

ARTICLE

# A Novel Method for Studying the Pharmacokinetics of [<sup>14</sup>C]Umeclidinium After Application to the Axilla or Palm of Healthy Male Subjects

T Pene Dumitrescu<sup>1,\*†</sup>, LL Santos<sup>2</sup>, SC Hughes<sup>3</sup>, AI Pereira<sup>3</sup>, GC Young<sup>3</sup>, E Hussey<sup>2,‡</sup>, P Charlton<sup>2,§</sup>, S Baptiste-Brown<sup>4</sup>, JS Stuart<sup>2</sup>, V Vincent<sup>5</sup>, SP van Marle<sup>6</sup> and VD Schmith<sup>1</sup>

Umeclidinium (UMEC), a long-acting muscarinic antagonist approved for chronic obstructive pulmonary disease (COPD), was investigated for primary hyperhidrosis as topical therapy. This study evaluated the pharmacokinetics, safety, and tolerability of a single dose of [<sup>14</sup>C]UMEC applied to either unoccluded axilla (UA), occluded axilla (OA), or occluded palm (OP) of healthy males. After 8 h the formulation was removed. [<sup>14</sup>C]UMEC plasma concentrations (Cp) were quantified by accelerator mass spectrometry. Occlusion increased systemic exposure by 3.8-fold. Due to UMEC absorption-limited pharmacokinetics, Cp data from the OA were combined with intravenous data from a phase I study. The data were described by a two-compartment population model with sequential zero and first-order absorption and linear elimination. Simulated systemic exposure following q.d. doses to axilla was similar to the exposure from the inhaled therapy, suggesting that systemic safety following dermal administration can be bridged to the inhaled program, and offering the potential for a reduced number of studies and/or subjects. *Clin Transl Sci* (2016) 9, 183–191; doi:10.1111/cts.12406; published online on 15 June 2016.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ Topical anticholinergics were reported to be effective in managing hyperhidrosis; however, no topical anticholinergic has achieved regulatory approval

### WHAT QUESTION DID THIS STUDY ADDRESS?

✓ The study objectives were:

- To characterize UMEC safety, tolerability, and pharmacokinetics following topical administration to the axilla or the palm.
- To use bridge systemic safety from the inhaled to the dermal route.

### WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?

✓ This study showed:

- Considerably greater absorption through axilla than through palm;
- Occlusion increases UMEC absorption through axilla;
- A Modeling and Simulation method to compare the systemic exposure following daily dermal doses to that following inhaled approved doses.

### HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND TRANSLATIONAL SCIENCE?

✓ This novel work enables pharmacokinetic-based systemic safety bridging across administration routes and therapeutic areas and provides a framework for the benefit-risk assessment for dermal UMEC.

Hyperhidrosis, a condition affecting ~3% of the US population, consists of excessive sweating beyond what is physiologically required to maintain normal thermal regulation, and is associated with an overactivity of the sympathetic nervous

system that controls the sweat glands, with acetylcholine acting as the major neurotransmitter.<sup>1</sup> Primary hyperhidrosis (excessive sweating with unknown etiology) is localized, symmetrical, and can affect the axilla, palms, soles of the

<sup>1</sup>Clinical Pharmacology Modeling and Simulation, GSK, Research Triangle Park, North Carolina, USA; <sup>2</sup>Stiefel, a GSK company, Research Triangle Park, North Carolina, USA; <sup>3</sup>Drug Metabolism and Pharmacokinetics, GSK, Ware, UK; <sup>4</sup>Clinical Pharmacology Sciences & Study Operations, GSK, King of Prussia, Pennsylvania, USA; <sup>5</sup>R&D Projects Clinical Platforms and Sciences, QSci, GSK, Bangalore, India; <sup>6</sup>Pharmaceutical Research Associates International Group, Zuidlaren, The Netherlands.

\*Correspondence: T Pene Dumitrescu (Teodora.x.pene-dumitrescu@gsk.com)

†Currently at Brickell Biotech, Miami, FL.

‡Currently at UCB BioSciences, Raleigh, NC.

§Currently at Nuventra Pharma Sciences, Durham, NC.

Received 25 January 2016; accepted 17 May 2016; published online on 15 June 2016. doi:10.1111/cts.12406

feet, and other areas. These symptoms result in substantial limitations in work, social interaction, physical activity, as well as emotional and psychological distress, leading to a debilitating effect on the quality of life of the patients, comparable to severe psoriasis, end-stage renal disease, rheumatoid arthritis, or multiple sclerosis.<sup>2,3</sup>

Current management of hyperhidrosis follows an incremental, stepwise approach that transitions from less-invasive therapies such as topical aluminum chloride, iontophoresis, or oral anticholinergics, to more invasive treatments such as botulinum toxin type A (BTX-A) injections<sup>3</sup> or surgery. In addition, anticholinergic agents such as glycopyrrolate have been reported to be effective as topical therapy in managing hyperhidrosis since 1978.<sup>4–8</sup> However, to date, no topical anticholinergics have achieved regulatory approval.

Umeclidinium (UMEC), a long-acting anticholinergic agent approved as inhaled therapy for chronic obstructive pulmonary disease (COPD) (INCRUSE ELLIPTA), is being repurposed as a potential new dermal therapy for patients with primary axillary or palmar hyperhidrosis. Dermal application may offer a high therapeutic benefit by maintaining a high concentration at the site of action while reducing the risk of adverse events by virtue of the low systemic exposure to drug-related material. In order to capitalize on the extensive systemic safety data and clinical experience with inhaled UMEC, an essential step is to determine UMEC pharmacokinetics following dermal administration.

