using a Diskus device (containing salmeterol as a component of Advair) or a pressurized metered-dose inhaler (pMDI) device (containing albuterol as Proventil HFA) using a double-dummy approach to maintain the blind. Patients were instructed in the different methods of inhalation for the two devices to ensure that study drug delivery was appropriate.

Study treatments were:

- FPS 100/50: one dose Advair Diskus 100/50 + one dose placebo Diskus + two doses placebo pMDI.
- FPS 200/100: two doses Advair Diskus 100/50 + two doses placebo pMDI.
- Albuterol 90: two doses placebo Diskus + one dose Proventil HFA + one dose placebo pMDI.
- Albuterol 180: two doses placebo Diskus + two doses Proventil HFA.
- Placebo treatment: two doses placebo Diskus + two doses placebo pMDI.

Patients were randomized equally to one of 10 treatment sequences (Williams design), receiving each of five single treatments on one of the five randomized study visits. Visits were separated by 3–10 days of washout.

Spirometry

Spirometry was performed at each study center using a pneumotach spirometer. Spirometry was conducted in accordance with American Thoracic Society (ATS) guidelines[25] and at each time point, the largest FEV_1 value from at least three acceptable efforts was used.

Methacholine challenge

Methacholine challenges were implemented following ATS guidelines for methacholine challenge tests,[26] with the following exception: methacholine was administered through a breath-actuated nebulizer (AeroEclipse® RBAN; Monaghan Medical Corporation, Plattsburgh, NY) rather than the English Wright nebulizer recommended in the guidelines (since the latter was unavailable to purchase at the time of this study). This necessitated a shorter nebulization time as the AeroEclipse RBAN is more efficient than the English Wright. Published data[27,28] suggested that a tidal breathing duration of 12–20 seconds using the AeroEclipse RBAN would deliver a similar amount of methacholine as 2 minutes tidal breathing with the English Wright nebulizer, hence a tidal breathing time of 20 seconds was used for this study. Medical-grade compressed air at a flow rate of 6–8 L/minute was used to power the nebulizer.

Serial approximate doubling methacholine concentrations (0.031, 0.063, 0.125, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32, 64, and 128 mg/mL) were administered in 2 mL aliquots of solution. These were preprepared by ASKE Solutions (Austin, TX) using Provocholine® manufactured by Methapharm, Inc., (Brantford, Ontario, Canada) and provided to the individual study centers. This continued until the FEV_1 decreased by 20% from baseline or the maximum concentration was given.

Statistical analyses

The sample size for the study was determined by simulating by subject data and fitting a linear mixed model between the low and the high dose of albuterol (90 and

180 μg), and between the low and high dose of Advair Diskus (100/50 and 200/100 μg). The simulations assumed that the primary endpoint (PC_{20}) was distributed log normally and the geometric mean PC_{20} at the high dose was double the geometric mean PC_{20} at the low dose. Since doses of albuterol and salmeterol were also doubled, this equated to a dose–response slope of 1.0 (on the natural log scale for both PC_{20} and dose). The simulations showed that 30 evaluable subjects would give at least 95% power for the slope for either treatment to be statistically significant at the 5% level (i.e., approximately 90% power for both slopes to simultaneously demonstrate statistical significance). To account for dropouts, 40 subjects (four per treatment sequence) were planned to be randomized. If the slope for either treatment was <1.0, the power of the study to achieve statistical significance for the slopes would be lower. However, rather than establish statistical significance, the principal aim of this study was to estimate the dose–response slope between two different doses of Advair to explore the feasibility of future LTE studies. A sample size of 40 subjects was considered to be sufficient to provide this information for this purpose and be practically achievable.

The PC_{20} was calculated as per ATS guidelines.[26]

Subjects not responding to the highest concentration of methacholine (128 mg/mL) were assigned a value of 256 mg/mL, twice the highest concentration administered.

PC_{20} was analyzed on the natural log scale using two separate linear regression models for each treatment (with additional factors to form a linear mixed model). Analyses were performed with placebo using dose as a continuous explanatory variable and without placebo using (natural) log dose as the explanatory variable (Table 1).

All analyses were stratified by subject (as a random effect nested within treatment sequence). Treatment period and treatment sequence were included as categorical fixed effects. Dose was included as an additional random effect in the analyses that included placebo (to form a random coefficients model). Analyses that did not include placebo only included subject as a random effect. Each analysis was performed separately for each treatment (Advair Diskus and albuterol) and at each time point (1, 6, and 10 hours postdose). A separate model was used at each time point, as this study was planned to identify a single time point that was most appropriate to use in a future pivotal local LTE study for salmeterol. The slope (b) and within-subject variance was assessed for each of the analyses without placebo. The former was defined as difference in response using log to base e, ($\log_e PC_{20}$) divided by difference in $\log_e$ (dose). The latter was taken as the root mean squared error (s), which is the residual error of the linear model.

The ratios of changes in PC_{20} between low and high doses of albuterol and Advair Diskus were estimated from each linear model by calculating the difference in $\log_e PC_{20}$ at each dose (high dose minus low dose) and exponentiating this difference. The 95% confidence intervals (CIs) for the log PC_{20} differences can be exponentiated to give 95% CIs for the PC_{20} ratios.

The sample size for a future LTE study designed to demonstrate BE for a generic FPS versus Advair Diskus was estimated using SAS software (Cary, NC). This was based on simulations using a four-period, four-sequence, four-treatment Williams design assuming true parallel slopes and common doubling doses for both Advair Diskus and the generic product. A Finney, 4-point, 2-by-2 parallel line bioassay method[29,30] was used to estimate RP, and Fieller's theorem was used to determine 90% CIs. Inputs for these simulations included a fixed intercept and between-subject variance (–0.6592, 0.8986 respectively) obtained from the linear mixed-model analysis carried out for the 1-hour postdose time point for Advair Diskus in this study. The slope and within-subject variance inputs for the simulations included estimated values from this study as well as published data on salmeterol, formoterol, and albuterol/salbutamol.[21–23,31–34,35–37] These simulations were performed using the following assumptions: an RP assessment on the dose–scale assuming a true RP = 1; 90% power for two-sided 90% CI within 0.80–1.25 BE limits. This was repeated for BE limits assuming 0.67–1.50 limits.

Results

Patients

A total of 68 patients was screened for this study with 46 patients determined to be eligible, randomized, and treated (patient demographics and baseline characteristics can be found in Table 2). All 46 subjects were included in the full analysis set. This is the primary dataset used in the analyses.

The majority of screen-failure patients were due to screening FEV_1 <70% predicted ($N=4$), a PC_{20} at visit 1 > 8 mg/mL ($N=6$), or a PC_{20} at visit 2 that increased less than fourfold or did not achieve PC_{20} at >128 mg/mL ($N=4$) following administration of albuterol. The other screen failures were due to screening after study enrollment was closed ($N=4$), withdrawal of consent ($N=3$), and uncontrolled diabetes ($N=1$).

Of the 46 patients that were randomized into the study (Fig. 1), 43 patients completed all five study periods. The three patients who withdrew early had baseline FEV_1 <70% predicted at visit 4 (one patient) or errors in the administration of the methacholine challenge at visit 2 (two patients; screening) that were identified after randomization. The methacholine challenge was stopped at visit 2 after study personnel had administered four times the concentration that led to a PC_{20} at visit 1 rather than continuing until the FEV_1 had decreased by at least 20% and a PC_{20} was determined. This meant that these patients were not eligible for the study.

Study results

Methacholine challenge test. Geometric mean PC_{20} responses to treatment are presented in Table 3. Compared with placebo, the geometric mean methacholine PC_{20} measured

TABLE 1. SUMMARY OF LINEAR MIXED MODELS USED

Analysis	*Dose variable*	*Random effects*	*Fixed effects*
With placebo	Dose, continuous	Dose, subject	Sequence, period
Without placebo	Log dose, continuous	Subject	Sequence, period

TABLE 2. DEMOGRAPHICS AND BASELINE CHARACTERISTICS

	N=46
Age, mean (range) years	37.6 (18–64)
Males, *n* (%)	22 (47.8)
Race, *n* (%)	
White	36 (78.3)
Black/African American	8 (17.4)
Other	2 (4.3)
BMI, mean (SD) kg/m^2	26.79 (4.94)
Smoking history, *n* (%)	
Never smoked	39 (84.8)
Exsmokers	7 (15.2)
Consumption by exsmokers, mean (range) pack-years	2.17 (0.1–9.0)
Spirometry, mean (SD)	
FEV_1, L	2.94 (0.70)
FEV_1% predicted normal	83.1 (9.70)
PC_{20}, geometric mean (GSD) mg/mL	0.517 (2.69)
Concomitant ICS use, *n* (%)	17 (37.0)

BMI, body mass index; FEV_1, forced expiratory volume in 1 second; GSD, geometric standard deviation; ICS, inhaled corticosteroid; PC_{20}, provocative concentration of methacholine producing a 20% fall; SD, standard deviation.

1 hour postdose was increased in 89% of patients following administration of either Advair Diskus 100/50 μg or Advair Diskus 200/100 μg, 82% of patients following albuterol 90 μg, and 91% of patients following albuterol 180 μg.

A small number of subjects ($N=5$) demonstrated a high degree of bronchoprotection and did not achieve a PC_{20} at the 128 mg/mL concentration during one or more challenges while taking Advair Diskus or albuterol and were assigned to a PC_{20} of 256 mg/mL for that challenge. Sensitivity analyses, including use of linear extrapolation to calculate the PC_{20}, demonstrated no difference from the primary analyses (data not shown).

Linear mixed-model analysis of the full dataset, including placebo demonstrated that high and low doses of both active treatments significantly increased PC_{20} values versus placebo. This increase in PC_{20} compared with placebo was significant for albuterol only at 1 hour postdose, but was significant at 1, 6, and 10 hours postdose for Advair Diskus. These results are consistent with the pharmacology of SABAs and LABAs.

To explore whether there was a statistically significant dose–response between low and high doses of both treatments, the analyses were performed just including the active treatments (Tables 4 and Tables 5).

The steepest dose–response between high and low dose was seen at 1 hour postdose for both albuterol and Advair (Tables 4 and 5) and the slope estimates for the two formulations were similar (0.374 and 0.310, respectively). However, the dose–response was not statistically significant for either formulation, with the lower ends of the 95% CI overlapping zero and the upper end of the CI exceeding two times the slope estimate (0.815 and 0.754, respectively). This lack of significance and wide CI is consistent with the fact that the study was not adequately powered to show statistical significance for slopes of this magnitude: a dose–response slope of 1.0 was assumed in the power calculation. Assuming a within-subject variance of ~0.5 and a power of 90%, a sample size of ~240 patients would be required to achieve statistical significance for slope of 0.31.

Maximal effect above basal placebo effect (E_{max}) model results suggest that the administered doses of albuterol and the salmeterol component of Advair exceeded their respective dose that produces half E_{max} (ED_{50}) and are in a region of the dose-response curve, where the slope becomes increasingly shallow (Table 6 and Figs. 2, 3).

Sample sizing for future studies

Computations of (s) (WS-SD), (b) (slope), and s/b values from our study and from the published literature (Table 7), and subsequent sample size estimations provided additional

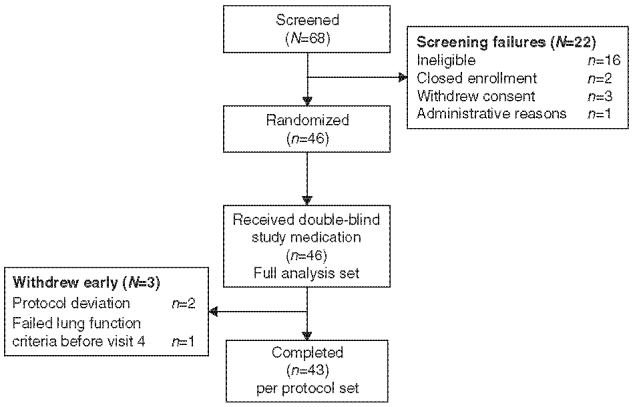

FIG. 1. Patient flow.

TABLE 3. METHACHOLINE PC_{20} (MG/ML)—1, 6, AND 10 HOURS POSTDOSE

Time postdose	*Placebo (N=43)*	*Advair Diskus 100/50 μg (N=44)*	*Advair Diskus 200/100 μg (N=44)*	*Albuterol HFA inhalation aerosol 90 μg (N=45)*	*Albuterol HFA inhalation aerosol 180 μg (N=44)*
1 hour					
N	43	44	44	45	44
Geometric mean	0.74	2.76	3.69	2.66	3.42
GSD	3.07	2.88	3.12	3.85	2.98
Median	0.77	2.36	2.75	2.31	2.59
Min, max	0.09, 6.89	0.38, 25.22	0.56, 256.00	0.33, 56.15	0.57, 47.62
6 hours					
N	42	44	44	44	41
Geometric mean	0.78	3.28	3.92	0.98	0.75
GSD	3.90	4.00	3.91	4.54	4.63
Median	0.98	2.98	3.45	0.93	0.62
Min, max	0.02, 7.84	0.27, 53.55	0.52, 256.00	0.11, 256.00	0.03, 256.00
10 hours					
N	37	42	44	41	38
Geometric mean	0.75	3.32	3.35	0.84	0.96
GSD	3.98	7.65	5.64	4.91	5.18
Median	0.866	2.31	2.62	0.67	0.87
Min, max	0.06, 12.51	0.08, 256.00	0.12, 256.00	0.03, 256.00	0.05, 256.00

GSD = exp(σ), where σ = SD on natural log scale.
GSD, geometric standard deviation; Min, max, minimum, maximum; PC_{20}, provocative concentration of methacholine producing a 20% fall; SD, standard deviation.

information concerning feasibility using methacholine challenge methods for assessing dose-scale LTE of a generic FPS compared with Advair Diskus. We judged sample sizes <100 to be practical and those >100 to be impractical.

The BE limits chosen (0.80–1.25 vs. 0.67–1.50) have a substantial influence on the sample size required for this kind of study. Using the wider BE limits (0.67–1.50), estimated sample sizes were <100 when s/b ratio was less than ~1.0 (Fig. 4). This level of s/b ratio is seen in four published studies that evaluated albuterol and formoterol products, but none evaluating salmeterol-containing products (Table 7). If the narrower BE limits (0.80–1.25) are chosen instead, the sample size is 2.5- to 3.0-fold higher for any given s/b ratio value. This would provide sample sizes <100 only when s/b is less than ~0.6. This level of s/b ratio was seen in three published studies that evaluated albuterol and formoterol products, and none evaluating salmeterol. Based on discussions some of the authors have had with the FDA, the 90% CI for RP may be required to be within the narrower 0.80–1.25 limits. While 0.67–1.50 is part of the guidance for albuterol,[13] this may not be acceptable for Advair.

Based on the literature, the s/b ratios associated with salmeterol (ranging from 2.07 to 3.48) are higher than those associated with studies evaluating albuterol (ranging from 0.27 to 1.09) and this results in larger sample sizes. This appears to be largely caused by lower values of slope, (b), in the salmeterol studies. These range from 0.28 to 0.75 in salmeterol studies, but from 0.72 to 0.98 in albuterol studies.

Consistent with this and based on the estimated (s), (b), and s/b values for salmeterol in our study, the estimated sample size is relatively high: ~1240 patients to give 90% power for demonstrating BE using the 0.80–1.25 BE limits, and 460 patients to give 90% power using the 0.67–1.50 BE limits. The primary endpoint used for calculating the estimated sample size was the 1-hour postdose time point; if the 6-hour time point was used, the s/b ratio is even higher with an estimated sample size for 0.80–1.25 BE limits of ~5000 patients and ~1900 patients for 0.67–1.50 BE limits. It is not possible to estimate a sample size using the 10-hour time point as the slope observed at that time point was negative (Table 5).

However, there is substantial uncertainty concerning the true value of (b) for salmeterol, given the wide CI for (b) in

TABLE 4. LINEAR MIXED MODEL RESULTS FOR ALBUTEROL (LOG_E PC_{20}; LOG_E DOSE; WITHOUT PLACEBO)

Time point	*90 μg*[a]	*180 μg*[a]	*Difference (95% CI)*	*Dose-Ratio*[b] *(95% CI)*	*Slope*[c] *(95% CI)*	*Model mean squared error*
1 hour	0.973	1.232	0.259 (–0.047 to 0.565)	1.296 (0.954–1.759)	0.374 (–0.068 to 0.815)	0.5036
6 hours	0.051	–0.244	–0.295 (–0.640 to 0.050)	0.744 (0.527–1.052)	–0.426 (–0.924 to 0.073)	0.5761
10 hours	–0.108	–0.068	0.040 (–0.304 to 0.384)	1.041 (0.738–1.468)	0.058 (–0.439 to 0.554)	0.5119

[a]Residual maximum likelihood estimates of mean $\log_e$ (PC_{20}).
[b]Ratio of geometric mean PC_{20} = antilog of difference in $\log_e$ PC_{20}.
[c]$\Delta\log_e(PC_{20})/\Delta\log_e(\text{dose})$.
CI, confidence interval; $\log_e$, log to base e; PC_{20}, provocative concentration of methacholine producing a 20% fall.