It is generally agreed that the extent of percutaneous absorption of a compound may differ in various anatomical locations depending on the thickness and composition of the stratum corneum, the size and number of follicles, sebum composition, etc.<sup>9–11</sup> Furthermore, numerous studies have reported that occlusion may enhance the skin permeability of various compounds.<sup>12</sup> The primary objectives of this study were to characterize the pharmacokinetics (PK) in the presence and absence of occlusion, and to develop a population PK model of UMEC following single-dose administration to the axilla or the palm. The secondary objectives were to determine the amount of UMEC potentially absorbed in the skin and to characterize the safety and tolerability of topical UMEC after single-dose administration to axilla or palm. Modeling and simulations (M&S) was used to predict plasma-concentration profiles after repeated q.d. doses, and to estimate the likelihood of exceeding the systemic exposure from the inhaled UMEC therapeutic dose, allowing bridging of the systemic safety to the inhaled program. A feasibility assessment based on in-house *in vitro* skin penetration data suggested that the established validated bio-analytical method using LC-MS/MS would provide insufficient sensitivity to quantify UMEC plasma concentrations following dermal administration. Therefore, a novel translational approach using <sup>14</sup>C-labeled drug applied dermally with detection by accelerator mass spectrometry (AMS) was proposed to quantify the anticipated lower plasma drug concentrations.

## MATERIALS AND METHODS

### Study design

This was a single-center, single-dose, open-label trial (ClinicalTrials.gov: NCT01934153, GSK study number 117157)

conducted by Pharmaceutical Research Associates International in The Netherlands, and sponsored by Stiefel, a GlaxoSmithKline company. The protocol specified up to four possible sequential cohorts: unoccluded axilla (UA), occluded axilla (OA), occluded palm OP, and unoccluded palm (UP), with six evaluable subjects per cohort that completed dosing and critical assessments. Interim analyses of preliminary safety and PK data were conducted after each cohort to assess whether the subsequent cohort required a decrease in dose, or whether it was feasible to obtain quantifiable concentrations.

### Subjects

A total of 18 male subjects (six subjects per cohort) were enrolled in the UA, OA, and OP cohorts. Due to the fact that there were no quantifiable concentrations obtained in the OP cohort, the UP cohort was not conducted. Eligibility criteria included age 30–55 years, with body mass index (BMI) between 18–27 kg/m<sup>2</sup>, with a history of smoking of less than five cigarettes/day within the last year, and lack of participation in a clinical trial with [<sup>14</sup>C]-labeled compounds within the last 12 months. Participants were required to refrain from smoking, the use of prescription/nonprescription drugs, consumption of red wine and citrus fruits, and excess alcohol and caffeine.

### Treatments

All subjects received a single dose of 165 mg (~666 kBq/18 μCi) of a 1.85% (w/w) solution of [<sup>14</sup>C]UMEC applied over a 40-cm<sup>2</sup> surface area of one axilla or one palm, for 8 h. The calculated net amount of active UMEC was ~3.06 mg. The whole body exposure to radiation (effective dose) and the local skin radiation dose were estimated to be 33 μSv and 43 mSv/cm<sup>2</sup>, respectively. The application area was covered with an unocclusive dome in the UA cohort to prevent contamination of surrounding areas, or with an occlusive dressing in the OA and OP cohorts. After 8 h, the site was washed multiple times and then tape stripped twice over the entire application area. All washings and tape strips were assessed for radioactivity. The dose selection strategy is provided in the **Supplementary Information**.

### Ethics

The study was approved by the local ethics committees and was conducted in accordance with the Declaration of Helsinki principles and was consistent with the International Conference of Harmonization guidelines for Good Clinical Practices. Informed consent was obtained from each subject. The study complied with the International Commission on Radiological Protection limits for whole body effective radiation dose of <1 mSv, and within the limit for local skin radiation dose of < 50 mSv/cm<sup>2</sup>.<sup>13,14</sup>

### Assessments

#### Safety

Safety assessments included the monitoring of adverse events (AEs), clinical laboratory assessments, vital signs, and 12-lead electrocardiograms. Skin tolerability (application site irritation) was assessed using the Skin Tolerability Assessment Scale (**Supplementary Information**).

### Analytical methods

[<sup>14</sup>C] UMEC was uniformly labeled with <sup>14</sup>C atoms in two six-member metabolically stable carbon rings of the biphenyl methyl part of the molecule.

The percent of the radioactive dose adhering to the protective dome, occlusive dressing, or recovered from the tape strips, or by washing of the skin surface was measured using liquid scintillation counting.

Using the <sup>14</sup>C label as a tracer, total radioactivity and parent UMEC radioactivity was measured in plasma. Plasma samples were collected at predose, 2, 4, 5, 6, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 16, 24, 30, 36, 48, 72 h postdose, and at the follow-up visit using AMS. Plasma samples were analyzed for both total radioactivity<sup>15</sup> and [<sup>14</sup>C] UMEC. The lower limit of quantification (LOQ) for total radioactivity was ~6.50 pg equiv UMEC/mL. [<sup>14</sup>C] UMEC analysis was carried out using a validated method based on protein precipitation, followed by high-pressure liquid chromatography (HPLC) and AMS analysis. The LOQ was 0.348 pg/mL using a 1,000 μL aliquot of EDTA plasma with the higher limit of quantification (HLQ) of 94.2 pg/mL.

### Pharmacokinetics (PK) and statistical methods

#### Safety and tolerability

Safety was evaluated based on the incidence, intensity, and type of AEs and clinical laboratory parameters, assessments of vital signs, ECGs, and skin tolerability (application site irritation).

#### Percent dose recovered

The percentage of the dose recovered from the tape strips and the total percentage of the dose recovered from the skin surface was compared across treatment groups by evaluating the differences and two-sided 90% confidence intervals.

#### Noncompartmental analysis

Total plasma radioactivity and [<sup>14</sup>C] UMEC concentrations were analyzed by NCA using the actual sampling times in WinNonlin v. 6.3.0 (Pharsight, St. Louis, MO). The following PK parameters were determined, where possible: maximum observed plasma concentration ( $C_{max}$ ), time to  $C_{max}$  ( $t_{max}$ ), area under the plasma concentration-time curve (AUC) from time 0 to the last quantifiable sample ( $AUC_{0-last}$ ), AUC from time zero to 12 h or 24 h ( $AUC_{0-12}$  and  $AUC_{0-24}$ , respectively), AUC from time zero to time infinity ( $AUC_{0-\infty}$ ), and apparent terminal phase half-life ( $t_{1/2}$ ).

When possible, following log-transformation,  $C_{max}$ , and AUC of [<sup>14</sup>C] UMEC (and total radioactivity) were analyzed using a mixed effects model with fixed effect terms for cohort. Point estimates and their associated 90% confidence intervals were constructed for the comparison, when possible (OA: UA). The point estimates and their associated 90% confidence intervals were then back-transformed to provide point estimates and 90% confidence intervals for the ratios. To compute point estimates and associated 90% confidence intervals for the median differences between cohorts,  $T_{max}$  of [<sup>14</sup>C] UMEC was separately analyzed with nonparametric methods.

### Population PK modeling

Population PK modeling was performed using nonlinear mixed effects modeling software Phoenix NLME v. 1.2 (Pharsight) and the first-order conditional estimation method with interaction. The handling of the nonquantifiable concentrations for both the NCA and population PK analyses is described in the **Supplementary Information**. The starting point of model development was a prior two-compartment model following i.v. infusion (unpublished data). Skin absorption was modeled in a stepwise manner using various structural models, including: first-order absorption with lag time; transit compartment absorption with transit rate constant  $K_{lag}$  (Erlang type absorption<sup>16,17</sup>); mixed Erlang and first-order absorption processes; Weibull absorption;<sup>18</sup> two parallel first-order absorption processes with lag time; and two sequential zero and first-order absorption processes.

An exponential variance model was used to describe the interindividual variability:

$$\Theta_i = \Theta_{TV} * \exp(\eta_i)$$

where  $\Theta_{TV}$  is the population mean parameter and  $\eta_i$  is assumed to be a random variable with zero mean and variance  $\omega^2$ . Random effects were initially modeled on clearance and volume of the central compartment. The requirement for additional random effects was investigated by sequentially adding and removing them from the model. The residual error was assumed to be proportional to the plasma concentrations. Due to the limited number of subjects, the investigation of covariate effects on parameters was not conducted.

#### Model selection and evaluation

Model selection was based on the goodness-of-fit graphical analysis, successful minimization, and estimation of covariance; physiological plausibility; Akaike's information criteria or a difference in the objective function value of more than 3.84 (for nested models). Predictive performance was assessed using visual predictive check (VPC) ( $n = 1,000$ ) and bootstrap ( $n = 200$ ).

#### Repeated dose simulations

In all, 3,000 PK profiles following 14 daily dermal doses were simulated with uncertainty using ModelRisk 5 (Vose Software) and Phoenix NLME 1.2, assuming that 2 mg/cm<sup>2</sup> of the 1.85% (w/w) formulation were applied to both axillas (occluded) in males and females once daily for 14 days. Simulation assumptions are presented in the **Supplementary Information**. Mean  $C_{max}$  and  $AUC_{0-\tau}$  (calculated assuming an LOQ of 10 pg/mL used in the inhaled studies) were compared to the exposure parameters from the 62.5 μg and 125 μg inhaled UMEC doses.<sup>19</sup>

## RESULTS

A total of 18 subjects were enrolled, six subjects per cohort in the UA, OA, and OP cohorts. Due to the lack of quantifiable concentrations in the OP cohort, the unoccluded palm (UP) cohort was not conducted. Demographic characteristics are summarized in **Table 1**. All subjects were male and between 30 and 55 years of age.

**Table 1** Subject demographics

Demographics	Unoccluded axilla n = 6	Occluded axilla n = 6	Ooccluded palm n = 6
Age in Years, Mean (SD) Range	39.3 (8.71) (32-51)	47.8 (9.02) (30-55)	45.7 (6.28) (38-52)
Sex, n (%)			
Male:	6 (100)	6 (100)	6 (100)
Height (cm), Mean (SD)	182.0 (7.69)	178.5 (6.53)	179.2 (5.34)
Weight (kg), Mean (SD)	81.1 (8.88)	82.9 (6.94)	79.3 (4.27)
BMI (kg/m <sup>2</sup> ), Mean (SD)	24.5 (2.12)	26.0 (0.808)	24.7 (1.88)
Ethnicity, n (%)			
Hispanic or Latino:	0	0	0
Not Hispanic or Latino:	6 (100)	6 (100)	6 (100)
Race, n (%)			
African American/African Heritage	1 (20)	0	0
White - White/Caucasian/European Heritage	5 (80)	6 (100)	6 (100)

#### Amount of radiolabeled drug potentially absorbed

At the end of the 8-h application period, the unabsorbed radiolabeled drug was removed from the skin surface by repeated washing, and the application area was tape stripped twice to remove the topmost layers of stratum corneum, and the radioactivity in the washings and strips measured. The median percentage of the total radiolabeled dose recovered from the tape strips was 0.6%, 1.20%, and 0.1% from the UA, OA, and OP cohorts, respectively (**Supplementary Table S1**). The median percentage of the total radioactivity recovered from the skin surface by washing was 80.5%, 74.4%, and 52.6% from the UA, OA, and OP cohorts, respectively (**Supplementary Table S1**). Thus, the amount of dose potentially absorbed was ~20–25% in the axilla cohorts and ~47% in the OP cohort.

#### Plasma concentrations and noncompartmental analysis of [<sup>14</sup>C]UMEC and total radioactivity

Changes in the plasma concentrations of [<sup>14</sup>C]UMEC over time in individual subjects are presented as semilogarithmic plots in **Figure 1a**, and those of total drug-related radioactivity in **Figure 1b**. The PK parameters calculated for [<sup>14</sup>C]UMEC and total radioactivity are shown in **Table 2** and **Supplementary Table S2**, respectively. Where possible, [<sup>14</sup>C]UMEC PK parameters from the UA cohort were compared with those from the OA cohort.