TABLE 5. LINEAR MIXED MODEL RESULTS FOR ADVAIR DISKUS (LOG_E PC_{20}; LOG_E DOSE; WITHOUT PLACEBO)

Time point	*100/50 μg*[a]	*200/100 μg*[a]	*Difference (95% CI)*	*Dose-ratio*[b] *(95% CI)*	*Slope*[c] *(95% CI)*	*Model mean squared error*
1 hour	1.101	1.316	0.215 (–0.094 to 0.523)	1.239 (0.911–1.687)	0.310 (–0.135 to 0.754)	0.4976
6 hours	1.276	1.382	0.106 (–0.205 to 0.417)	1.112 (0.815–1.518)	0.153 (–0.296 to 0.602)	0.5054
10 hours	1.289	1.242	–0.047 (–0.520 to 0.427)	0.954 (0.594–1.532)	–0.068 (–0.750 to 0.615)	1.1018

[a]Residual maximum likelihood estimates of mean log_e (PC_{20}).
[b]Ratio of geometric mean PC_{20} = antilog of difference in log_e PC_{20}.
[c]$\Delta log_e(PC_{20})/\Delta log_e(dose)$.
CI, confidence interval; log_e, log to base e; PC_{20}, provocative concentration of methacholine producing a 20% fall.

our study (–0.135–0.754) and the similarly broad range of estimated values of (b) obtained from the literature. If the true value of (b) is in fact greater than our estimated value, this would reduce the estimated sample size. For example, if the upper confidence limit for (b) in our study (0.754) is substituted for the estimated (b) value, this decreases the s/b ratio to 0.94 and the sample size to 212 and 80 for BE limits of 0.80–1.25 and 0.67–1.50, respectively.

High variability (s) can also increase the s/b ratio and required sample size. While the value of (s) observed in our study is lower than that obtained from many published studies (Table 7), still lower values obtained from other studies suggest the potential for substantial room for improvement. This would further reduce the sample size. For example, if the value of (b) is again assumed to be 0.754 and variability (s) is assumed to be 0.60 (a modest reduction in the value observed in our study), the estimated sample size decreases to 152 and 57 for BE limits of 0.80–1.25 and 0.67–1.50, respectively.

Safety evaluation

During the study, 13 of 46 (28.3%) patients experienced 16 adverse events (AEs) (placebo, $n=0$; 100/50 μg Advair Diskus, $n=3$; 200/100 μg Advair Diskus, $n=2$; 90 μg albuterol, $n=6$; 180 μg albuterol, $n=5$). AEs reported by one or more patients were flushing and headache. Reported AEs of flushing were possibly related to methacholine administration as all these occurred in both the Advair Diskus or albuterol treatment periods. Higher concentrations of methacholine were typically administered to these subjects to achieve a PC_{20}, thus increasing the possibility of an effect of methacholine on systemic muscarinic receptors. The events of flushing occurred during/following administration of the methacholine challenges and resolved rapidly thereafter. No AEs were classified as severe, all being mild or moderate in intensity.

TABLE 6. E_{MAX} MODEL RESULTS AT 1 HOUR POSTDOSE, LOG_E PC_{20} AS OUTCOME

Treatment	*E_0 (SE)*	*E_{max} (SE)*	*ED_{50} (SE)*
Albuterol (MDI)	–0.30 (0.18)	1.90 (0.70)	44.14 (61.69)
Advair® Diskus®	–0.30 (0.18)	2.05 (0.70)	28.26 (33.37)

E_0, basal (placebo) effect; E_{max}, maximal effect above E_0; ED_{50}, dose that produces half E_{max}; log_e, log to base e; MDI, metered-dose inhaler; PC_{20}, provocative concentration of methacholine producing a 20% fall; SE, standard error.

No serious AEs, no treatment discontinuations due to an AE, and no clinically significant changes in laboratory evaluations, 12-lead ECGs, or vital signs were observed during the study.

Discussion

Both salmeterol (as part of a combination with fluticasone propionate) and albuterol demonstrated the bronchoprotective effect against bronchospasm induced by the oral inhalation of methacholine. All active study treatments demonstrated statistically significant differences from placebo at 1 hour after dosing. As expected, only the salmeterol-containing treatments demonstrated long-acting effects.

Our Monte Carlo simulations and clinical study results provide insight into the feasibility of using a clinical bioassay with methacholine PC_{20} as the outcome measure for evaluating dose–scale LTE of generic and brand name orally inhaled formulations containing salmeterol.

Simulation results indicate that the choice of BE limits for a dose–axis LTE study is a critical factor in determining the feasibility of using this clinical bioassay model. When BE limits of 0.80–1.25 are applied, sample sizes required to

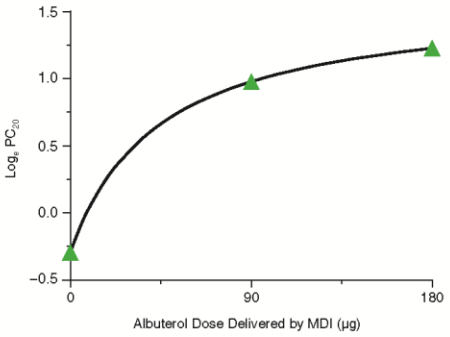

FIG. 2. E_{max} model results of effects of albuterol on log_e PC_{20} at 1 hour postdose. E_{max}, maximal effect above basal placebo effect; log_e, log to base e; MDI, metered-dose inhaler; PC_{20}, provocative concentration of methacholine producing a 20% fall in the forced expiratory volume in 1 second (FEV_1).

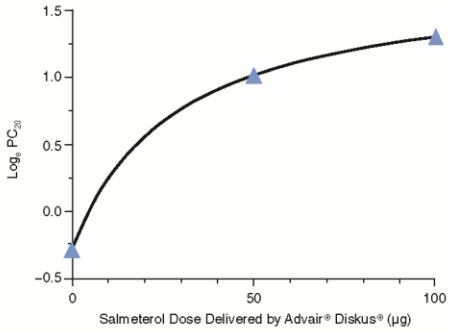

FIG. 3. E_{max} model result of effects of Advair® Diskus® on $\log_e PC_{20}$ at 1 hour postdose. E_{max}, maximal effect above basal placebo effect; $\log_e$, log to base e; PC_{20}, provocative concentration of methacholine producing a 20% fall in the forced expiratory volume in 1 second (FEV_1).

provide 90% power are ~2.5–3.0-fold higher than when BE limits of 0.67–1.50 are applied (Fig. 4). Thus, the choice of the narrower BE limits will substantially increase the cost and reduce the feasibility of successfully using this clinical bioassay.

It has long been recognized that s/b ratio is an important factor in determining sample size–the lower the s/b value, the lower the sample size. The s/b value observed for salmeterol in our study is high relative to those reported for formoterol and albuterol (Table 7). This leads to an estimated sample size of 1240 and 460 for BE limits of 0.80–1.25 and 0.67–1.50, respectively. Our data are consistent with salmeterol s/b ratios reported in the literature, which are also high (>2.0). Given the complexity of conducting this type of study, we judge sample sizes this large to be impractical.

The literature suggests that s/b ratios reported for both formoterol and albuterol will be associated with practical sample sizes (Table 7 and Fig. 4). The s/b value obtained by Prabhakaran et al.[22] for formoterol was 0.27, yielding a sample size of 18 and 7 for the two sets of equivalence limits. However, the s/b obtained from Palmqvist et al.[21] for formoterol was much higher (2.19), yielding sample sizes of 1162 and 425. Parameswaran et al.[33] obtained an s/b ratio of 0.27 for albuterol, yielding low and practical sample sizes of 20 and 8, respectively, for the two sets of equivalence limits, whereas Ahrens et al.[31] obtained an s/b of 1.02 giving sample sizes of 249 and 92. The difference between these two albuterol studies was largely due to differences in (s), variability, rather than (b), slope.

The higher s/b values estimated for salmeterol in our study and in the literature are at least in part due to lower values of (b) (i.e., a shallower slope) than are seen for albuterol and formoterol in the literature (Table 7). Our mean estimate of (b) for salmeterol was 0.31 (95% CI, –0.135 to 0.754), whereas published mean estimates of (b) observed for formoterol and albuterol ranged from 0.623 to 1.121, and 0.722 to 0.976, respectively.

Given the wide CI for our (b) estimate for salmeterol (–0.135 to 0.754), and similarly wide range of values of (b) available in the published literature (0.276–0.749), the "true value" of (b) for salmeterol may be substantially higher or lower than our estimated value of 0.31. This will impact the sample size required. For example, if the higher upper CI limit for (b) is assumed, this reduces the s/b ratio to 0.94, and the sample size to 212 and 80 for BE limits of 0.80–1.25, and 0.67–1.50, respectively. This makes this methacholine-based bioassay methodology appear to be more feasible, at least when using the wider BE limits. The (b) value could, of course, also be near the lower CI limit, which would clearly be associated with impractically large sample sizes. We do not have CIs available for the mean estimates of (b) in the literature, but it is likely they would also be wide. Thus, more precise estimates of the value of (b) for salmeterol are needed.

The slope may be influenced by the subjects selected for the study. When designing the study, we considered who the most appropriate population of subjects would be to increase the likelihood of observing a dose–response. Thus, our study

TABLE 7. VARIABILITY AND DOSE–RESPONSE SLOPE COMPARISONS OF STUDIES

						Log$_e$			*Estimated sample size*	
Study	*Treatment*	*Dose (µg)*	*n*	*# sites*	*SLOPE (b)*	*WSV (s^2)*	*WS-SD (s)*	*s/b*	*0.80–1.25*	*0.67–1.50*
Current study	Salmeterol	50–100	46	5	0.310	0.50	0.71	2.29	1240	460
Higham[34]	Salmeterol	25–100	16	1	0.350	0.53	0.73	2.07	1041	382
Palmqvist[21]	Salmeterol	50–250	15	1	0.276	0.92	0.96	3.48	2900	1069
Langley[35]	Salmeterol	50	33	1	NA	0.93	0.96	NA	NA	NA
Derom[23]	Salmeterol	50–100	12	1	0.749		NA		NA	NA
Prabhakaran[22]	Formoterol	12–24	10	1	1.121	0.09	0.30	0.27	18	7
Palmqvist[21]	Formoterol	12–60	15	1	0.623	1.86	1.37	2.19	1162	425
Current study	Albuterol	90–180	46	5	0.374	0.50	0.71	1.90	858	316
Ahrens[31]	Albuterol	90–270	24	1	0.722	0.54	0.74	1.02	249	92
Creticos[32]	Albuterol	90–180	13	1	0.976	0.27	0.51	0.53	68	26
Parameswaran[33]	Albuterol[a]	100–200	18	1	0.871	0.06	0.24	0.27	20	8
Higham[34]	Albuterol[a]	100–400	16	1	0.800	0.76	0.87	1.09	285	103
Giannini[36]	Albuterol[a]	100	18	1	NA	0.96	0.98	NA	NA	NA
Inman[37]	Albuterol[a]	200	20	1	NA	0.28	0.53	NA	NA	NA

[a]Described in the literature using the INN (salbutamol) and using the doses associated with this.

b, slope; INN, international nonproprietary name; $\log_e$, log to base e; NA, not available; s, WS-SD; s/b, WS-SD/slope; WSV, within-subject variance; WS-SD, within-subject standard deviation.

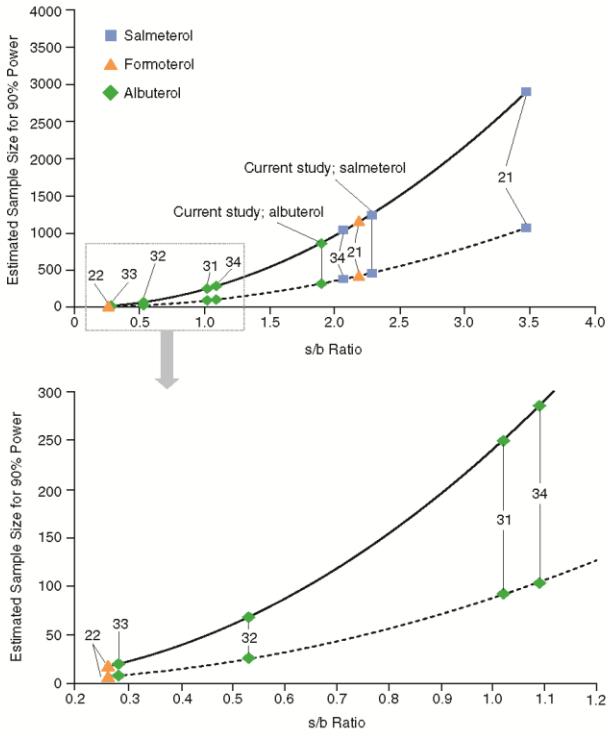

FIG. 4. Sample sizing for a bioequivalence study. Solid line = 0.80–1.25 BE limits. Dashed line = 0.67–1.50 BE limits. BE, bioequivalence; $\log_e$, log to base e; *s*/b, WS-SD/slope; WS-SD, within-subject standard deviation.

was designed to select subjects following the points of consideration raised in Prabhakaran et al.[22] as described in the materials and methods section.

The dose–response slope is likely primarily influenced by the pharmacology and approved doses of the particular drug studied. The E_{max} model is useful in considering how and why this may be true. The ED_{50} and the E_{max} of a particular drug, and the doses of the drug that are commercially available will all influence the steepness of the observed dose–response. First, the shallow dose–response observed for salmeterol might be because of the position of the lowest commercially available dose of salmeterol available as Advair 50 μg, on its dose–response curve. Specifically, the 50 and 100 μg salmeterol doses may be above the ED_{50}, where the dose–response curve becomes progressively more shallow, while commercially available doses of albuterol and formoterol may lie lower on the curve relative to the ED_{50}, where the curve is steeper and approximately linear. In support of this hypothesis, the estimated ED_{50} for salmeterol from our study was 28.26 μg making the 50 μg low dose 1.77 times the ED_{50}. In contrast, the ED_{50s} we estimated for albuterol based on data from the literature (were 71, 120, and 130 μg[32–34]), making the 90 μg low dose of albuterol near to or below the ED_{50}. We were unable to identify formoterol data suitable for fitting an E_{max} model. Second, the shallow salmeterol slope may be due to an E_{max} value that is lower than that for formoterol or albuterol. If the E_{max} is lower, this would make the slope at all points on the curve more shallow, including between 50 and 100 μg of salmeterol. These findings for salmeterol are compatible with those of Palmqvist et al.[21]; those authors did not demonstrate a dose–response between 50 μg of salmeterol and higher doses, yet they did show a dose–response between different clinically relevant doses of formoterol. Graphic representation of data presented in that article suggests that the response to formoterol approaches an E_{max} plateau at a substantially higher response level than does to salmeterol. Similarly, the E_{max} value we calculated for salmeterol in this study (2.05) is considerably lower than that we calculated based on the literature for albuterol (3.7, 4.0, 3.4[32–34]). The difference in E_{max} between formoterol, albuterol, and salmeterol would not be surprising, since these drugs have different intrinsic activities at the beta 2 receptor site.[38,39] These two possibilities are not mutually exclusive.

The measure of variability of a study, (s), also has a substantial effect on s/b ratio and the subsequent sample size required for an LTE study. Variability can be influenced by choices made during the process of study design, and it is important to control as many of the factors that may influence variability as possible. Single-center studies conducted by Prabhakaran et al.[22] and Parameswaran et al.[33] serve as the gold standard for how much it is possible to reduce within-subject variability with values of (s) of 0.30 and 0.24, respectively. However, it is challenging to achieve this level of control of variability in a larger, multicenter study. Accordingly, the value of (s) in our study was substantially higher (0.71) than those studies. Still, in the authors' experience, it is possible to reduce (s) to levels below this with careful attention to measures intended to reduce variability.

Specific sources of variability were considered during the design of our study, and measures were undertaken to reduce within-subject variability:

- Methacholine solutions were centrally prepared and provided to each study center as kits for each study visit, with each concentration in a separate labeled vial. The methacholine solutions were analyzed at a central laboratory to ensure that the correct concentration of methacholine was in each vial before shipment.
- All centers were instructed to follow ATS guidelines for quality of spirometry.

The authors recommend that additional steps intended to reduce variability be considered in the design of future studies. To maximize quality of spirometry in multicenter trials utilizing methacholine challenge, standardized spirometry equipment should be used at each study center, and real-time programmatic quality control feedback should be provided to ensure that centers are following the ATS guidance and maximize the quality scores for spirometry associated with methacholine challenge. Avoidance of enrollment of a subject during periods when their asthma is worsened by the inhalation of allergens may also reduce variability. Avoidance of LABA use within 3 weeks of enrollment (as per our study) and of frequent SABA use during the study may both reduce variability and enhance the ability to detect a dose–response by minimizing the influence of beta-agonist tolerance.

Another factor that could impact the variability (s) of a methacholine challenge-based LTE study is the method of administering the methacholine. The English Wright nebulizer, with 2 minutes of tidal volume breathing, was commercially unavailable for use in our study. Hence, we administered methacholine using the AeroEclipse RBAN with tidal breathing for 20 seconds. This was based upon reports published just before conduct of the study indicating that this would deliver a similar dose as 2 minutes of tidal breathing through the English Wright nebulizer.[27] The short duration of tidal breathing in our study could have increased variability of the delivered methacholine dose, as subjects would likely only take a small and variable number of breaths in a 20-second period of time.[40,41] Unfortunately, variability associated with the PC_{20} values obtained using these two nebulizers has not been directly compared. If future studies are conducted using the AeroEclipse RBAN it may be more appropriate to reduce the starting and final concentrations of methacholine as suggested by Coates et al.[42] to enable a 1-minute tidal breathing time rather than utilizing a short tidal breathing time. However, changes to the starting concentrations of methacholine will necessitate qualification of the lower concentrations of methacholine by their manufacturers.

More recently, a vibrating mesh nebulizer (Aerogen® Solo Nebulizer; Galway, Ireland) has been proposed as a replacement for the English Wright nebulizer.[43] In that study, the PC_{20} from each nebulizer was confirmed by repeated measures. Repeatability of the PC_{20} (indicated by intraclass correlation coefficient) for the Solo nebulizer was comparable with that of the English Wright nebulizer, as was the estimated dose delivered (provocative dose of methacholine causing a 20% drop in FEV_1). The most recent guidelines for the conduct of methacholine challenge studies[41] recommend tidal breathing of at least 1 minute to reduce the variability due to different breathing patterns. As the Solo nebulizer used by Davis et al.[43] required tidal breathing for 91–166 seconds to deliver the full methacholine dose, this nebulizer may produce lower variability of methacholine delivery than would the AeroEclipse RBAN used in our study. Thus, use of the Solo nebulizer may be preferred for future studies.

Additional factors to consider in the design of a dose–scale LTE study are the assumed true value for RP and the fold difference between high and low doses of the drug being tested. In this discussion, a value of RP = 1 (the best case) and a twofold difference in dose have been assumed. It is also possible to assume acceptably small differences in RP between products (RP = 0.95, for example), which will tend to increase sample size. In general, the effect of including a larger difference between high and low doses (for example, three- or fourfold difference rather than the twofold difference used in this study) depends on the shape of the dose–response curve. If the dose–response is approximately linear across this three- or fourfold range, then including the wider dose range in the study will reduce the required sample size. If, however, the dose–response begins to plateau beyond a twofold range, then including a wider range will decrease dose–response slope and increase the sample size. Given that the lowest possible dose of salmeterol, one inhalation containing 50 μg, appears to already be above the ED_{50}, a high dose that is three- or fourfold higher than this will be closer to the E_{max} asymptote, making the apparent linear slope (b) lower, the s/b ratio higher, and the resulting sample size larger.

The above discussion focuses on the effects of salmeterol 1 hour after dosing. Because salmeterol is a long-acting bronchodilator, the assessment of LTE between two salmeterol-containing products would ideally reflect peak effect and duration of action. While our results demonstrated that it may be difficult to use a methacholine challenge to assess LTE at the 1-hour time point (timed to coincide with peak effect), the use of data from the 6- or 10-hour time points to compare potency (to assess duration of action) would be even more difficult. Based on our 6-hour data, we estimated a sample size of $\sim$5000 subjects to establish LTE (using 0.80–1.25 BE limits, an estimated slope of 0.153, and a WS-SD [i.e. "s"] of 0.7109) or $\sim$1900 subjects using 0.67–1.50 BE limits. Conducting studies with sample sizes as large as these would clearly be impractical. Based on our 10-hour data, we could not estimate a sample size because the observed slope was negative (the low dose produced a greater response than the high dose). These results suggest that use of methacholine challenge methodology for comparing the duration of action of salmeterol products is not feasible. Alternative methods would be needed to assess

salmeterol LTE over the entire dosing interval. This may be the reason that current FDA guidance requires assessment of bronchodilation over salmeterol's entire dosing interval (serial FEV_1 over 12 hours after the first dose).[44]

The albuterol arm was included in our study as an intended positive control for identification of dose–response. Significant response to a twofold difference between low and high doses of albuterol has previously been demonstrated by Ahrens et al.,[31] Creticos et al.,[32] and Parameswaran et al.[33] (with estimated (b) values of 0.722, 0.976, and 0.871, respectively). However, as the main focus in our study was to assess the dose–response of salmeterol, the first methacholine challenge began at 1 hour poststudy drug dose. This was necessary to allow for salmeterol's comparatively slower onset of effect. This likely meant that the peak effect of albuterol had already occurred, and albuterol effects were already declining before this challenge was completed. In these other reported studies, the methacholine challenges were initiated 10–15 minutes postalbuterol administration and completed 15–30 minutes later.[32–33] Thus, the lower-than-expected slope observed for albuterol in our study (b=0.374) is likely due to the inherent design of the study rather than reflecting the pharmacology of albuterol.

In summary, results of the current study suggest that the dose–response slope (b) for salmeterol-induced protection against methacholine-induced bronchospasm, estimated to be 0.310, is shallower than slopes observed in the literature for albuterol and formoterol. As a result, successful use of this method for assessing dose–scale LTE of salmeterol products may be difficult.

This slope estimate and its relatively wide 95% CI (–0.135 to 0.754) are consistent with the relatively wide range of slopes available in the literature for salmeterol.[21,23,34] To resolve uncertainty concerning the "true" value of the slope for salmeterol, more precise estimates are needed.

Use of methacholine challenge to document LTE of test and reference orally inhaled salmeterol formulations 1 hour after dosing could be feasible, with sample sizes <100, if certain conditions are present: 0.67–1.50 BE limits are assumed, the "true" value of (b) is near the upper end of the CI for our estimate (and the upper end of the range of values observed in the literature), and careful attention is paid to reducing variability in the study results.

In the absence of all of these conditions, the study will require an impractically large sample size (>100 subjects). In this case, alternate models for demonstrating LTE, such as those presented in current FDA guidance,[44] will be needed.

Acknowledgments

The study was funded by Mylan, Inc. (Canonsburg, PA) provided funding for medical writing and editorial support. The authors wish to acknowledge Roger Hill, PhD, for medical writing and Paula Stuckart for editorial assistance in the preparation of the article (Ashfield Health care Communications, Middletown, CT). The authors acknowledge Bruce Williams (Mylan) for clinical operations related to the study, and also the investigators at the other clinical sites.

Author Disclosure Statement

R. Allan, S. Haughie, and J. Ward are employees of Mylan and have stock ownership in Mylan; R. Ahrens and S. Singh have no conflicts of interest.

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Received on December 10, 2018
in final form, May 9, 2019

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2.3 Local Therapeutic Equivalence Study

2.3.1 ATS 2019

Allan R, Kerwin EM, White MV, Miller SD, Haughie S, Ward JK, Ng D. Pulmonary Therapeutic Bioequivalence of Wixela™ Inhub™ and Advair® Diskus® in Adults With Asthma. American Journal of Respiratory and Critical Care Medicine 2019; 199: A2205. https://doi.org/10.1164/ajrccm-conference.2019.199.1_meetingabstracts.a2205

P786

Pulmonary Therapeutic Bioequivalence of Wixela™ Inhub™ and Advair Diskus® in Adults with Asthma

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BACKGROUND

- A combination of oral inhaled corticosteroids (ICS) and long-acting β_2-adrenergic agonists (LABAs) is recommended for patients with asthma not controlled with ICS alone and for patients with chronic obstructive pulmonary disease (COPD) at high risk for exacerbations[1-3]
- Fluticasone propionate (FP)/salmeterol dry powder inhaler is a widely prescribed ICS/LABA fixed-dose combination drug, marketed in the United States as Advair Diskus®[4,5]
- Wixela™ Inhub™ was developed as a substitutable generic equivalent to Advair Diskus®
- Study objective: To confirm the local (pulmonary) bioequivalence of Wixela Inhub (test) and Advair Diskus (reference) in adult patients with asthma.

METHODS

- Randomized, double-blind, placebo-controlled, parallel-group study (NCT02245672) in adults with mild-to-moderate asthma using forced expiratory volume in 1 second (FEV_1) to compare the therapeutic effect of FP/salmeterol (100/50 µg twice daily) following inhalation via test or reference products
- The study design consisted of a 3- to 4-week, single-blind, placebo run-in period followed by a 4-week, double-blind treatment period
- Patients discontinued their regular asthma medication (including ICS and LABAs) at screening, except for study-provided, short-acting β-agonist (SABA; prn)
- The study protocol was approved by Quorum Institutional Review Board and all patients provided written informed consent.

Key Inclusion Criteria

- Age ≥18 years with diagnosis of asthma ≥12 weeks according to National Asthma Education and Prevention Program guidelines[1]
- Baseline FEV_1 of 50% to 85% of predicted after ≥6 hours without short-acting bronchodilator use
- Improvement of ≥12% FEV_1 within 30 minutes of 360 µg albuterol
- Current nonsmokers (for >12 months and with smoking history of ≤10 pack-years).

Key Exclusion Criteria

- Respiratory condition other than asthma or allergic rhinitis
- Hospitalization for asthma within the past year
- Asthma exacerbation within the preceding 3 months
- Respiratory tract, sinus, or ear infection within the preceding 4 weeks.

Treatments

- Wixela Inhub 100/50 (test), Advair Diskus 100/50 (reference), or placebo administered in a double-blind, double-dummy manner
- 1 inhalation twice daily from each of their assigned inhalers for 4 weeks.

Assessments

Efficacy

- Spirometry assessments were completed at screening (Day −28), at run-in (Days −3 to −7), at −0.5, 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours on Day 1 of treatment, prior to dosing on Day 15, and on Day 29
  - To establish bioequivalence of the LABA component (salmeterol), the FEV_1 area under the effect curve over 12 hours (FEV_1 $AUEC_{0-12}$) after the first study dose on Day 1 was evaluated
  - The ICS component (FP) was evaluated by measuring trough FEV_1 after 28 days of twice-daily dosing.

Safety

- Safety assessments included adverse events and laboratory safety tests, vital signs (blood pressure and pulse rate), and electrocardiograms.

Statistical Analyses

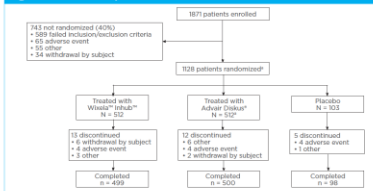
- Linear analysis of covariance (ANCOVA) models were fitted for each end point. Least squares (LS) means were derived for each treatment, and LS mean differences were calculated for test versus placebo for each efficacy end point
- Both test and reference treatments had to show statistically significant superiority (P <0.05) compared with placebo
- LS means from the ANCOVA models for both efficacy end points were used to generate LS mean test versus reference ratios for which 90% confidence intervals (CIs) were calculated using Fieller's theorem
- The 90% CIs for the test/reference ratios of LS means of both FEV_1 AUC_{0-12} on Day 1 and trough FEV_1 on Day 29 were required to be within the range of 0.80 to 1.25 for salmeterol and FP, respectively.

RESULTS

Patients

- A total of 1127 patients were randomized to receive test, reference, or placebo at 97 sites in the United States (**Figure 1**)

Figure 1. Patient Disposition



*One of the patients randomized to the reference product (Advair Diskus®) was not treated because of a failure to meet the inclusion/exclusion criteria, resulting in 512 patients treated with Advair Diskus and 1127 total treated patients.

- Compliance was ≥95% across groups
- Baseline characteristics were comparable across treatment groups (**Table 1**).

Table 1. Baseline Patient Demographics

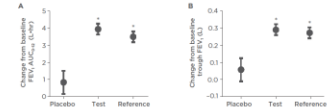
	T 100/50 µg (n = 512)	R 100/50 µg (n = 512)	Placebo (n = 103)	Total (N = 1127)
Age, mean (range)				
Years	42.6 (18-84)	42.5 (18-81)	43.5 (18-77)	42.6 (18-84)
Males				
n	206 (40.2)	203 (39.6)	39 (37.9)	448 (39.8)
Race, n (%)				
White	378 (73.8)	372 (72.7)	73 (70.9)	823 (73.0)
Black	92 (18.0)	98 (19.1)	22 (21.4)	212 (18.8)
Other	42 (8.2)	42 (8.2)	8 (7.8)	92 (8.2)
Duration of asthma, years				
Mean (range)	26.9 (0.3-79.8)	27.1 (0.8-70.7)	28.3 (0.6-65.8)	27.1 (0.3-79.8)
Prior asthma medication, n (%)				
ICS	86 (16.8)	97 (18.9)	20 (19.4)	203 (18.0)
ICS/LABA	189 (36.9)	175 (34.2)	40 (38.8)	404 (35.8)
Prebronchodilator spirometry				
n	512	511	103	1126
FEV_1, mean (SD), L	2.33 (0.61)	2.32 (0.61)	2.28 (0.59)	2.32 (0.61)
FEV_1, mean (SD), % predicted	69.92 (8.64)	70.05 (8.83)	69.48 (9.03)	69.94 (8.76)
FEV_1 reversibility				
n	511	511	103	1125
Improvement, mean (SD), %	23.23 (15.37)	24.43 (16.80)	23.97 (16.88)	23.84 (16.17)

FEV_1 indicates forced expiratory volume in 1 second; ICS, inhaled corticosteroids; LABA, long-acting β-agonist; R, reference product (Advair Diskus®); SD, standard deviation; T, test product (Wixela™ Inhub™).

Efficacy

- Compared with placebo, test and reference both increased Day 1 FEV_1 AUC_{0-12} (LS mean increase: 3.134 L·hr and 2.677 L·hr, respectively; P <0.0001; **Figure 2A**) and Day 29 trough FEV_1 (LS mean increase: 0.235 L and 0.215 L, respectively; P <0.0001; **Figure 2B**)

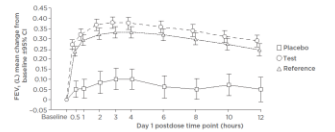
Figure 2. Day 1 (A) and Day 29 (B) Improvement in Lung Function Following Treatment with Test and Reference Fluticasone Propionate/Salmeterol and Placebo[a]



Baseline-subtracted data presented as least squares mean ±95% confidence interval.
[a]Test indicates Wixela™ Inhub™ 100/50 µg; reference, Advair Diskus® 100/50 µg. *Difference from placebo, P <0.0001.
AUC_{0-12} indicates area under the effect curve over 12 hours; FEV_1, forced expiratory volume in 1 second.

- Test and reference demonstrated similar FEV_1 responses, with overlapping 95% CIs over the 12 hours of serial spirometry measures made on Day 1, with clear separation from placebo (**Figure 3**)

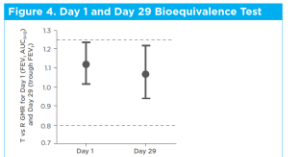
Figure 3. Change from Baseline in FEV, Over Time on Day 1 with Placebo, Test, and Reference Fluticasone Propionate/Salmeterol[a]



[a]Test FP/S indicates Wixela™ Inhub™; reference FP/S, Advair Diskus®.
Data are mean ±95% CIs.
CI indicates confidence interval; FEV_1, forced expiratory volume in 1 second; FP/S, fluticasone propionate/salmeterol.

- LS mean test/reference ratios (90% CIs) for Day 1 FEV_1 AUC_{0-12} and Day 29 trough FEV_1 were 1.120 (1.016, 1.237) and 1.069 (0.938, 1.220), respectively, indicating bioequivalence for both end points (**Figure 4**).