Following administration of [<sup>14</sup>C]UMEC to the axilla, a slow increase in plasma concentrations was observed in the majority of the subjects in the axilla cohorts, followed by a slow apparent mono- or biphasic decline (**Figure 1a**). The majority of the subjects in the UA cohort had incomplete plasma concentration–time profiles, while plasma concentrations in the OA cohort were quantifiable out to 72 h in most cases, and 315 h in one subject. All samples from

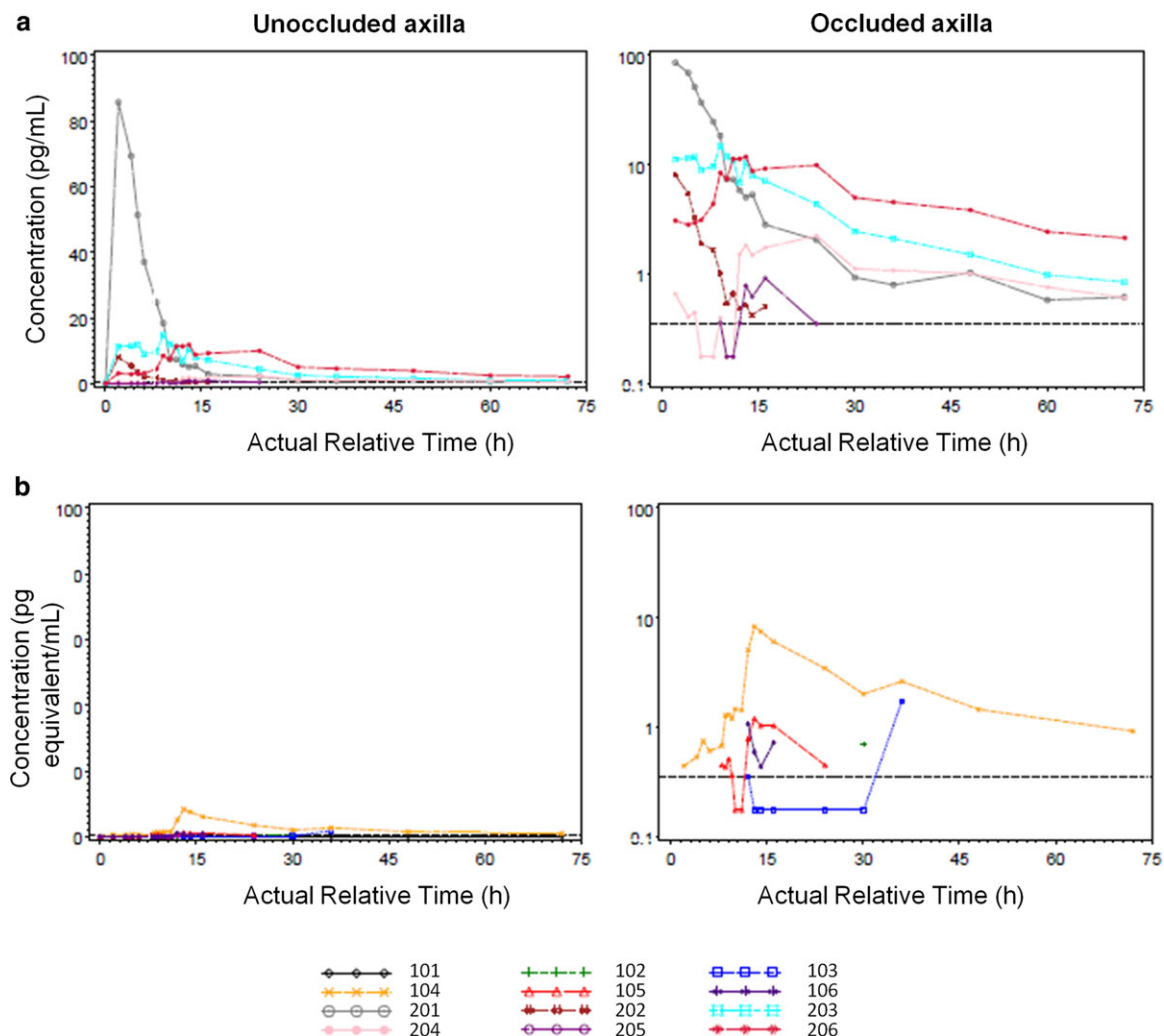
the OP cohort had nonquantifiable (NQ) concentrations of UMEC (LOQ was 0.348 pg/mL). Because UMEC has absorption rate limited pharmacokinetics, the terminal phase represents absorption, rather than elimination.<sup>20</sup> Median (range)  $t_{max}$  values were 13 h (12 h – 36 h) in UA, and 11 h (2 h – 24 h) in OA (**Table 2**). In a subset of two subjects in the OA group the maximum concentration of UMEC in plasma was reached by 2 h after dosing. There was a very high degree of variability observed for most of the key PK parameters, with %CVb values ranging between 121% (for  $C_{max}$ ) (**Table 2**) to 1,322% (for  $AUC_{0-12}$ ) (data not shown). Due to highly variable or NQ concentrations in the terminal phase, calculation of the  $t_{1/2}$  and  $AUC_{0-\infty}$  was possible in only one subject in the OA group. The comparison of PK parameters for the OA and UA cohorts showed that occlusion increased absorption by 3.8-fold (90% confidence interval [CI]: 0.732–20.0) based on  $C_{max}$ , and by 6.5-fold (90% CI: 0.590–70.6) based on  $AUC_{0-24}$ . The results of the comparison based on  $AUC_{0-24}$  are more difficult to interpret given that  $AUC_{0-\infty}$  could not be calculated.

Due to the higher LOQ for total radioactivity compared to the [<sup>14</sup>C]UMEC (6.5 vs. 0.348 pg/mL, respectively), the majority of the total radioactivity levels in the UA cohort were NQ, with only one subject having two quantifiable concentrations (**Figure 1b**). In the OA cohort, total radioactivity levels were quantifiable up to 24 h. The time course of plasma total radioactivity levels in the subjects with quantifiable levels tracked the corresponding plasma concentrations of [<sup>14</sup>C]UMEC up to the assay LOQ. Median (range)  $t_{max}$  values were 11.5 h (2 h – 24 h) in the OA cohort (**Table S2**). There was a high degree of variability observed for most of the key PK parameters, with %CVb values ranging between 126% (for  $C_{max}$ ) (**Table S2**) to 306% (for  $AUC_{0-12}$ , data not shown). Due to highly variable or NQ levels in the terminal elimination phase, calculation of the  $t_{1/2}$  and  $AUC_{0-\infty}$  was possible in only one subject in the OA group.