Figure 4. Day 1 and Day 29 Bioequivalence Test



T/R FEV_1 LS mean ratio ±90% CI for both Day 1 (FEV_1 AUC_{0-12}) and Day 29 (trough FEV_1) coprimary end points were both within the standard bioequivalence limits, shown as dotted lines.
AUC_{0-12} indicates area under the effect curve over 12 hours postdose; CI, confidence interval; GMR, geometric mean ratios; FEV_1, forced expiratory volume in 1 second; LS, least squares; R, reference product (Advair Diskus®); T, test product (Wixela™ Inhub™).

Safety

- Both test and reference products were well-tolerated
- Adverse events were generally mild and occurred with similar frequency across active treatment groups (**Table 2**)
- No clinically significant changes to laboratory safety tests, vital signs, or electrocardiograms were observed.

Table 2. Treatment-Emergent Adverse Events[a] by System Organ Class and Preferred Term (Safety Set)

	T 100/50 µg (n = 512)	R 100/50 µg (n = 512)	Placebo (n = 103)
Any treatment-emergent adverse events	72 (14.1)	75 (14.6)	15 (14.6)
Infections and infestations	34 (6.6)	38 (7.4)	5 (4.9)
Upper respiratory tract infection	7 (1.4)	11 (2.1)	0
Nasopharyngitis	3 (0.6)	7 (1.4)	2 (1.9)
Respiratory, thoracic, and mediastinal disorders	15 (2.9)	25 (4.9)	7 (6.8)
Asthma	7 (1.4)	10 (2.0)	5 (4.9)
Oropharyngeal pain	3 (0.6)	5 (1.0)	1 (1.0)
Gastrointestinal disorders	6 (1.2)	5 (1.0)	1 (1.0)
Nervous system disorders	6 (1.2)	6 (1.2)	0
Headache	3 (0.6)	5 (1.0)	0
Musculoskeletal and connective tissue disorders	4 (0.8)	4 (0.8)	1 (1.0)
Injury, poisoning, and procedural complications	2 (0.4)	3 (0.6)	1 (1.0)
Cardiac disorders	3 (0.6)	0	1 (1.0)

[a]Reported in ≥1% of patients in the overall study population and/or ≥1% of any treatment group.
R indicates reference product (Advair Diskus®); T, test product (Wixela™ Inhub™).

CONCLUSIONS

- Wixela Inhub 100/50 demonstrated local (pulmonary) bioequivalence to Advair Diskus based on clinical end points
- Wixela Inhub demonstrated a comparable safety profile to Advair Diskus
- Wixela Inhub is approved by the US Food and Drug Administration as a substitutable generic equivalent to Advair Diskus for the treatment of asthma and COPD.

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DISCLOSURE: Richard Allan, Scott Haughie, Jonathan K. Ward, and Dik Ng are employees of Mylan Pharma UK Ltd., Sandwich, Kent, United Kingdom. Edward M. Kerwin is a consultant for Amphastar, AstraZeneca, Boehringer Ingelheim, Forest, GlaxoSmithKline, Mylan, Novartis, Sunovion, Teva, and Theravance Biopharma. Martha V. White is a consultant for Mylan Specialty L.P. and Merck; board member for the Allergy & Asthma Network Mothers of Asthmatics; received grants, fees, or support from Mylan Specialty L.P., Merck, CSL Behring, AstraZeneca, Shire, Salix, Novartis, Teva, GlaxoSmithKline, Dyax, and Meda. S. David Miller has nothing to disclose.

2.3.2 Journal of Aerosol Medicine and Pulmonary Drug Delivery 2020

Ng D, Kerwin EM, White MV, Miller SD, Haughie S, Ward JK, Allan R. Clinical Bioequivalence of Wixela Inhub and Advair Diskus in Adults With Asthma. Journal of aerosol medicine and pulmonary drug delivery. 2020 Apr;33(2):99-107. PubMed PMID: 31634023. Epub 2019/10/22. eng.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2019.1547

JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 33, Number 2, 2020
Mary Ann Liebert, Inc.
Pp. 99–107
DOI: 10.1089/jamp.2019.1547

Clinical Bioequivalence of Wixela Inhub and Advair Diskus in Adults With Asthma

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Abstract

Background: Wixela® Inhub® is a dry powder inhaler approved as a generic equivalent to Advair® Diskus® (fluticasone propionate [FP]/salmeterol fixed-dose combination) for patients with asthma or chronic obstructive pulmonary disease (COPD). This study aimed at confirming the local (lung) therapeutic equivalence of both the FP and salmeterol components of Wixela Inhub (test [T]) to Advair Diskus (reference [R]) after inhalation.
Methods: This randomized, double-blind, double-dummy, placebo-controlled, parallel-group study in patients ≥18 years with mild-to-moderate persistent asthma compared the local therapeutic equivalence (using forced expiratory volume in 1 second [FEV_1]) of FP/salmeterol (100/50 μg) after inhaled delivery via T and R.
Results: Randomized patients ($N = 1127$) received T ($n = 512$), R ($n = 512$), or placebo ($n = 103$). T and R significantly increased day 1 FEV_1 area under the effect curve over 12 hours of the change from baseline ($AUC_{[0-12]}$) and day 29 trough FEV_1 over placebo, indicating that these endpoints were sufficiently sensitive for evaluation of bioequivalence. On day 1, T and R each increased FEV_1 $AUC_{(0-12)}$ over placebo (3.134 L•h [T], 2.677 L•h [R]; each $p < 0.0001$). Following twice-daily dosing for 28 days, T and R also each increased trough FEV_1 (measured on day 29) over placebo (235 mL [T], 215 mL [R]; each $p < 0.0001$). Least-squares mean T/R ratios (90% confidence intervals) for day 1 FEV_1 $AUC_{(0-12)}$ and day 29 trough FEV_1 were 1.120 (1.016–1.237) and 1.069 (0.938–1.220), respectively, indicating that T and R were bioequivalent for both co-primary endpoints. FP/salmeterol was well tolerated when administered via either T or R.
Conclusions: These results demonstrate that the therapeutic effects of Wixela Inhub are bioequivalent to Advair Diskus in the lung. Wixela Inhub represents a therapeutically equivalent new FP/salmeterol treatment option for use in the treatment of asthma and COPD.

Keywords: Advair Diskus, Wixela Inhub, fluticasone propionate, salmeterol, local bioequivalence, generic drugs

Introduction

Inhaled corticosteroids (ICS) and long-acting β_2-adrenergic agonists (LABA) are widely used, safe, and effective anti-inflammatory and bronchodilator agents, respectively, for the treatment of asthma and chronic obstructive pulmonary disease (COPD).[1,2] Current guidelines recommend the administration of fixed-dose ICS/LABA combination drugs as maintenance therapy in asthma and COPD.[3–5] Advair Diskus (GlaxoSmithKline, Research Triangle Park, NC) is a widely prescribed ICS/LABA combination drug (fluticasone propionate [FP]/salmeterol [as xinafoate]; FPS) for asthmatic patients not controlled with ICS alone and for COPD patients at high risk of exacerbations.[1,2] With the expiration of the US patent for Advair Diskus in 2016, several generic versions are currently advancing toward regulatory approval.[6–9] The most advanced of these, in terms of drug development stage in the United States, is Wixela Inhub, composed of FPS inhalation powder (Mylan, Inc., Canonsburg, PA) predispensed in a multidose inhaler (Inhub; Mylan, Inc.),[10,11] which was recently approved by the US Food and Drug Administration (FDA).[12]

[1]Mylan Pharma UK Ltd., Sandwich, Kent, United Kingdom.
[2]Clinical Research Institute of Southern Oregon, Medford, Oregon.
[3]Institute for Asthma and Allergy, Wheaton, Maryland.
[4]Northeast Medical Research Associates, Inc., North Dartmouth, Massachusetts.

The FDA guidelines for the development of generic FPS inhalers require, as part of a weight of evidence approach (together with *in vitro* pharmaceutical equivalence and systemic pharmacokinetic bioequivalence), local (lung) therapeutic equivalence studies that, in total, demonstrate therapeutic equivalence to Advair Diskus.[13] Clinical development of Wixela Inhub followed these guidelines, and recent studies confirmed the pharmacokinetic bioequivalence of single doses of Wixela Inhub for each of the three authorized Advair Diskus dose strengths.[14] Here, we report the results of the FDA-mandated local therapeutic equivalence study (NCT02245672) in adult patients with asthma.

The objective of this study was to compare the clinical efficacy of the FP and salmeterol components of Wixela Inhub 100/50 μg and Advair Diskus 100/50 μg by using spirometry. To evaluate bioequivalence of the bronchodilator component (salmeterol), forced expiratory volume in 1 second (FEV_1) was measured repeatedly for 12 hours after the first study dose. The anti-inflammatory component (FP) was then evaluated by measuring trough FEV_1 after 28 days of twice-daily dosing.

Materials and Methods

In this article, "test product" (T) and "reference product" (R) are defined as follows: T is Wixela Inhub (FPS administered via the Inhub inhaler), and R is Advair Diskus.

Study design and conduct

This multicenter, randomized, double-blind, double-dummy, placebo-controlled, parallel-group study was conducted between October 22, 2014 and July 10, 2015 at 101 U.S. centers. The study consisted of a 21–28-day single-blind, placebo run-in period followed by a 4-week double-blind treatment period. The primary objective was to assess the local therapeutic equivalence of T and R using spirometry.

The study conformed to appropriate ethical guidelines and was conducted in accordance with the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for good clinical practice[15] and the code of ethics of the World Medical Association's Declaration of Helsinki.[16] Quorum Institutional Review Board approved the study protocol, and all patients provided written informed consent.

Patients and treatments

Consistent with the FDA guidelines for a clinical endpoint study to assess local therapeutic equivalence of FPS products,[13] key inclusion criteria included age ≥18 years with diagnosis of asthma ≥12 weeks according to National Asthma Education and Prevention Program guidelines[3]; a mean baseline FEV_1 of 50%–85% predicted after ≥6 hours without short-acting bronchodilator use; postbronchodilator reversibility (percent improvement) of ≥12% within 30 minutes of 360 μg albuterol; and current nonsmokers (with no smoking history within the past 12 months and a total smoking history of ≤10 pack-years). Patients were excluded if they had a respiratory condition or another severe progressive disease other than asthma and allergic rhinitis, were hospitalized for asthma within the past year or had an asthma exacerbation within the preceding 3 months, or had a respiratory tract, sinus, or ear infection within the preceding 4 weeks.

After completion of the placebo run-in period of 21–28 days (all subjects receiving placebo for Wixela Inhub, one inhalation twice daily), eligible patients were randomly assigned to one of three groups (T, R, or placebo) in a 5:5:1 ratio by using a subject identification number assigned via an automated interactive voice-/web-response system. Each treatment was administered in a double-blind, double-dummy manner (with placebo inhalers matched to T or R used for the placebo group and to maintain the blind in the active treatment groups). Patients were required to take one inhalation twice daily from each of their assigned inhalers for 4 weeks.

Advair Diskus and Wixela Inhub contained qualitatively and quantitatively equivalent formulations of both active pharmaceutical ingredients (a fixed-dose combination of micronized crystalline FP and salmeterol [as xinafoate]) and inactive excipients (lactose monohydrate). The Diskus and Inhub inhalers were medium resistance passive dry powder inhalers, contained 60 premetered doses of FP and salmeterol, and had the same operating procedures.[17]

The pharmaceutical performance of multiple commercial batches of R (Advair Diskus) was tested to characterize the performance using *in vitro* methods, including measures of delivered dose and aerodynamic particle size distribution.[18] The single batch of Advair Diskus used in the study was representative of the median of the Advair Diskus commercial batch population.

The T drug (Wixela Inhub) was also tested to characterize performance by using *in vitro* methods. The two batches of Wixela Inhub used in the study were manufactured at commercial scale, representative of the product in terms of *in vitro* performance, and were age-matched to be within 3 months of the batch of Advair Diskus used in the study.

The placebo for Advair Diskus used commercial stock of Advair Diskus inhalers. Specifically, these were opened under good manufacturing practice (GMP) conditions, the blister strips containing FP and salmeterol were replaced with strips containing lactose, and the inhalers were subsequently closed and packaged for clinical trial use. The placebo for Wixela Inhub used Inhub inhalers containing lactose alone.

Assessments

Spirometry assessments were completed at screening (day −28); at run-in (day −3 to −7); at −0.5, 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours on day 1 of treatment, before dosing on day 15, and on day 29. Primary endpoints were the area under the effect curve over 12 hours (FEV_1 $AUC_{(0-12)}$) for the change from baseline (CFB) in FEV_1 on day 1, the first day of treatment, and CFB in trough FEV_1 on day 29 after 4 weeks of dosing. Safety assessments included adverse events (AEs) and laboratory safety tests, vital signs (blood pressure and pulse rate), and electrocardiograms.

Statistical analysis

The safety set was defined as all randomly assigned patients who had taken ≥1 dose of study drug and for whom postdose safety data were available. The full analysis set

(FAS) was defined as all randomly assigned patients who had taken ≥1 dose of study drug and had provided data for either co-primary efficacy endpoint (FEV_1 $AUC_{(0-12)}$ or day 29 CFB in trough FEV_1). The per-protocol set (PPS) was defined as all patients in the FAS who had not violated or deviated from the protocol in a manner that could have affected the outcome of the FEV_1 assessments for both co-primary efficacy endpoints. The FAS was the primary analysis set used to establish assay sensitivity (the ability to discriminate both T and R treatments from placebo), whereas the PPS was the primary analysis set used to establish bioequivalence between T and R treatments.

The original sample size for this study was calculated by assuming a 92% between-subject coefficient of variation (CV; expected mean and standard deviation [SD] for CFB in trough FEV_1 on day 29 of 0.51 and 0.47 L, respectively). This led to an estimated sample size of 380 subjects for T and 380 subjects for R to give 90% power to demonstrate clinical bioequivalence (T/R ratio and 90% confidence interval [CI] wholly contained within the 0.80–1.25 limits) between T and R, assuming a true T/R ratio of 1.0. The original sample size for the placebo group ($n=76$) was based on performing a two-sided significance test at the 5% level with 99.9% power, an SD of 0.47 L, and a true mean difference from each active arm of 0.3 L for CFB in trough FEV_1 on day 29 (allocation ratio of 5:1 for active to placebo). Therefore, the total number of subjects required to complete the study was 836 subjects (380 [T], 380 [R], and 76 [placebo]). This was rounded up to 935 subjects required to be randomized (425 [T], 425 [R], and 85 [placebo]) to allow for ~10% dropout postrandomization. The process was repeated for the FEV_1 $AUC_{(0-12)}$ endpoint.

As sample size assumptions were based on historical reports of the effect of Advair Diskus in similar but not identical patient populations,[19–21] a blinded sample size re-estimation (BSSR), which was prespecified in the protocol, was conducted for this study when 286 subjects had completed the study. The assumptions made about the CV for the original sample size calculation for CFB in trough FEV_1 on day 29 were not supported by the aggregate data used for the BSSR. Therefore, the sample size was recalculated and revised accordingly. The total sample size to complete the study was revised from 836 to 990 subjects (450 [T]+450 [R]+90 [placebo]) requiring ~1100 subjects to be randomized (the maximum allowable in the protocol). The revised sample size for the active treatment arms in this study was based on at least 81% power and assumed 112% between-subject CV (expected mean and SD for CFB in trough FEV_1 on day 29 of 0.26 and 0.29 L, respectively, as assessed from the BSSR results). The sample size for the placebo group ($n=90$) was chosen to maintain the allocation ratio (5:5:1).

Determination of assay sensitivity was required for the bioequivalence results to be valid. To evaluate assay sensitivity, comparisons of T versus placebo and R versus

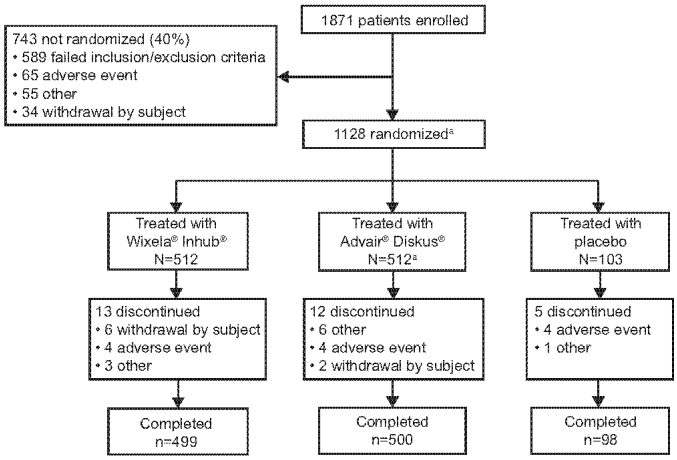

FIG. 1. Patient flow. One of the patients randomized to the reference product (Advair Diskus) was not treated because of a failure to meet the inclusion/exclusion criteria, which resulted in 512 patients treated with Advair Diskus and 1127 total treated patients. All 1127 patients receiving a study treatment (Wixela Inhub [T], Advair Diskus [R], or placebo) were analyzed for safety. The FAS consisted of 1122 patients (509 [R], 511 [T], and 102 [placebo]); 5 treated patients (3 [T], 1 [R], and 1 [placebo]) were excluded from the FAS due to being enrolled into the study twice and so the second participations were excluded from the FAS. The PPS consisted of 1105 patients (502 [T and R], 101 [placebo]); 17 patients (7 [T], 9 [R], and 1 [placebo]) were excluded from the PPS due to ≥1 significant protocol deviation. FAS, full analysis set; PPS, per protocol set; R, reference product (Advair Diskus); T, test product (Wixela Inhub).

placebo were performed for day 1 FEV_1 $AUC_{(0–12)}$ and day 29 trough FEV_1. A linear analysis of covariance (ANCOVA) model was fitted for each endpoint. Least-squares (LS) means were derived for each treatment, and LS mean differences were calculated for T versus placebo and for R versus placebo for each efficacy endpoint. Assay sensitivity was demonstrated if the *p*-values for all four comparisons (active treatment versus placebo for each FEV_1 efficacy endpoint) were each less than 0.05.

To assess bioequivalence, LS means (one for T and one for R) from the ANCOVA models were used to generate T/R ratios for LS means for FEV_1 $AUC_{(0–12)}$ and trough FEV_1 efficacy endpoints. Overall, 90% CIs were calculated by using Fieller's theorem.[22] To demonstrate bioequivalence, the 90% CIs for the FEV_1 $AUC_{(0–12)}$ and trough FEV_1 T/R ratios were each required to be wholly contained within the interval 0.80–1.25 (i.e., 80%–125%).

Results

Patients

Of the 1871 enrolled patients, 1127 (60%) were randomized and treated, with 512 patients each receiving T and R and 103 patients receiving placebo (Fig. 1). The most common reason for exclusion of enrolled patients was failure to meet baseline spirometry criteria. All 1127 patients receiving a study treatment were analyzed in the safety set. The FAS consisted of 1122 patients (509 [R], 511 [T], and 102 [placebo]) whereas the PPS consisted of 1105 patients (502 [T], 502 [R], and 101 [placebo]). Of the randomized patients, 97% ($n=1097$) completed the 4-week treatment period.

Baseline demographic and clinical characteristics were well matched across treatment groups (Table 1). In the total study population (safety set), mean age was 42.6 years, 40% of patients were male, mean duration (range) of asthma was 27.1 (0.3–79.8) years, mean (SD) FEV_1 percent predicted was 69.94 (8.76), and mean percent improvement in FEV_1 postbronchodilator was 23.84 (16.17). A total of 607 (54%) participants were taking ICS or ICS/LABA medication for maintenance of their asthma before entering the washout period of the study. Overall, 96% of patients in the safety set were compliant (i.e., within 75%–125% of per-protocol inhaler use) with treatment during the double-blind phase, and compliance was comparable for T (96%), R (95%), and placebo (97%).

Efficacy

Both active treatments substantially improved day 1 FEV_1 by the first time point measured (mean CFB, T, 270 mL; R, 237 mL at 30 minutes postdose; Fig. 2); there was minimal improvement with placebo (mean CFB 52 mL). The maximum increase in FEV_1 was observed at 3 hours postdose (mean CFB, T, 379 mL; R, 333 mL; placebo, 101 mL). T and R demonstrated similar FEV_1 responses, with overlapping 95% CIs over the 12 hours of serial spirometry measures made on day 1 with clear separation from placebo (Fig. 2). LS mean increases in day 1 FEV_1 $AUC_{(0–12)}$ were comparable for T and R (3.953 and 3.496 L•h, respectively) and less for placebo 0.819 L•h (Fig. 3A and Table 2 [FAS]).

Both active treatments also substantially improved day 29 trough FEV_1. The LS mean increases in CFB in trough FEV_1 after twice-daily dosing for 28 days were 293 mL (T), 272 mL (R), and 58 mL (placebo) (Fig. 3B and Table 2 [FAS]).

Table 1. Baseline Demographic and Clinical Characteristics (Safety Set)

Characteristic	*T (n=512)*	*R (n=512)*	*Placebo (n=103)*	*Total (n=1127)*
Age, mean (range), years	42.6 (18–84)	42.5 (18–81)	43.5 (18–77)	42.6 (18–84)
Males, *n* (%)	206 (40.2)	203 (39.6)	39 (37.9)	448 (39.8)
Race, *n* (%)				
White	378 (73.8)	372 (72.7)	73 (70.9)	823 (73.0)
Black/African American	92 (18.0)	98 (19.1)	22 (21.4)	212 (18.8)
Other	42 (8.2)	42 (8.2)	8 (7.8)	92 (8.2)
BMI, mean (SD), kg/m^2	29.4 (6.0)	29.1 (5.9)	29.4 (5.9)	29.3 (5.9)
Duration of asthma, mean (range), years	26.9 (0.3–79.8)	27.1 (0.8–70.7)	28.3 (0.6–65.8)	27.1 (0.3–79.8)
Prior asthma medication, *n* (%)				
ICS or ICS/LABA	275 (53.7)	272 (53.1)	60 (58.3)	607 (53.9)
ICS	86 (16.8)	97 (18.9)	20 (19.4)	203 (18.0)
ICS/LABA	189 (36.9)	175 (34.2)	40 (38.8)	404 (35.8)
Prebronchodilator spirometry				
n	512	511	103	1126
FEV_1, mean (SD), L	2.33 (0.61)	2.32 (0.61)	2.28 (0.59)	2.32 (0.61)
FVC, mean (SD), L	3.46 (1.00)	3.41 (0.95)	3.42 (0.94)	3.43 (0.97)
FEV_1/FVC, mean (SD), %	68.60 (9.05)	69.97 (9.30)	67.88 (9.39)	68.70 (9.19)
FEV_1, mean (SD), % predicted	69.92 (8.64)	70.05 (8.83)	69.48 (9.03)	69.94 (8.76)
FEV_1 reversibility				
n	511	511	103	1125
Improvement, mean (SD), %	23.23 (15.37)	24.43 (16.80)	23.97 (16.88)	23.84 (16.17)
Reversibility, mean (SD), L	0.53 (0.29)	0.55 (0.32)	0.53 (0.30)	0.54 (0.30)

BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting β-agonist; R, reference product (Advair Diskus); SD, standard deviation; T, test product (Wixela Inhub).

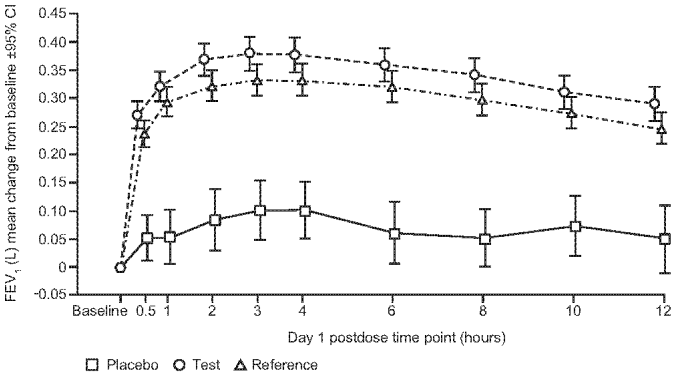

FIG. 2. Change from baseline in FEV_1 over time on day 1 with placebo (open squares), test (open circles), and reference (open triangles) FPS. Test FPS, Wixela Inhub; reference FPS, Advair Diskus. Data are mean and 95% CI. CI, confidence interval; FEV_1, forced expiratory volume in 1 second. FPS, fluticasone propionate/salmeterol.

LS mean increases over placebo in day 1 FEV_1 $AUC_{(0-12)}$ were 3.134 L•h (T) and 2.677 L•h (R), each $p < 0.0001$ versus placebo (Table 2 [FAS]), demonstrating clear clinical efficacy for the first dose of both active treatments. Both active treatments also significantly increased trough FEV_1 over placebo after twice-daily dosing for 28 days with day 29 CFB in trough FEV_1 of 235 mL [T] and 215 mL [R], each $p < 0.0001$ (Table 2 [FAS]).

As both T and R significantly increased day 1 FEV_1 $AUC_{(0-12)}$ and day 29 trough FEV_1 over placebo ($p < 0.0001$; Table 2), the prespecified primary analysis criteria for assay sensitivity were met.

Bioequivalence was then assessed, and the T/R ratios for LS means (90% CIs) for day 1 FEV_1 $AUC_{(0-12)}$ and day 29 trough FEV_1 were 1.120 (1.016–1.237) and 1.069 (0.938–1.220), respectively (Table 2 [PPS]). As the 90% CIs for day 1 FEV_1 $AUC_{(0-12)}$ and day 29 CFB in trough FEV_1 were between 0.80 and 1.25 (Fig. 4) for both endpoints, this indicated that T and R were bioequivalent on both endpoints.

Safety

Treatment-emergent AEs occurred in 14.4% of patients in the safety set, with individual AEs displaying a similar incidence across the three treatment groups (Table 3). The percentage of asthma-related AEs was higher in the placebo group (4.9%) and lower and comparable in both active treatment groups (T, 1.4%; R, 2.0%). The percentage of discontinuations was also higher in the placebo group (4.9%) compared with the active treatment groups (T, 2.5%; R, 2.3%). The most commonly reported AEs were infections and respiratory disorders. No serious AEs or deaths occurred during the study period. AEs associated with FPS, such as oral candidiasis and dysphonia, occurred with a similarly low incidence in the T and R groups (candidiasis: 0.8% vs. 0.4%; dysphonia: 0.2% vs. 0.6%, respectively) and did not occur at all in the placebo group. A very low incidence (<1%) of AEs categorized as cardiac disorders was observed, all of which were mild in intensity, did not require

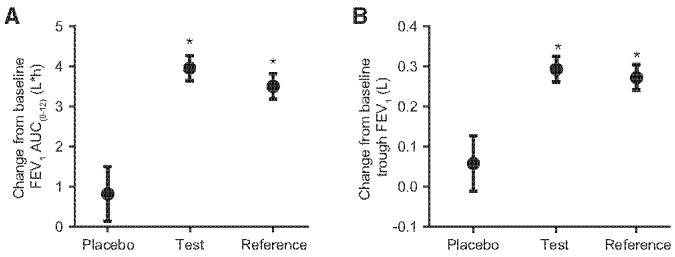

FIG. 3. Day 1 **(A)** and day 29 **(B)** improvement in lung function after treatment with test and reference FPS and placebo. Baseline-subtracted data presented as LS mean and 95% CI. Test, Wixela Inhub; Reference, Advair Diskus. *Difference from placebo $p < 0.0001$. $AUC_{(0-12)}$, area under the effect curve over 12 hours; LS, least squares.

Table 2. Change from Baseline in FEV_1 $AUC_{(0–12)}$ on Day 1 and Trough FEV_1 on Day 29 (FAS and PPS)

	FAS (primary analysis for assay sensitivity)			*PPS (primary analysis for bioequivalence)*		
Treatment	*T*	*R*	*Placebo*	*T*	*R*	*Placebo*
Day 1 change from baseline in FEV_1 $AUC_{(0–12)}$						
Model-adjusted FEV_1 $AUC_{(0–12)}$,[a] L•h						
n	508	510	102	497	494	94
LS mean (SE)	3.953 (0.161)	3.496 (0.160)	0.819 (0.348)	3.973 (0.170)	3.541 (0.159)	0.840 (0.298)
95% CI	3.638 to 4.269	3.183 to 3.809	0.137 to 1.501	3.639 to 4.308	3.228 to 3.854	0.248 to 1.432
Difference from placebo[a]						
LS mean (SE)	3.134 (0.376)	2.677 (0.377)		3.133 (0.335)	2.701 (0.331)	
95% CI	2.396 to 3.872	1.938 to 3.417		2.472 to 3.795	2.047 to 3.3556	
p	<0.0001	<0.0001		<0.0001	<0.0001	
Equivalence test						
LS mean FEV_1 T/R ratio	1.130			1.120		
90% CI	1.025 to 1.246			1.016 to 1.237		
Day 29 change from baseline in trough FEV_1						
Model-adjusted change from baseline,[a] L						
n	504	505	100	498	497	99
LS mean (SE)	0.293 (0.016)	0.272 (0.016)	0.058 (0.035)	0.291 (0.016)	0.273 (0.016)	0.057 (0.036)
95% CI	0.261 to 0.325	0.241 to 0.304	–0.012 to 0.127	0.259 to 0.323	0.241 to 0.305	–0.012 to 0.127
Difference from placebo[b]						
LS mean (SE)	0.235 (0.038)	0.215 (0.038)		0.234 (0.038)	0.215 (0.038)	
95% CI	0.161 to 0.310	0.140 to 0.289		0.159 to 0.309	0.140 to 0.291	
p	<0.0001	<0.0001		<0.0001	<0.0001	
Equivalence test						
LS mean trough FEV_1 T/R ratio	1.079			1.069		
90% CI	0.948 to 1.230			0.938 to 1.220		

[a]Based on ANCOVA.
[b]Difference of LS means (T minus placebo, R minus placebo) from the ANCOVA model.
ANCOVA, analysis of covariance; $AUC_{(0–12)}$, area under the effect curve over 12 hours postdose; CI, confidence interval; FAS, full analysis set; FEV_1, forced expiratory volume in 1 second; LS, least squares; PPS, per protocol set; R, reference product (Advair Diskus); SE, standard error; T, test product (Wixela Inhub).

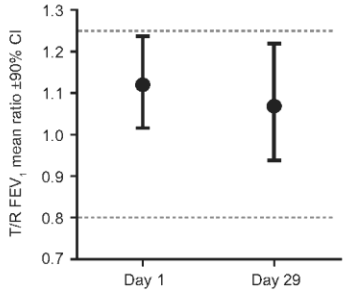

FIG. 4. Day 1 and day 29 bioequivalence test. T/R FEV_1 LS mean ratio and 90% CI for both day 1 (FEV_1 $AUC_{(0–12)}$) and day 29 (trough FEV_1) co-primary endpoints were within the standard bioequivalence limits, shown as dotted lines. $AUC_{(0–12)}$, area under the effect curve over 12 hours post-dose; R, reference product (Advair Diskus); T, test product (Wixela Inhub).

intervention, and did not result in patients being withdrawn from the study. There were no clinically significant changes to laboratory safety tests, vital signs, or electrocardiograms.

Discussion

Wixela Inhub was recently approved by the FDA as a fixed-dose FPS powder for oral inhalation to provide a generic equivalent to Advair Diskus. This study, recommended by the FDA for the clinical development of generic inhaled drugs containing FP and salmeterol powder,[13] confirmed the local therapeutic equivalence of both the FP and salmeterol components of Wixela Inhub (T) after inhalation of 100/50 μg FPS, the lowest approved dose strength for Advair Diskus (R). Further, FPS administered as Wixela Inhub demonstrated a comparable safety profile to Advair Diskus.

A direct comparison of these results with those reported for the original Advair Diskus pivotal trials can be challenging, not only due to the expected limitations inherent in comparing between studies[23] but also because of fundamental changes in the management of asthma itself over the past 20 years.[24] As more asthma patients are treated with ICS and ICS/LABA therapy, the patients willing and able to participate in placebo-controlled studies may have a milder phenotype of asthma than those from 20 years ago and, hence, the opportunity to observe the magnitude of changes in lung function previously reported may be limited. A total of 54% of participants were taking ICS or ICS/LABA medication before the washout period in this study, of whom approximately one-third were taking an ICS without a LABA, and the remaining two-thirds were taking an ICS with a LABA. Thus, although the results (day 1 CFB in FEV_1 $AUC_{(0–12)}$: 3.95 L•h [T] and 3.50 L•h [R]; day 29 CFB in trough FEV_1: 293 mL [T] and 272 mL [R]) are lower in absolute magnitude of lung function improvement than those originally reported for the same dose of Advair Diskus (5.81 L•h and 510 mL, respectively),[19] the findings are otherwise consistent.