#### Population pharmacokinetic modeling

Due to the high number of NQ concentrations in the UA and OP cohorts, only data from the OA cohort were used in the population PK modeling. In addition, the data from the OA cohort were considered to be most representative of the clinical setting, where the axilla is in a semi-occluded environment. To distinguish between the absorption and elimination rate constants, plasma data from the OA cohort were combined with concentration–time data following 30 min i.v. infusion of 65 μg UMEC from a prior study in nine subjects (ClinicalTrials.gov Identifier NCT01110018).

A summary of the key population PK models is included in the **Supplementary Information**. A two-compartment model with two sequential absorption processes (a zero and first-order absorption with lag time) and elimination from the central compartment was found to best describe the combined data set (**Figure 2**). Interindividual variability was identified on clearance (CL), volume of distribution of the central compartment ( $V_1$ ), intercompartmental clearance (CL2), first-order absorption rate constant ( $K_a$ ), and lag time (tlag).



**Figure 1** Spaghetti semilogarithmic plots of individual subjects plasma [<sup>14</sup>C]UMEC (a) and plasma total radioactivity concentration (b) vs. time profiles for unoccluded axilla (left) and occluded axilla (right). The dotted lines represent the lower limit of quantification of 0.348 pg/mL for [<sup>14</sup>C]umeclidinium (a), and 6.50 pg equivalent UMEC/mL for the total radioactivity (b). Concentrations below the limit of quantification (BLQ) were set to zero if they occurred before the first measurable concentration in a profile, or to ½ the LOQ if they occurred after a measurable concentration and prior to the C<sub>max</sub>. All of the consecutive BLQ values occurring at the end of the concentration–time profiles were set to missing.

Residual variability was described using a proportional error model.

Pharmacokinetic parameters along with corresponding relative standard error (%RSE) and interindividual variability (expressed as a coefficient of variation, %CV) are summarized in **Table 3**. The goodness-of-fit plots (**Figure 3**), bootstrapping, and VPC confirmed that the final model had good model performance and was adequate for clinical trial simulations.

#### Exposure coverage compared to the marketed 62.5 μg inhaled dose

The population PK model was further used to predict the systemic exposure following repeated daily dermal doses of 2 mg/cm<sup>2</sup> of the 1.85% formulation to both occluded axillas at steady state. **Figure 4** shows that systemic UMEC concentrations at steady state following daily administration

to both axillas are predicted to be lower than the maximum systemic steady state concentrations from the 62.5 μg and 125 μg inhaled doses (**Figure 4**).<sup>19</sup> In addition, the predicted mean (90% prediction interval) C<sub>max</sub> ratio between the inhaled 62.5 μg and the dermal dose to both axillas was 2.85 (0.36, 22.4). The exposure ratio based on AUC<sub>0-τ</sub> (1.04[0.14, 7.01]) is confounded given that AUC<sub>0-τ</sub> could not be calculated in >47.4% of the simulated subjects, as expected for dermal products.

#### Safety and tolerability

One AE (skin irritation of mild intensity) was considered to be related to study treatment, and no SAEs were reported following a single dose of UMEC topically administered to the UA, OA, or OP of healthy male subjects. No clinical chemistry or hematology abnormalities met criteria for clinical importance, and no safety concerns were identified based on

**Table 2** Summary of selected plasma PK parameters for [<sup>14</sup>C]umeclidinium derived using noncompartmental analysis

Application site	Unoccluded axilla (n = 6)	Occluded axilla (n = 6)
$C_{max}$ (pg equiv/mL)		
n	4	6
Geom mean (%CVb)	2.07 (120.8)	7.92 (333.1)
$T_{max}$ (h)		
n	4	6
Median [range]	13.0 [12.0 – 36.0]	11.0 [2.0 – 24.0]
$AUC_{(0-t^a)}$ (pg equiv·hr /mL)		
n	4	6
Geom. mean (%CVb)	15 (395.3)	113.7 (407.0)
$AUC_{(0-\infty)}$ (pg equiv·hr /mL)		
n	0	1 <sup>b</sup>
Geom. mean (%CVb)	NC (NC)	702.5 (NC)
$t_{1/2}$ (h)		
n	0	1 <sup>b</sup>
Median [range]	NC [NC]	109 [NC]

NC, not calculable; No PK parameters could be calculated for the OP cohort as there were no quantifiable concentrations.

<sup>a</sup>Where t ranged from 16.02 h to 72.02 in the UA cohort, and ranged from 16.02 h to 315.02 h in the OA cohort.

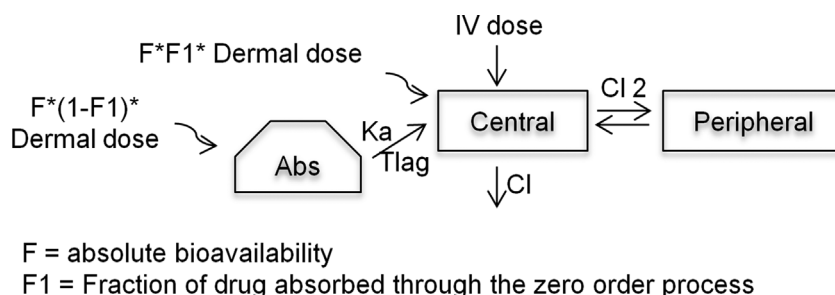
<sup>b</sup>No measures of distribution are appropriate when n = 1; observed value is presented.

evaluations of vital signs or ECGs. Mild or moderate skin irritation was identified in 9 out of 12 subjects with axillary application, with one skin irritation episode reported as an AE (mild intensity) as described above. A summary of the safety

results and a discussion of safety parameters that met the prespecified criteria for clinical importance are presented in the **Supplementary Information**.