The day 1 and day 29 spirometry data were also comparable with those reported for the OT329 SOLIS bioequivalence study, which used an almost identical study design.[7] For example, the day 1 CFB in FEV_1 $AUC_{(0–12)}$ for T (3.95 L•h) and R (3.50 L•h) were similar with respect to the magnitude of change with the corresponding day 1 values for OT329 SOLIS and Advair Diskus (3.72 and 3.55 L•h, respectively). In addition, the day 29 placebo-corrected CFB in trough FEV_1 for T (235 mL) and R (215 mL) in this study are more similar to the corresponding day 29 values for OT329 SOLIS and Advair Diskus (168 and 163 mL, respectively) than to historical studies. The consistency of spirometry data across these more recently conducted studies suggests that the study design is robust, and the results are reproducible and representative of treatment effects in this population of asthma patients.

The design of this study was consistent with other FPS local therapeutic equivalence studies,[7,9,21] and they adhered to the FDA guidelines for evaluation of local therapeutic equivalence for FPS products.[13] The use of the lowest of three dose strengths of the FP component approved for Advair Diskus was appropriate, because it was the most likely to identify any treatment differences in FP between T and R and consistent with the FDA guidance. The use of higher dose strengths, which elicit maximal responses of FPS in many patients,[25] might have masked potential differences between T and R and

TABLE 3. TREATMENT-EMERGENT ADVERSE EVENTS BY SYSTEM ORGAN CLASS AND PREFERRED TERM (SAFETY SET)

Patients with AE, n *(%)*	*T* (n=*512*)	*R* (n=*512*)	*Placebo* (n=*103*)
Any treatment-emergent AE	72 (14.1)	75 (14.6)	15 (14.6)
Infections and infestations	34 (6.6)	38 (7.4)	5 (4.9)
Upper respiratory tract infection	7 (1.4)	11 (2.1)	0
Nasopharyngitis	3 (0.6)	7 (1.4)	2 (1.9)
Respiratory, thoracic, and mediastinal disorders	15 (2.9)	25 (4.9)	7 (6.8)
Asthma	7 (1.4)	10 (2.0)	5 (4.9)
Oropharyngeal pain	3 (0.6)	5 (1.0)	1 (1.0)
Gastrointestinal disorders	6 (1.2)	5 (1.0)	1 (1.0)
Nervous system disorders	6 (1.2)	6 (1.2)	0
Headache	3 (0.6)	5 (1.0)	0
Musculoskeletal and connective tissue disorders	4 (0.8)	4 (0.8)	1 (1.0)
Injury, poisoning, and procedural complications	2 (0.4)	3 (0.6)	1 (1.0)
Cardiac disorders	3 (0.6)	0	1 (1.0)

Reported in ≥1% patients in the overall study population and/or ≥1% of any treatment group.
AE, adverse event; R, reference product (Advair Diskus); T, test product (Wixela Inhub).

resulted in erroneous conclusions. We acknowledge, however, that international regulatory agencies may have different requirements for study designs and doses to be studied for the demonstration of local therapeutic equivalence.[26]

Due to the difference in physical appearance of T and R, each treatment was administered twice daily for 28 days in a double-blind manner, using the double-dummy technique[27] with placebo inhalers matched to T or R. This can be considered a gold standard for clinical trials and contrasts with the bioequivalence study for OT329 SOLIS, in which the placebo treatment group received the placebo for the SOLIS inhaler only and hence the T and R were not blinded between each other.[7] Use of a double-dummy technique, which increases the robustness of conclusions of randomized trials of experimental interventions,[28] is a strength of this study. This robust double-dummy study design was also employed in local therapeutic equivalence trials of a novel dry powder FPS inhaler (AirFluSal® Forspiro®; Sandoz International GmbH, Holzkirchen, Germany)[9] and a chlorofluorocarbon-free metered-dose FPS inhaler.[21]

The results of the primary analyses were corroborated by secondary analyses (assay sensitivity [PPS] and bioequivalence [FAS]) that showed that (1) day 1 FEV_1 $AUC_{(0-12)}$ and day 29 trough FEV_1 endpoints were significantly superior to placebo for both T and R ($p < 0.0001$) and (2) T was bioequivalent to R for both co-primary endpoints.

The demonstration of local therapeutic equivalence using spirometry endpoints in this article is also supported by previously presented data on pharmacokinetic bioequivalence to all three-dose strengths of Advair Diskus (100/50, 250/50, and 500/50 μg FP/S) (Haughie et al., 2019), *in vitro* equivalence (e.g., emitted dose) at all three dose strengths,[18] as well as meeting all of the FDA requirements for device equivalence.[17]

In conclusion, Wixela Inhub, which was recently approved by the FDA, will represent a new generic-equivalent FPS treatment option for asthmatic patients whose symptoms are uncontrolled with ICS alone and COPD patients at high risk of exacerbations.

Acknowledgments

The authors acknowledge the study investigators and Bruce Williams (Mylan Pharma UK Ltd.) for clinical operations support. The authors also wish to acknowledge Roger Hill, PhD, for medical writing, and Paula Stuckart for editorial assistance in the preparation of this article (Ashfield Healthcare Communications, Middletown, CT).

Author Disclosure Statement

D.N., S.H., J.K.W., and R.A. are employees of Mylan and have stock ownership in Mylan.

E.M.K. has participated in consulting, advisory boards, speaker panels, or received travel reimbursement for Amphastar, Astra Zeneca, Boehringer Ingelheim, GlaxoSmithKline, Mylan, Novartis, Oriel, Pearl, Sunovion, Teva, and Theravance. He has conducted multicenter clinical research trials for ~40 pharmaceutical companies.

M.V.W. reports grants from Mylan, during the conduct of the study; personal fees from Mylan; grants from Sanofi-Aventis; and grants from Genentech, outside the submitted work.

S.D.M. has no disclosures to declare.

Funding Information

This study was funded by Mylan, Inc. (Canonsburg, PA) who also provided funding for medical writing and editorial support.

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Received on May 17, 2019
in final form, August 27, 2019

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2.4 PK Bioequivalence Studies

2.4.1 ATS 2019

Ward JK, Wood N, Allan R, Haughie S. Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses of Advair Diskus® and Wixela™ Inhub™: Results of 3 Pharmacokinetic Equivalence Studies. American Journal of Respiratory and Critical Care Medicine 2019; 199: A2208. https://doi.org/10.1164/ajrccm-conference.2019.199.1_meetingabstracts.a2208

P789

Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses of Advair Diskus® and Wixela™ Inhub™: Results of 3 Pharmacokinetic Equivalence Studies

Jonathan K. Ward,[1] Nolan Wood,[2] Richard Allan,[1] Scott Haughie[1]

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BACKGROUND

- A combination of oral inhaled corticosteroids (ICS) and long-acting β_2-adrenergic agonists (LABAs) is recommended for patients with asthma not controlled with ICS alone and for patients with chronic obstructive pulmonary disease (COPD) at high risk for exacerbations[1,2]
- Fluticasone propionate (FP)/salmeterol dry powder inhaler is a widely prescribed ICS/LABA fixed-dose combination drug, marketed as Advair Diskus®
- Wixela™ Inhub™ was developed according to US Food and Drug Administration guidance[3] and was recently approved as a substitutable generic equivalent to Advair Diskus[3,4]
- Study objective: To confirm the pharmacokinetic (PK) bioequivalence (BE) of FP/salmeterol following single doses of Wixela Inhub (test) and Advair Diskus (reference).

METHODS

- Three open-label, randomized, 2-way crossover, single-dose studies in healthy adults (N = 66 each) compared the systemic exposure of a single-dose strength of FP/salmeterol (Study 1: 100/50, Study 2: 250/50, and Study 3: 500/50 µg) following oral inhalation from the test and reference.

Subjects

- Eligible subjects included healthy adults aged 18 to 55 years, with a body mass index of 18 to 30.5 kg/m^2 and weight >45 kg
- Subjects were excluded if they had abnormal lung function (forced expiratory volume in 1 second [FEV_1] <80% predicted), were current smokers or ex-smokers who had given up for <6 months or had a smoking history of ≥10 pack-years.

Treatments

- Each FP/salmeterol dose strength was administered as 3 inhalations, resulting in total FP/salmeterol doses of 300/150 µg (Study 1), 750/150 µg (Study 2), and 1500/150 µg (Study 3)
- Doses were administered after an 8-hour fast.

Pharmacokinetic Assessments and End Points

- Plasma samples were obtained predose and from 2 minutes to 48 hours postdose
- Drug concentrations were analyzed using validated high-performance liquid chromatography tandem mass spectrometry
- Primary end points were area under the plasma concentration-time curve from time 0 to last measurable concentration (AUC_{0-t}) and the maximum observed plasma drug concentration (C_{max}) for both FP and salmeterol
- PK parameters were calculated for all subjects who completed at least 1 treatment period
- The BE acceptance criteria specified that the 90% confidence intervals of the geometric mean test/reference ratios should be within 0.80 to 1.25 for all 4 primary end points
- BE assessments were performed using a predefined PK analysis set (subjects who completed both periods, had calculable values for ≥1 of the primary end points, and did not have any adverse events or protocol deviations that would affect PK).

Safety

- The safety population comprised all randomized subjects
- Safety assessments included assessment of adverse events, electrocardiograms, vital signs (blood pressure and pulse rate), cardiac telemetry, and laboratory safety tests.

Statistical Analyses

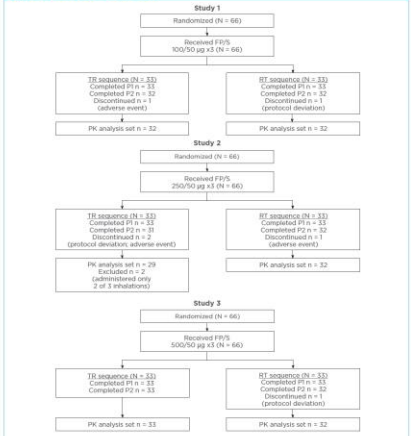
- Sample size calculations were based on salmeterol C_{max} (end point exhibiting greatest variability) and indicated that 62 subjects were required for the PK analysis set; therefore, 66 subjects were randomized per study
- PK parameters and adverse events were summarized using descriptive statistics
- Primary end points were analyzed on the natural log scale using analysis of variance (ANOVA) with terms for sequence, subject within sequence, period, and treatment.

RESULTS

Subjects

- Of the 198 randomized subjects across the 3 studies (**Figure 1**):
  - All subjects completed treatment period 1 and were analyzed for safety and PK parameters
  - A total of 192 subjects also completed treatment period 2, and 190 were included in the PK analysis set

Figure 1. Patient Disposition



FP/S indicates fluticasone propionate/salmeterol; P1, treatment period 1; P2, treatment period 2; PK, pharmacokinetic; R, reference product (Advair Diskus®); T, test product (Wixela™ Inhub™).

- Key baseline characteristics were comparable across the 3 studies (**Table 1**).

Table 1. Subject Baseline Characteristics

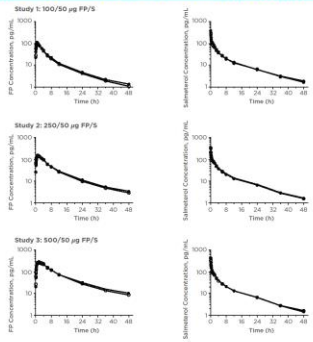
	Study 1 FP/S 100/50 µg (N = 66)	Study 2 FP/S 250/50 µg (N = 66)	Study 3 FP/S 500/50 µg (N = 66)
Age, n (%)			
Females	29 (43.9)	36 (54.5)	42 (63.6)
Males	37 (56.1)	30 (45.5)	24 (36.4)
Age, years			
Mean (range)	33.8 (19-53)	37.7 (18-55)	35.7 (19-53)
Race, n (%)			
White	40 (60.6)	51 (77.3)	47 (71.2)
Black	23 (34.8)	14 (21.2)	17 (25.8)
Other	3 (4.5)	1 (1.5)	2 (3.0)
BMI, kg/m^2			
Mean ± SD	26.3 ± 2.7	26.3 ± 3.3	25.8 ± 2.5
Tobacco history, n (%)			
Never used	54 (81.8)	57 (86.4)	58 (87.9)
Past use of tobacco	12 (18.2)	9 (13.6)	8 (12.1)

BMI indicates body mass index; FP/S, fluticasone propionate/salmeterol; SD, standard deviation.

Pharmacokinetic Assessments

- In each study, plasma concentration versus time data for test and reference were comparable (**Figure 2**)

Figure 2. Plasma FP and Salmeterol versus Time Data Following Administration of FP/S to Healthy Subjects (Test or Reference) in Studies 1, 2, and 3



Data presented are arithmetic mean plasma concentration (semilog scale; n = 62-66) following administration of FP/S to healthy subjects (test [closed circles] or reference [open circles]).
H indicates hours; FP, fluticasone propionate; S, salmeterol; R, reference (Advair Diskus®); T, test (Wixela™ Inhub™).

- Wixela Inhub met the BE acceptance criteria at each dose strength (**Table 2**).

Table 2. Bioequivalence of FP and Salmeterol (Pharmacokinetic Analysis Set)

Treatment	AUC_{0-t} (pg·h/mL)	AUC_{0-t} T/R ratio (90% CI)	C_{max} (pg/mL)	C_{max} T/R ratio (90% CI)
Study 1: FP/Salmeterol 100/50 µg (n = 64)				
FP				
Wixela Inhub	600.3	1.041 (1.00, 1.08)	103.7	0.979 (0.87, 0.96)
Advair Diskus	576.4		112.9	
Salmeterol				
Wixela Inhub	696.4	1.079 (1.04, 1.11)	347.7	0.998 (0.94, 1.04)
Advair Diskus	644.9		348.3	
Study 2: FP/Salmeterol 250/50 µg (n = 61)				
FP				
Wixela Inhub	1251	1.074 (1.02, 1.13)	164.2	1.009 (0.95, 1.07)
Advair Diskus	1164		162.7	
Salmeterol				
Wixela Inhub	641.2	1.029 (0.99, 1.07)	296.2	0.934 (0.87, 1.00)
Advair Diskus	623.3		317.4	
Study 3: FP/Salmeterol 500/50 µg (n = 65)				
FP				
Wixela Inhub	2689	0.966 (0.92, 1.00)	252.8	0.897 (0.86, 0.93)
Advair Diskus	2783		281.8	
Salmeterol				
Wixela Inhub	672.3	1.003 (0.96, 1.04)	334.9	0.863 (0.81, 0.91)
Advair Diskus	670.8		388.6	

Data presented are geometric mean, geometric mean ratio, and 90% confidence interval for the ratio (back-transformed from ANOVA T and R least squares means; T-R mean differences and 90% CI for the difference).
AUC_{0-t} indicates area under the concentration-time curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum plasma concentration; FP, fluticasone propionate; R, reference; T, test.

Safety

- FP/salmeterol at all doses was well-tolerated for both test and reference
- Adverse events were generally mild and occurred with similar frequency in the test and reference groups.

CONCLUSIONS

- Wixela Inhub demonstrated systemic PK BE to Advair Diskus at all dose strengths
- PK BE provides direct evidence of equivalent systemic safety and indirect evidence for equivalent pulmonary deposition
- Wixela Inhub represents a generic equivalent FP/salmeterol treatment option that can be substituted for Advair Diskus in subjects with asthma (>4 years) whose symptoms are uncontrolled with ICS alone and in subjects with COPD at high risk for exacerbations.

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DISCLOSURE: Jonathan K. Ward, Richard Allan, and Scott Haughie are employees of Mylan Pharma UK Ltd., Sandwich, Kent, United Kingdom. Nolan Wood was former employee of Mylan Pharma UK Ltd. currently employed by Certara Strategic Consulting, London, UK.

American Thoracic Society International Conference; Dallas, TX, USA; May 17-22, 2019

2.4.2 Journal of Aerosol Medicine and Pulmonary Drug Delivery 2020

Haughie S, Allan R, Wood N, Ward J. Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses from Advair Diskus and Wixela Inhub: Results of Three Pharmacokinetic Bioequivalence Studies. Journal of aerosol medicine and pulmonary drug delivery. 2020 Feb;33(1):34-42. PubMed PMID: 31364911. Epub 2019/08/01. eng.

Final publication is available from Mary Ann Liebert, Inc., publishers https://doi.org/10.1089/jamp.2019.1537

JOURNAL OF AEROSOL MEDICINE AND PULMONARY DRUG DELIVERY
Volume 33, Number 1, 2020
Mary Ann Liebert, Inc.
Pp. 34–42
DOI: 10.1089/jamp.2019.1537

Equivalent Systemic Exposure to Fluticasone Propionate/Salmeterol Following Single Inhaled Doses from Advair Diskus and Wixela Inhub: Results of Three Pharmacokinetic Bioequivalence Studies

Scott Haughie, MSc,[1] Richard Allan, BSc,[1] Nolan Wood, PhD,[2] and Jon Ward, PhD[1]

Abstract

Background: Wixela® Inhub® was developed to deliver inhaled fluticasone propionate/salmeterol (FP/S) combination as a substitutable generic equivalent to Advair® Diskus®. These studies aimed to confirm the pharmacokinetic bioequivalence (BE) of FP/S after single doses of Wixela Inhub (test [T]) and Advair Diskus (reference [R]).