## DISCUSSION

A novel study was conducted to characterize the safety, tolerability, and PKs of topical [<sup>14</sup>C]UMEC after single-dose administration to the axilla or palm in healthy male subjects. Minimal UMEC was absorbed into the systemic circulation with sporadic quantifiable plasma concentrations in four of six subjects receiving [<sup>14</sup>C]UMEC to the unoccluded axilla, while all six subjects receiving [<sup>14</sup>C]UMEC to the occluded axilla had quantifiable plasma concentrations with high intersubject variability. There was no measurable UMEC absorbed into the systemic circulation when a single dose was applied to the occluded palm. The safety, tolerability, and PK from this study, along with clinical trial simulations, showed that predicted exposures to UMEC applied to the axilla after repeat daily dosing are similar to those observed with inhaled dosing in patients with COPD;<sup>19</sup> thus, the systemic long-term safety data from the inhaled therapeutic dose are applicable to the dermal indication.<sup>21</sup> Therefore, it is anticipated that the systemic side effects that can be associated with oral anticholinergics (e.g., dry mouth, blurred vision, constipation, urinary retention, etc.) will be minimized when UMEC is administered directly to the axilla or palm. The results from noncompartmental and compartmental modeling are discussed in more detail below.



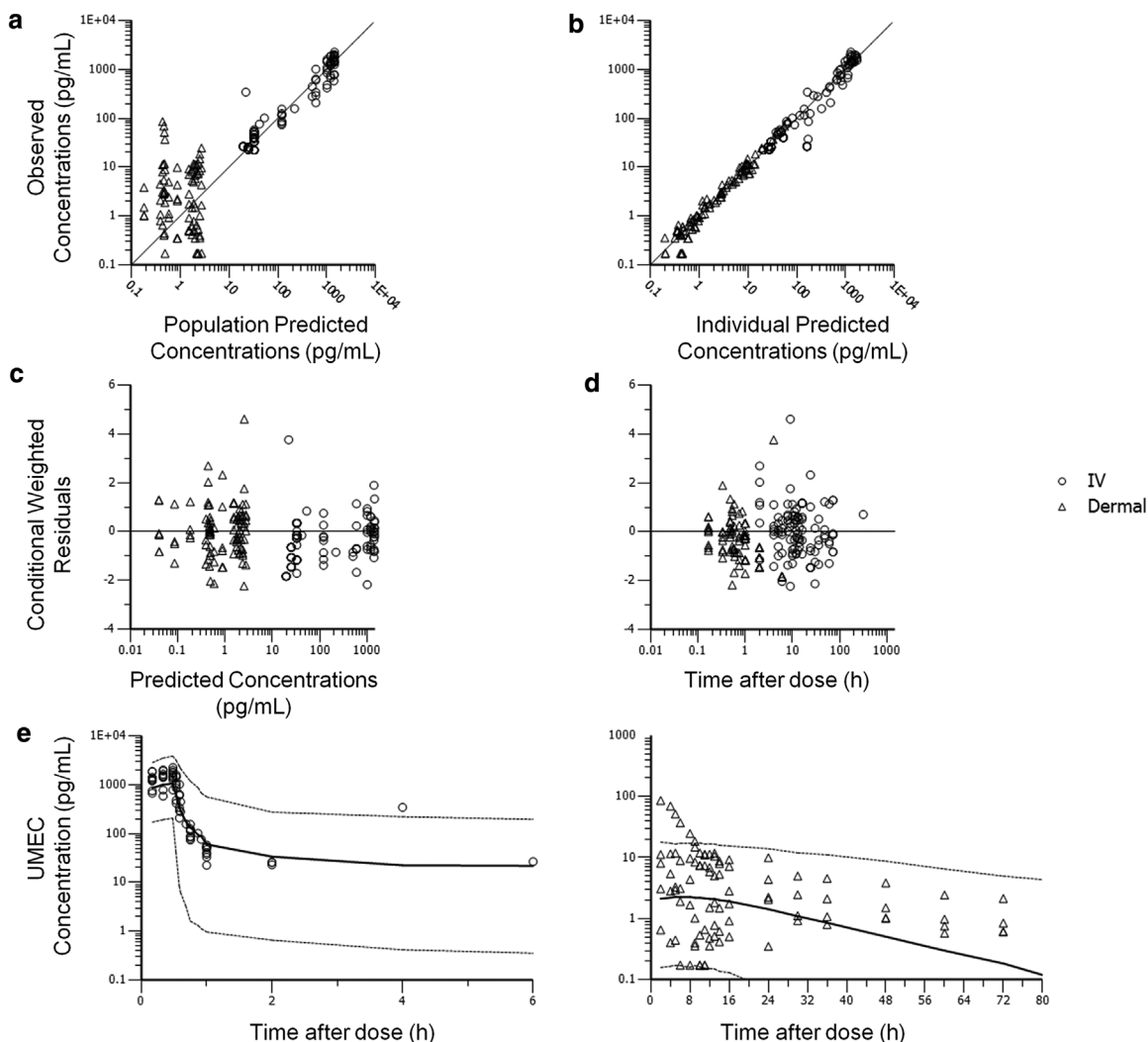
- F = absolute bioavailability
- F1 = Fraction of drug absorbed through the zero order process

**Figure 2** Population pharmacokinetic model structure.

**Table 3** Population pharmacokinetic parameter estimates for the final model

Parameter (units)	Parameter description	Model parameter estimates	Bootstrap median (RSE%)	Interindividual variability, CV% (RSE%)
V1 (L)	Volume of central compartment	7.56	7.61 (15.4%)	50.2 (85.3%)
CL (L/h)	Elimination clearance	55.0	47.5 (23.0%)	98.6 (51.4%)
V2 (L)	Volume of peripheral compartment	194.7	255.1 (57.7%)	
CL2 (L/h)	Intercompartmental Clearance	40.9	41.6 (30.8%)	104 (48.8%)
Ka (1/h)	First-order absorption rate constant	0.0934	0.0910 (45.4%)	139 (74.8%)
F1	Fraction of the bioavailable drug absorbed through a zero order process.	0.312	0.171 (76.6%)	
F	Absolute plasma bioavailability following administration to occluded axilla	0.00275	0.00217 (53.4%)	
Tlag (h)	Lag time for the first-order absorption process	10.6	4.71 (49.1%)	141 (73.3%)
Residual error, CV%		40.6%	35.9%	

No of bootstraps = 200; CV, coefficient of variation; RSE, relative standard error calculated as (standard error of the estimate/final parameter estimate) \* 100.