Methods: Three open-label, randomized, two-way crossover, single-dose studies in healthy subjects ($N = 66$ each) compared the systemic exposure of FP and salmeterol after inhalation from three dose strengths of FP/S (100/50, 250/50, or 500/50 μg) delivered from T and R. Primary BE endpoints were the area under the plasma concentration-time curve from time = 0 to the last measurable concentration ($AUC_{(0\text{-}t)}$) and the maximum observed plasma concentration (C_{max}) for both FP and S. The BE acceptance criteria specified that the 90% confidence intervals (CIs) of the geometric mean T/R ratios for $AUC_{(0\text{-}t)}$ and C_{max} can be contained within 0.80–1.25 for both FP and salmeterol.

Results: Wixela Inhub met the acceptance criteria for BE for FP and salmeterol at each dose strength. Estimated $AUC_{(0\text{-}t)}$ and C_{max} geometric mean ratios (T/R [90% CI]) for FP were, respectively, 1.04 (1.00–1.08) and 0.92 (0.87–0.96) for 100/50 μg FP/S, 1.07 (1.02–1.13) and 1.01 (0.95–1.07) for 250/50 μg, and 0.97 (0.92, 1.00) and 0.90 (0.86–0.93) for 500/50 μg. Estimated $AUC_{(0\text{-}t)}$ and C_{max} ratios for salmeterol were, respectively, 1.08 (1.04–1.11) and 1.00 (0.94–1.04) for 100/50 μg FP/S, 1.03 (0.99–1.07) and 0.93 (0.87–1.00) for 250/50 μg, and 1.00 (0.96–1.04) and 0.86 (0.81–0.91) for 500/50 μg. FP/S at all doses via both T and R was comparably well tolerated.

Conclusions: Wixela Inhub was bioequivalent to Advair Diskus at all three dose strengths for both FP and S, providing direct evidence of equivalent systemic safety and indirect evidence for equivalent pulmonary deposition.

Keywords: Advair Diskus, asthma, COPD, fluticasone propionate, pharmacokinetic bioequivalence, salmeterol, Wixela Inhub

Introduction

A COMBINATION OF ORAL INHALED CORTICOSTEROIDS (ICS) and long-acting β_2-adrenergic agonists (LABAs) is recommended for patients with asthma not controlled with ICS alone and for patients with chronic obstructive pulmonary disease (COPD) at high risk of exacerbations.[1–4] Fluticasone propionate/salmeterol (FP/S) dry powder inhaler is a widely prescribed ICS/LABA fixed-dose combination drug, marketed in the United States as Advair® Diskus® (GlaxoSmithKline, Research Triangle Park, NC). Advair Diskus is available in three strengths, described according to the variable nominal FP dose and acknowledging the fixed 50 μg nominal dose of salmeterol base in each strength in μg:

[1]Mylan Pharma UK Ltd., Sandwich, United Kingdom.
[2]Certara UK Ltd., London, United Kingdom.

100/50, 250/50, and 500/50. All three strengths are licensed for the twice-daily treatment of adult and adolescent asthma, the 100/50 μg strength for the management of pediatric asthma (≥4 years), and the 250/50 μg strength for the treatment of COPD.

With the expiration of U.S. patent protection for Advair Diskus in 2016,[5] generic versions of the drug are progressing toward approval of an abbreviated new drug application (ANDA) by the U.S. Food and Drug Administration (FDA).[6,7] The most advanced generic is Wixela® Inhub® (previously known as MGR001; Mylan, Inc., Canonsburg, PA), which delivers FP/S from a novel multidose dry powder inhaler (Inhub® device, previously known as CRC749).[8,9] A clinical development program for Wixela Inhub has been completed, and an ANDA has recently been approved.[10] As part of the clinical development plan for a substitutable generic equivalent of FP/S, the FDA requires the conduct of a pharmacokinetic (PK) bioequivalence (BE) study for each of the dose strengths approved for Advair Diskus.[11] Here, we report the results of three PK BE studies conducted in support of the development of Wixela Inhub.

The studies were all conducted in healthy male and female volunteers, with one study for each at the 100/50, 250/50, and 500/50 μg FP/S dose strengths. The objective of each study was to confirm the systemic PK BE of FP and salmeterol after oral inhalation of single doses of Wixela Inhub and Advair Diskus.

Materials and Methods

In this article, "test product" (T) and "reference product" (R)[11] are defined as follows: T is Wixela Inhub (FP/S administered via the Inhub device), R is Advair Diskus. Both products contained 60 premetered individual doses. Each dose of Advair Diskus comprised a white powder mix of micronized FP (100, 250, or 500 μg) and micronized salmeterol xinafoate salt (72.5 μg, equivalent to 50 μg of salmeterol base) in a 12.5 mg of formulation containing lactose monohydrate (as an excipient). The formulation contained within Wixela Inhub is qualitatively and quantitatively equivalent to that contained within Advair Diskus in terms of both active (FP and salmeterol [as xinafoate]) and inactive (lactose monohydrate) ingredients.

Study design and conduct

Three open-label, randomized, two-way crossover studies were conducted at a single clinical center in the United States between April 2015 and July 2017, each under a separate protocol. Each study compared the systemic exposure of FP and salmeterol after FP/S administration of T and R at one of the three Advair Diskus dose strengths (FP/S 100/50, 250/50, or 500/50 μg) authorized in the United States. Study 1 evaluated FP/S 100/50 μg, study 2 evaluated FP/S 250/50 μg, and study 3 evaluated FP/S 500/50 μg. The objective of each study was to confirm the PK BE of both FP and salmeterol after oral inhalation of single doses of T and R.

The studies conformed to appropriate ethical guidelines and were conducted in accordance with the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guideline for good clinical practice[12] and the code of ethics of the World Medical Association's Declaration of Helsinki.[13] Each study protocol was approved by an appropriate institutional review board, and all patients provided written informed consent.

Study subjects and treatments

Each study was conducted in 66 healthy male and female subjects who received single orally inhaled doses of both T and R, one per study period, with a minimum 7-day washout in between. Subjects were excluded if they had used any prescription or nonprescription drugs within 7 days of the start of the study, had abnormal lung function (forced expiratory volume in 1 second <80% of predicted), or were current smokers, ex-smokers who had given up smoking for <6 months, and/or had a smoking history of ≥10 pack-years.

Each study had an identical two-treatment, two-period crossover design (2×2), with subjects randomized equally to each treatment sequence. To obtain adequate plasma FP and salmeterol levels, each FP/S dose strength was administered as three inhalations, resulting in total FP/S doses of 300/150 μg (study 1), 750/150 μg (study 2), and 1500/150 μg (study 3). This was implemented to ensure that both FP and salmeterol were detectable for at least three half-lives for each analyte after dosing, thus allowing an appropriate estimation of the PK parameters. In addition, the use of three inhalations was expected to reduce variability and ensure a variation coefficient (CV) of <30% for all of the BE endpoints. Based on the performance of the reference product determined in an exploratory PK study, a prospective agreement was obtained from the US FDA that the use of three inhalations was appropriate based on their stated requirement that the dose chosen should comprise the "Minimum number of inhalations that is sufficient to characterize a PK profile by using a sensitive analytical method."[11] To ensure consistent dosing, subjects received inhalation training on day −1 and day 1 (before treatment). Treatments were administered after an 8-hour fast, and standard meals were provided at ~4 and 9–10 hours postdose and appropriate times thereafter. Water was allowed *ad libitum* throughout the study except during the period from 1 hour before dose through to 1 hour postdose.

PK assessments and endpoints

For each study, plasma samples were obtained for each treatment period before dosing and at 2, 5, 10, 15, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose. Sampling started at 2 minutes postdose to ensure that peak plasma concentrations of salmeterol were adequately captured, and continued up to 48 hours postdose to ensure coverage of at least three times the terminal elimination half-life ($T_{1/2}$) estimates of the FP and salmeterol components. Drug concentrations were analyzed using validated high-performance liquid chromatography tandem mass spectrometry with a lower limit of quantification of 1 pg/mL.

Primary PK endpoints were area under the plasma concentration-time curve from time=0 to the last measurable plasma drug concentration ($AUC_{(0\text{-}t)}$) and maximum observed plasma drug concentration (C_{max}) for both FP and salmeterol. PK parameters were derived by using

noncompartmental methods using Phoenix® WinNonlin® (version 6.3; Certara L.P. [Pharsight], St. Louis, MO). Safety assessments included adverse events (AEs), electrocardiograms, vital signs (blood pressure and pulse rate), cardiac telemetry, and laboratory safety tests.

Statistical methods

The safety population comprised all randomized subjects who received at least one treatment. PK parameters were calculated for all subjects who completed at least one treatment period, and those subjects who had calculable values for at least one of the primary PK parameters in at least one period were included in the PK parameter set. The statistical analysis of BE was conducted using a predefined PK analysis set (subjects who completed both treatment periods, had calculable values for at least one of the primary endpoints in both periods, and did not experience any protocol deviations or AEs that would affect PK).

Sample size calculations were based on salmeterol C_{max}, since from previous Mylan studies (data on file), this PK parameter exhibits a greater within-subject standard deviation (WSD) than salmeterol $AUC_{(0\text{-}t)}$, FP C_{max}, or FP $AUC_{(0\text{-}t)}$ (values for salmeterol C_{max} WSD in the range 0.22–0.28 on the natural log scale). Using a WSD of 0.22 and a one-sided significance level $\alpha = 0.05$, sample size calculations indicated that 62 subjects in the PK analysis set gave 90% power (true difference in means of log[0.9]), and a WSD of 0.28 gave 80% power (true difference in means of log[1.1]) to demonstrate BE. Thus, a total of 66 subjects were randomized in each study to give at least 62 in the PK analysis set.

Primary endpoints ($AUC_{(0\text{-}t)}$ and C_{max}) were analyzed by using analysis of variance (ANOVA), allowing for variation due to sequence, subject within sequence, period, and treatment. The analysis was performed on the natural log scale. Least-squares mean differences (plus standard errors and 90% confidence intervals [CIs]) were produced on the log scale and exponentiated to give ratios of geometric means and associated 90% CIs on the original scale. To demonstrate BE at each dose strength,[11] the 90% CIs of the T to R geometric mean ratios for $AUC_{(0\text{-}t)}$ and C_{max} were each required to be wholly contained within the interval 0.80–1.25 (i.e., 80%–125%) for both the FP and salmeterol components. PK parameters and AEs were summarized by using descriptive statistics. All statistical analyses were conducted by using SAS® version 9.3 (Cary, NC).

Results

Subjects

For each of the three studies ($N = 66$ for each study), all subjects completed treatment period 1 and were analyzed for safety and PK parameters; of the 198 randomized subjects across the three studies, 192 subjects also completed treatment period 2 and 190 were included in the PK analysis set (Fig. 1). Of the six subjects who did not complete treatment period 2, three subjects discontinued due to an AE and three subjects discontinued due to protocol deviations. Key baseline characteristics (age, body mass index, and tobacco history) were comparable across the three studies (Table 1).

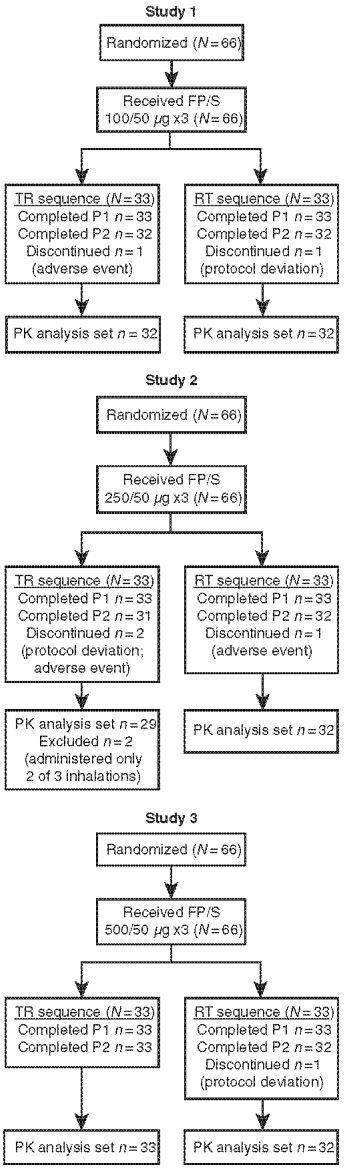

FIG. 1. Subject disposition.

Table 1. Subject Baseline Characteristics (Safety Population)

	Study 1 FP/S 100/50 μg (N=66)	*Study 2 FP/S 250/50 μg (N=66)*	*Study 3 FP/S 500/50 μg (N=66)*
Age, *n* (%)			
Females	29 (43.9)	36 (54.5)	42 (63.6)
Males	37 (56.1)	30 (45.5)	24 (36.4)
Age, mean (range), years	33.8 (19–53)	37.7 (18–55)	35.7 (19–53)
Race, *n* (%)			
White	40 (60.6)	51 (77.3)	47 (71.2)
Black or African American	23 (34.8)	14 (21.2)	17 (25.8)
Other	3 (4.5)	1 (1.5)	2 (3.0)
BMI, mean ± SD, kg/m^2	26.3 ± 2.7	26.3 ± (3.3)	25.8 ± 2.5
Tobacco history, *n* (%)			
Never used	54 (81.8)	57 (86.4)	58 (87.9)
Past use of tobacco	12 (18.2)	9 (13.6)	8 (12.1)

BMI, body mass index; FP/S, fluticasone propionate/salmeterol; SD, standard deviation.

PK BE assessments

Fluticasone propionate. In each study, the plasma FP concentration versus time data for T and R were comparable (Fig. 2, left panels); thus, the FP PK parameters for T and R were also comparable (Table 2). FP was rapidly absorbed, with T_{max} values ranging from 0.75 hours (study 1) to 1.5 hours (study 3). Mean C_{max} values were dose–dependent, increasing from 109 pg/mL (study 1) to 290 pg/mL (study 3). Mean total systemic FP exposure ($AUC_{(0\text{-}t)}$) was similarly dose dependent, increasing from 609 pg•h/mL (study 1) to 2919 pg•h/mL (study 3). Mean $T_{1/2}$ values were similar across each dose strength, ranging from 9.95 hours (study 1) to 12.23 hours (study 3).

For each of the FP/S dose strengths, the geometric mean T/R ratios and 90% CIs were between 0.80 and 1.25 for FP $AUC_{(0\text{-}t)}$ and FP C_{max} (Table 3), indicating that T and R were bioequivalent for the FP component.

Salmeterol. In each study, the plasma salmeterol concentration versus time data for T and R were comparable (Fig. 2, right panels); thus, the salmeterol PK parameters for T and R were also comparable (Table 2). Salmeterol was very rapidly absorbed, with a T_{max} value of 5 minutes across the three studies. Mean C_{max} values were consistent across studies, ranging from 319 pg/mL (study 2) to 418 pg/mL (study 3). Mean $AUC_{(0\text{-}t)}$ was similarly consistent, ranging from 677 pg•h/mL (study 1) to 724 pg•h/mL (study 3). Mean $T_{1/2}$ values were also consistent, ranging from 11.21 hours (study 3) to 12.21 hours (study 1).

For each of the FP/S dose strengths, the 90% CI of the geometric mean T/R ratios for salmeterol $AUC_{(0\text{-}t)}$ and salmeterol C_{max} were between 0.80 and 1.25 (Table 3), indicating that T and R were bioequivalent for the salmeterol component.

Safety results

FP/S was well tolerated for both T and R in all studies with no clinically significant changes in electrocardiograms, vital signs (blood pressure and pulse rate), cardiac telemetry, or laboratory safety tests. AEs were generally mild and occurred with similar frequencies in T- and R-treated subjects in all studies (Table 4). The most commonly reported AE was headache. One subject experienced a serious AE, classified as dyspnea of moderate severity, that occurred after completion of treatment with R in study 1 (100/50 μg dose strength) and was considered by the investigator not to be treatment related. One subject treated with T in study 1 experienced an upper respiratory tract infection of mild severity (considered by the investigator to be unrelated to treatment) that led to discontinuation.