**Figure 3** Goodness-of-fit and diagnostic plots by administration route for the final model. (a) Observed vs. population predicted concentrations. (b) Observed vs. individual predicted concentrations. (c) Conditional weighted residuals vs. predicted concentrations. (d) Conditional weighted residuals vs. time. (e) Visual predicted check: concentration vs. time after dose. Dotted, dashed, and full lines represent the 5<sup>th</sup>, median, and 95<sup>th</sup> percentiles of simulated concentrations, respectively (1,000 simulations). Triangles represent the dermal route, circles represent the IV route.

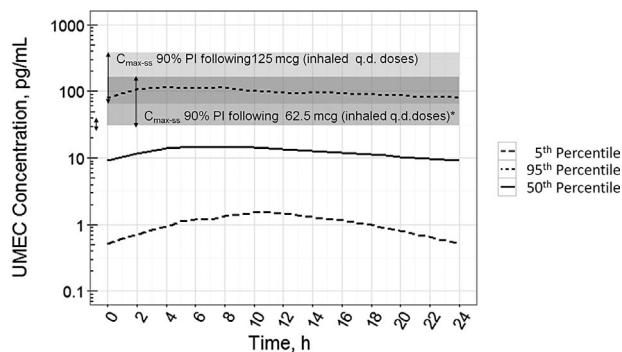
The absorption of UMEC following dermal dosing is slow and variable. The maximum observed plasma concentrations ( $C_{max}$ ) occurred 13 or 11 h postdose after administration to the unoccluded and occluded axilla, respectively, followed by a slow apparent mono- or biphasic decline. Because UMEC has absorption-rate limited PK, it is clear that absorption of [<sup>14</sup>C]UMEC occurred for up to at least 72 h in most patients and up to 13 days in one subject following administration of a single dose to the occluded axilla. As expected, the  $C_{max}$  was higher after administration to the occluded axilla than the unoccluded axilla, with a ratio of 3.8-fold (90% CI of 0.732, 19.947).

A two-compartment population PK model with linear elimination and sequential zero and first-order absorption processes described the disposition of UMEC following administration to occluded axilla when combined with data

following a 30-min i.v. infusion of 65  $\mu$ g UMEC from a prior study. Limitations of this model include the small number of subjects included in the data set and the inability to include population-specific covariates.

Total plasma radioactivity level-time profiles (when quantifiable) were similar in shape and concentrations of [<sup>14</sup>C]UMEC, down to the assay LOQ. This suggests that most of the circulating total drug related radioactivity may be attributed to the parent [<sup>14</sup>C]UMEC.

Approximately 80% or 75% of the radioactive dose was recovered from the skin surface in the unoccluded and occluded axilla cohorts, respectively, suggesting that 20–25% of the radiolabeled dose was potentially absorbed into the systemic circulation. This is an overestimate, given that the absolute bioavailability of unchanged UMEC was 0.22% based on the population PK model. Following administration



\* Marketed inhaled umeclidinium 62.5 µg dose (INCRUSE® ELLIPTA®)

**Figure 4** Predicted steady state UMEC plasma concentrations following daily doses to both axillars compared to  $C_{max}$  from the 62.5 µg and 125 µg inhaled doses in COPD patients. The dotted, full, and dashed lines represent the 5<sup>th</sup>, median, and the 95<sup>th</sup> percentiles of the simulated steady state concentrations following daily administration to both axillars, respectively (3,000 simulations). The gray ribbons represent the 90% prediction interval (PI) of the population-pharmacokinetic model-based steady state  $C_{max}$  predictions for UMEC following repeated daily inhaled doses of 62.5 µg or 125 µg doses in COPD patients.

to unoccluded or occluded axilla, the average percent of the radioactive dose recovered from the tape strips (representing the amount of drug in the topmost layers of the stratum corneum) was 0.6% and 1.2%, respectively. Since 20–25% of the radioactivity was not recovered in the washes or the tape strips, it is likely that the stratum corneum acts as a significant depot for UMEC.

Following administration to occluded palm, the average percent of the radioactive dose recovered from the tape strips was 0.1%. The average total percentage of the applied radioactive dose recovered from the palm after 8 h was 52.6%, suggesting that ~47% of the dose was potentially absorbed. This is also an overestimate, given that plasma concentrations of [<sup>14</sup>C]UMEC were nonquantifiable, confirming an absolute bioavailability of <1%. The difference between the percent of dose potentially absorbed in the axillary skin (20–25%) vs. ~47% in the palm may be due to the much thicker stratum corneum layer of the palm (~400 µm) vs. other sites (10–20 µm).<sup>22</sup> The planned administration of UMEC to the unoccluded palm cohort was not conducted.

To conclude, this study highlights the merit of the highly sensitive AMS [<sup>14</sup>C] measurement method as a framework for the detection of low levels of exposure following dermal application. This work enables model-based pharmacokinetic bridging of the systemic safety across different routes of administration and therapeutic areas; therefore, this study, along with safety data from long-term dosing of inhaled UMEC, provides a framework for the assessment of the benefit–risk profile for dermal UMEC.

**Acknowledgments.** We sincerely thank the Pharmaceutical Research Associates (PRA) clinical research unit in The Netherlands for

their assistance in the conduct of the study. This work was funded by GSK.

**Author Contributions.** T.P.D. wrote the article; E.H., P.C., S.B.-B., J.S.S., and V.S. designed the research; L.L.S., P.C., and S.V.M. performed the research; T.P.D., S.C.H., A.P., G.C.Y., and V.V. analyzed the data.