Discussion

Wixela Inhub is being developed as a generic equivalent to Advair Diskus. These studies, one for each of the three authorized Advair Diskus dose strengths, confirmed the PK BE of both FP and salmeterol components after oral inhalation of single doses of Wixela Inhub and Advair Diskus. For the FP/S 100/50 μg, 250/50 μg, and 500/50 μg dose strengths, BE criteria were fully met for both FP and salmeterol for each primary endpoint ($AUC_{(0\text{-}t)}$ and C_{max}), in accordance with regulatory guidance.[11]

PK parameters for FP and salmeterol after treatment with FP/S were consistent with published data on Advair Diskus[14–18]; however, the higher total FP/S doses of the current studies (300/150, 750/150, and 1500/150 μg) complicate a direct comparison of PK parameters with those from previous studies, which used lower total doses (100/50 and 250/50 μg). The use of higher total FP/S doses (three inhalations) in the current studies allowed for a thorough understanding of the PK profile of both FP and salmeterol from both Wixela Inhub and Advair Diskus, including an assurance that the plasma concentrations of each analyte were readily detectable to at least 12 hours postdose, and thus enabled a complete comparison of both T and R. In addition, the use of three inhalations of FP/S is associated with less variability of exposure, particularly for the 100/50 μg strength (within subject CV <30%), compared with the same dose administered with one inhalation (within subject CV >30% [Mylan data on file]).

Within each dose strength, FP and salmeterol PK parameters for T and R were similar. Peak plasma concentrations of FP and salmeterol occurred at 1–2 hours and 5 minutes, respectively, as previously reported.[18,19] The mean $T_{1/2}$ estimated for FP in these studies (11.01 hours)

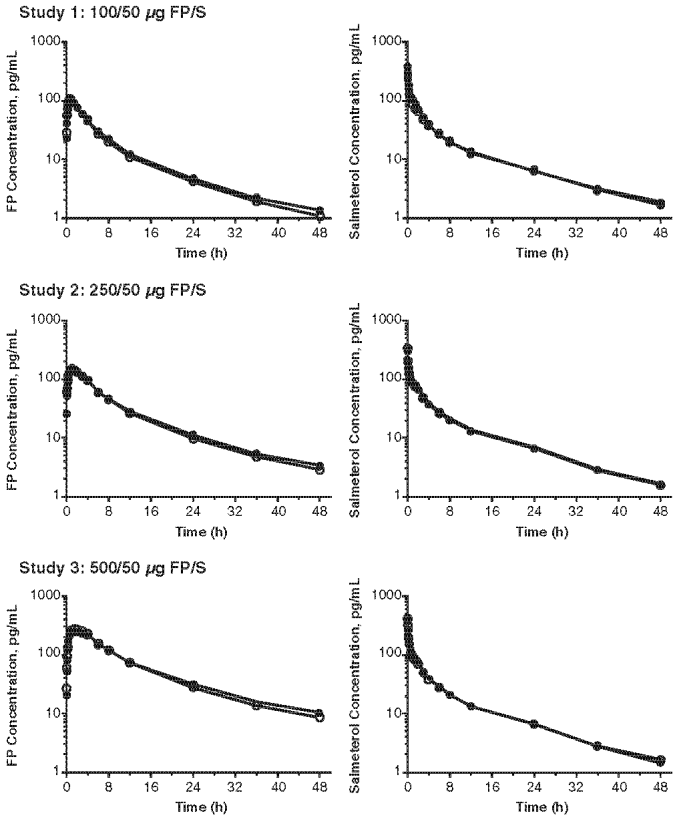

FIG. 2. Plasma FP (left panels) and plasma salmeterol (right panels) versus time data after administration of FP/S to healthy subjects (T [closed circles] or R [open circles]) in studies 1, 2, and 3. Data presented are arithmetic mean plasma concentration (semilog scale) (n=62–66). FP/S, fluticasone propionate/salmeterol.

was longer than some previous reports (4.7 hours[17] and 7.8 hours[18]) and similar to other estimates (11.4 hours[20] and 12.5 hours[21]). This increased $T_{1/2}$ could be a reflection of the fact that in this study three inhalations of FP/S were given, which meant that concentrations were sufficient to allow thorough characterization of the terminal phase. Therefore, the reported half-lives in this study are considered accurate.

Burmeister-Getz et al.[21] have reported that for Advair Diskus 100/50 μg, the inherent variability of R means it is not likely that a 2×2 comparison of a generic FP/S with Advair Diskus would achieve BE according to current FDA standards; our studies are counter to this position. It is recognized that variability exists in the PK response to Advair Diskus; however, if appropriate *in vitro* assessments such as fine particle mass (FPM) are performed across a large range of batches of Advair Diskus, it is possible to characterize the population of Advair Diskus batches. While remaining within the pharmaceutical specification for Advair Diskus, batches of R that are near the extremes of the distribution exist (e.g., a batch that has high FPM and another batch that has low FPM), and if such batches are compared, they can be shown not to be bioequivalent in a standard human PK study (Mylan data on file). However, if the comparison between the same two batches is corrected for the FPM content, they can be shown to be bioequivalent by using the same study data.

The Burmeister-Getz et al.[21] study did not report the key *in vitro* characteristics of the batches of Advair Diskus used, reporting only the age of the batches, which is not an

Table 2. Fluticasone Propionate and Salmeterol Pharmacokinetic Parameters by Study (Pharmacokinetic Parameter Set)

	Study 1		*Study 2*		*Study 3*	
Treatment[a]	*FP/S 100/50 μg*		*FP/S 250/50 μg*		*FP/S 500/50 μg*	
Device	*T* (n=65)	*R* (n=65)	*T* (n=65)	*R* (n=64)	*T* (n=65)	*R* (n=66)
FP PK parameters						
T_{max}, h	0.75 (0.08–1.50)	0.75 (0.08–1.50)	1.00 (0.08–3.00)	1.00 (0.33–3.01)	1.50 (0.33–4.00)	1.50 (0.33–4.00)
C_{max}, pg/mL	109.7±36.2	118.6±35.5	170.0±53.7	173.6±56.4	261.6±69.1	290.9±74.0
$AUC_{(0\text{-}t)}$, pg•h/mL	638±201	609±179	1298±418	1237±379	2851±970	2919±831
$T_{1/2}$, h	10.18±2.46[b]	9.95±2.77[d]	11.24±1.80[e]	10.38±1.71[f]	12.23±2.68[c]	10.57±1.90
Salmeterol PK parameters						
T_{max}, h	0.08 (0.03–1.50)	0.08 (0.03–2.00)	0.08 (0.04–1.01)	0.08 (0.05–2.01)	0.08 (0.03–1.00)	0.08 (0.03–1.50)
C_{max}, pg/mL	385.4±162.8	379.3±143.9	319.5±137.8	352.8±158.1	376.6±181.3	418.0±145.8
$AUC_{(0\text{-}t)}$, pg•h/mL	727±223	677±245	700±340	686±324	724±297	708±236
$T_{1/2}$, h	11.87±1.54[c]	12.21±1.93	11.55±1.71	11.66±1.86[e]	11.21±2.01	11.56±1.86[g]

Data are shown as arithmetic mean±standard deviation for all parameters except T_{max}, which is shown as median (range).

[a]Three inhalations in each study, resulting in total FP/S doses of 300/150 μg (study 1), 750/150 μg (study 2), and 1500/150 μg (study 3).

[b]$n=62$.

[c]$n=64$.

[d]$n=60$.

[e]$n=63$.

[f]$n=59$.

[g]$n=65$.

$AUC_{(0\text{-}t)}$, area under the concentration-time curve from time=0 to the last measurable concentration; C_{max}, maximum plasma concentration; FP, fluticasone propionate; FP/S, fluticasone propionate/salmeterol; h, hours; PK, pharmacokinetic; R, reference product (Advair® Diskus®); T, test product (Wixela® Inhub®); $T_{1/2}$, terminal elimination half-life; T_{max}, time to maximum plasma concentration.

Table 3. Bioequivalence of Fluticasone Propionate and Salmeterol (Pharmacokinetic Analysis Set)

Treatment[a]	*$AUC_{(0\text{-}t)}$ (pg·h/mL)*	*$AUC_{(0\text{-}t)}$ T/R ratio (90% CI)*[a]	*C_{max} (pg/mL)*	*C_{max} T/R ratio (90% CI)*[b]
Study 1: FP/S 100/50 μg (*n*=64)				
Fluticasone propionate				
T	600.3	1.04 (1.00–1.08)	103.7	0.92 (0.87–0.96)
R	576.4		112.9	
Salmeterol				
T	696.4	1.08 (1.04–1.11)	347.7	1.00 (0.94–1.04)
R	644.9		348.3	
Study 2: FP/S 250/50 μg (*n*=61)				
Fluticasone propionate				
T	1251	1.07 (1.02–1.13)	164.2	1.01 (0.95–1.07)
R	1164		162.7	
Salmeterol				
T	641.2	1.03 (0.99–1.07)	296.2	0.93 (0.87–1.00)
R	623.3		317.4	
Study 3: FP/S 500/50 μg (*n*=65)				
Fluticasone propionate				
T	2689	0.97 (0.92–1.00)	252.8	0.90 (0.86–0.93)
R	2783		281.8	
Salmeterol				
T	672.3	1.00 (0.96–1.04)	334.9	0.86 (0.81–0.91)
R	670.8		388.6	

Data presented as natural-log transformed geometric mean (based on least squares mean).
[a]Three inhalations were administered in each study, resulting in total FP/S doses of 300/150 μg (study 1), 750/150 μg (study 2), and 1500/150 μg (study 3).
[b]T and R were bioequivalent if the 90% CIs of the T to R geometric mean ratio were >0.80 and <1.25.
$AUC_{(0\text{-}t)}$, area under the concentration-time curve from time=0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum plasma concentration; FP/S, fluticasone propionate/salmeterol; PK, pharmacokinetic; R, reference product (Advair® Diskus®); T, test product (Wixela® Inhub®).

adequate indicator of pharmaceutical performance of the product. However, if batches of both T and R are well matched for *in vitro* parameters, and the R batch is representative of the Advair Diskus population, then PK BE can be achieved as demonstrated in these studies for all dose strengths of FP/S.

In addition, Burmeister-Getz et al.[19] have reported that variability of product batches may lead to an increase in type 1 error rate beyond the accepted 5% level by using the standard 2×2 crossover design. The assumption underlying this finding is that batches of T and R included in a PK study are chosen entirely at random (i.e., selected from any point

Table 4. Safety Overview (Safety Population)

Treatment[a]	*Study 1*		*Study 2*		*Study 3*	
	100/50 μg		*250/50 μg*		*500/50 μg*	
Device	*T* (n=65)	*R* (n=65)	*T* (n=65)	*R* (n=64)	*T* (n=65)	*R* (n=66)
Subjects reporting ≥1 treatment-emergent AE, *n* (%)	4 (6.2)	9 (13.8)	11 (16.9)	5 (7.8)	4 (6.2)	0
Subjects reporting ≥1 serious AE, *n* (%)	0	1 (1.5)[b]	0	0	0	0
Subjects reporting ≥1 AE leading to study discontinuation, n (%)	1 (1.5)[c]	0	1 (1.5)	1 (1.6)	0	0
Most commonly reported AEs[d], *n* (%)						
Headache	1 (1.5)	2 (3.1)	0	2 (3.1)	0	0
Dizziness	0	1 (1.5)	1 (1.5)	0 (0.0)	0	0
Vessel puncture site pain	0	0	3 (4.6)	2 (3.1)	0	0
Presyncope	1 (1.5)	1 (1.5)	2 (3.1)	0 (0.0)	0	0

[a]Three inhalations were administered in each study, resulting in total FP/S doses of 300/150 μg (study 1), 750/150 μg (study 2), and 1500/150 μg (study 3).
[b]One case of moderate dyspnea was reported 2 days 7 hours after FP/S administration and after completion of all study procedures; this AE was considered by the investigator as not related to study treatment.
[c]One nonserious case of mild upper respiratory tract infection led to discontinuation of this subject before period 2; this AE was considered by the investigator as not related to study treatment.
[d]AEs (preferred terms) reported by two or more subjects with any treatment.
AE, adverse event; FP/S, fluticasone propionate/salmeterol; R, reference product (Advair® Diskus®); T, test product (Wixela® Inhub®).

of the distribution for each product). The source of the inflated type 1 error is the overlap of the distribution of the individual T and R batches around the respective T and R averages. In general, the greater the interbatch variability, the wider this overlap, and the greater the chance of the erroneous finding that batches are bioequivalent, even if the product averages are not bioequivalent.

Recently, the same authors[22] presented the results of a PK study utilizing a multibatch design, which demonstrated BE for OT329 Solis 100/50 μg versus Advair Diskus 100/50 μg. This study design was not consistent with FDA guidance[11] but represented a novel, unprecedented approach to demonstrating PK BE, which was presumably developed to resolve the authors' concerns about the properties of standard PK BE designs raised in earlier publications. Although this and other multibatch approaches are still very much in their infancy,[21] we believe that the standard PK BE designs we have utilized, in combination with a thorough understanding of the *in vitro* characteristics that drive interbatch variability of both T and R, and well-established statistical analysis methods, still represent a robust and reliable assessment of the PK BE of FP/S combination products, in line with FDA guidance.

The recruitment of healthy subjects instead of subjects with asthma or COPD enabled a comprehensive assessment of systemic exposure of FP and salmeterol without the potential confounding factors such as variable and compromised pulmonary function or the use of concomitant medications, all of which could have a direct influence on the absorption, distribution, metabolism, and excretion of the study drugs. As the use of healthy subjects allows for consistent disease status and no requirement to modulate a patient's treatment regime, it is possible to conduct crossover design studies that enable a within-subject comparison of exposure, and thus the variability of a study is reduced substantially. Likely for these reasons, the use of healthy volunteers is reflected in regulatory guidance,[11] and healthy volunteers were recently used for similar BE studies.[14,23] In addition, a meta-analysis[24] demonstrated that although the apparent bioavailability of FP in healthy subjects is greater by ≈2-3-fold versus asthma subjects, there is conservation of the relative bioavailability when comparing the delivery of FP from different inhalation devices (i.e., the difference in exposure for FP delivered from Diskhaler® [GlaxoSmithKline, Research Triangle Park, NC]) and Diskus® was ~15% in both asthmatic and healthy subjects). This conservation of relative bioavailability suggests that if generic FP/S demonstrates PK BE to Advair Diskus in healthy subjects, it would likely also demonstrate PK BE in a patient population.

As many AEs associated with FP and salmeterol are related to systemic exposure to these products, demonstrating equivalent exposure indirectly demonstrates that a generic ICS/LABA should have a safety profile generally similar to the originator's product. This is particularly true of orally inhaled products that have poor systemic bioavailability such as FP, as the measured systemic exposure would be almost entirely related to the lung dose of the drug. The AEs observed for both T and R in the current studies were consistent in nature and frequency with those reported for Advair Diskus.[18] All AEs were mild or moderate in severity and of low incidence compared with the most commonly reported AEs according to the Advair Diskus prescribing information.[18]

In conclusion, our investigation confirmed that Wixela Inhub demonstrated systemic PK BE to Advair Diskus at all FP/S dose strengths using a consistent approach, with standard study designs for all FP/S dose strengths. Moreover, a study using clinical efficacy endpoints recommended by the FDA[11] has recently been completed as part of the Wixela Inhub clinical development plan (NCT02245672). That study demonstrated local (lung) BE of Wixela Inhub and Advair Diskus in patients with asthma based on the effects of both active treatments on lung function endpoints (forced expiratory volume in 1 second) measured after the first dose and 4 weeks of dosing that were both superior to placebo and statistically equivalent to each other.[25] Wixela Inhub, therefore, represents a substitutable generic equivalent FP/S treatment option for subjects with asthma whose symptoms are uncontrolled with ICS alone and for subjects with COPD at high risk of exacerbations.

Acknowledgments

Mylan, Inc., (Canonsburg, Pennsylvania, USA) provided funding for medical writing and editorial support. The authors wish to acknowledge Roger Hill, PhD, for medical writing and Paula Stuckart for editorial assistance in the preparation of this article (Ashfield Healthcare Communications, Middletown, CT).

The authors acknowledge Helen Bridger and Bruce Williams (Mylan) for clinical operations related to the studies.

The authors acknowledge Cynthia A. Zamora, MD, Worldwide Clinical Trials Early Phase Services, LLC, 2455 NE Loop 410, Suite 150, San Antonio, Texas, USA, 78217, who was the clinical site principal investigator for these studies.

Author Disclosure Statement

Authors S.H., R.A., and J.W. are employees of Mylan and have stock ownership in Mylan.

Author N.W. is a former employee of Mylan.

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Received on March 27, 2019
in final form, June 17, 2019

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