**Conflict of Interest.** T.P.D., L.S., S. H., A.P., G.Y., S.B.-B., J.S., and V.V. are employees of GlaxoSmithKline and hold stocks and/or stock options. E.H., P.C. and V.D.S. were employees of GlaxoSmithKline at the time of the study conduct and hold stocks and/or stock options. S.P.V.M. declared no conflict of interest.

- Strutton, D.R., Kowalski, J.W., Glaser, D.A. & Stang, P.E. US prevalence of hyperhidrosis and impact on individuals with axillary hyperhidrosis: results from a national survey. *J. Am. Acad. Dermatol.* **51**, 241–248 (2004).
- Lin, T.S. & Fang, H.Y. Transthoracic endoscopic sympathectomy in the treatment of palmar hyperhidrosis—with emphasis on perioperative management (1,360 case analyses). *Surg. Neurol.* **52**, 453–457 (1999).
- Swartling, C., Naver, H. & Lindberg, M. Botulinum A toxin improves life quality in severe primary focal hyperhidrosis. *Eur. J. Neurol.* **8**, 247–252 (2001).
- Hays, L.L. The Frey syndrome: a review and double blind evaluation of the topical use of a new anticholinergic agent. *Laryngoscope.* **88**, 1796–1824 (1978).
- Hyun, M.Y., Son, I.P., Lee, Y., Choi, H.G., Park, K.Y., Li, K. et al. Efficacy and safety of topical glycopyrrolate in patients with facial hyperhidrosis: a randomized, multicentre, double-blinded, placebo-controlled, split-face study. *J. Eur. Acad. Dermatol. Venereol.* **29**, 278–282 (2015).
- Kim, W.O., Kil, H.K., Yoon, D.M. & Cho, M.J. Treatment of compensatory gustatory hyperhidrosis with topical glycopyrrolate. *Yonsei Med. J.* **44**, 579–582 (2003).
- May, J.S. & McGuirt, W.F. Frey's syndrome: treatment with topical glycopyrrolate. *Head Neck.* **11**, 85–89 (1989).
- Shaw, J.E., Abbott, C.A., Tindle, K., Hollis, S. & Boulton, A.J. A randomised controlled trial of topical glycopyrrolate, the first specific treatment for diabetic gustatory sweating. *Diabetologia.* **40**, 299–301 (1997).
- Idson, B. Percutaneous absorption. *J. Pharm. Sci.* **64**, 901–924 (1975).
- Roberts, M.S., Favretto, W.A., Meyer, A., Reckmann, M. & Wongseelashote, T. Topical bioavailability of methyl salicylate. *Aust. N. Z. J. Med.* **12**, 303–305 (1982).
- Scheuplein, R.J. & Blank, I.H. Permeability of the skin. *Physiol. Rev.* **51**, 702–747 (1971).
- Zhai, H. & Maibach, H.I. Effects of skin occlusion on percutaneous absorption: an overview. *Skin Pharmacol. Appl. Skin Physiol.* **14**, 1–10 (2001).
- International Commission on Radiological Protection. Radiological Protection in Biomedical Research. ICRP Publication 62. *Ann. ICRP.* **22**, (1992).
- International Commission on Radiological Protection. The 2007 Recommendations of the International Commission on Radiological Protection. Radiological Protection in Biomedical Research. ICRP Publication 103. *Ann. ICRP.* **37**, (2007).
- Young, G.C., Corless, S., Felgate, C.C. & Colthup, P.V. Comparison of a 250 kV single-stage accelerator mass spectrometer with a 5 MV tandem accelerator mass spectrometer—fitness for purpose in bioanalysis. *Rapid Commun. Mass Spectrom.* **22**, 4035–4042 (2008).
- Rousseau, A., Leger, F., Le, M.Y., Saint-Marcoux, F., Paintaud, G., Buchler, M. et al. Population pharmacokinetic modeling of oral cyclosporin using NONMEM: comparison of absorption pharmacokinetic models and design of a Bayesian estimator. *Ther. Drug Monit.* **26**, 23–30 (2004).
- Krejcie, T.C., Jacques, J.A., Avram, M.J., Niemann, C.U., Shanks, C.A. & Henthorn, T.K. Use of parallel Erlang density functions to analyze first-pass pulmonary uptake of multiple indicators in dogs. *J. Pharmacokinetic. Biopharm.* **24**, 569–588 (1996).
- Zhou, H. Pharmacokinetic strategies in deciphering atypical drug absorption profiles. *J. Clin. Pharmacol.* **43**, 211–227 (2003).
- Goyal, N., Beerah, M., Kalberg, C., Church, A., Kilbridge, S. & Mehta, R. Population pharmacokinetics of inhaled umeclidinium and vilanterol in patients with chronic obstructive pulmonary disease. *Clin. Pharmacokinetic.* **53**, 637–648 (2014).
- Kelleher, D., Hughes, S., Mehta, R., Tombs, L., Kelly, K. & Church, A. Absorption, distribution, metabolism, and elimination (ADME) of umeclidinium (UMEC) in healthy adults. *Eur. Respir. J.* **40** (Suppl. 56), 384s (2012).
- Donohue, J.F., Niewoehner, D., Brooks, J., O'Dell, D. & Church, A. Safety and tolerability of once-daily umeclidinium/vilanterol 125/25 mcg and umeclidinium 125 mcg in patients with chronic obstructive pulmonary disease: results from a 52-week, randomized, double-blind, placebo-controlled study. *Respir. Res.* **15**, 78 (2014).
- Rushmer, R.F., Buettner, K.J., Short, J.M. & Odland, G.F. The skin. *Science.* **154**, 343–348 (1966).



© 2016 The Authors. *Clinical and Translational Science* published by Wiley Periodicals, Inc. on behalf of American Society for Clinical Pharmacology and Therapeutics. This is an open access article under the terms of the

Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Supplementary information accompanies this paper on the *Clinical and Translational Science* website.  
([http://onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1752-8062](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-8062))