Supplementary Information

Title: Limited Systemic Exposure with Topical Glycopyrronium Tosylate in Primary Axillary Hyperhidrosis

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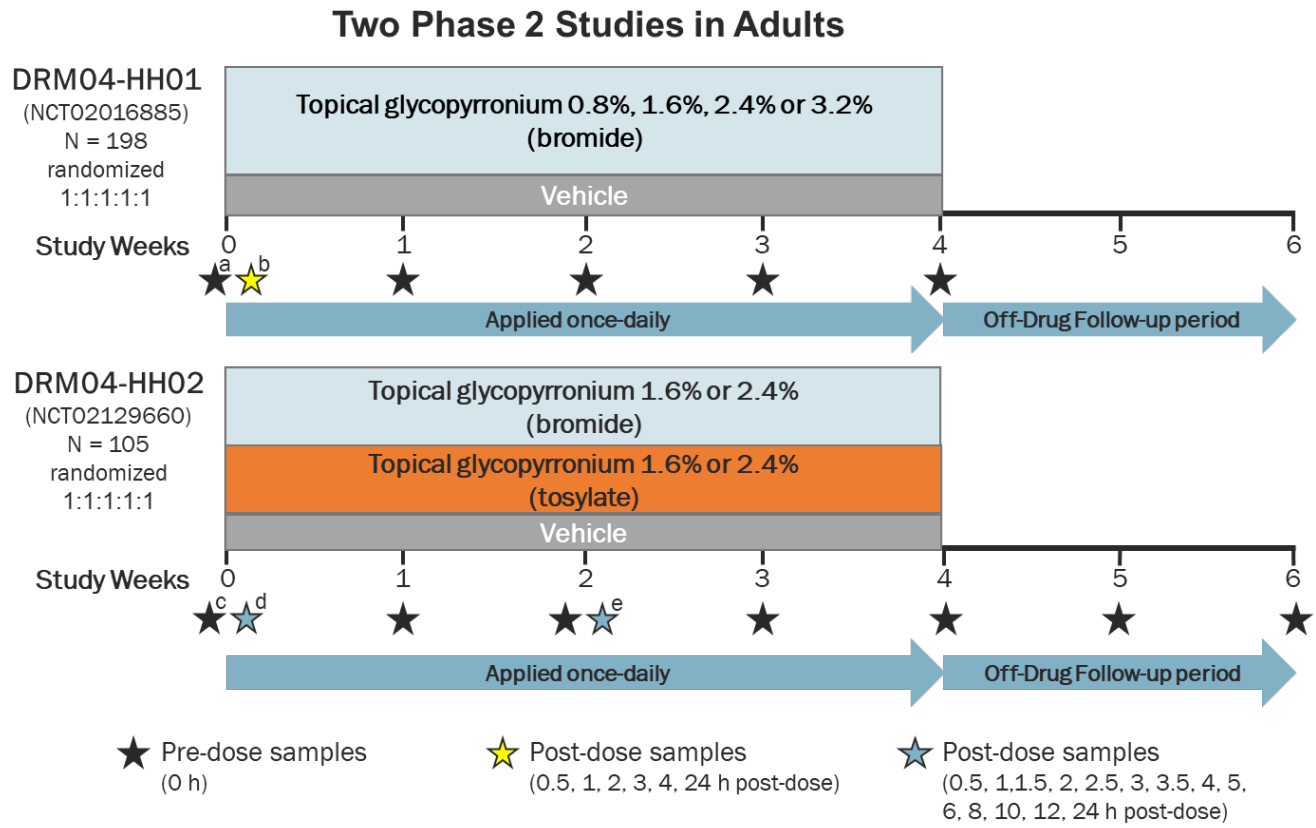
Supplemental Methods

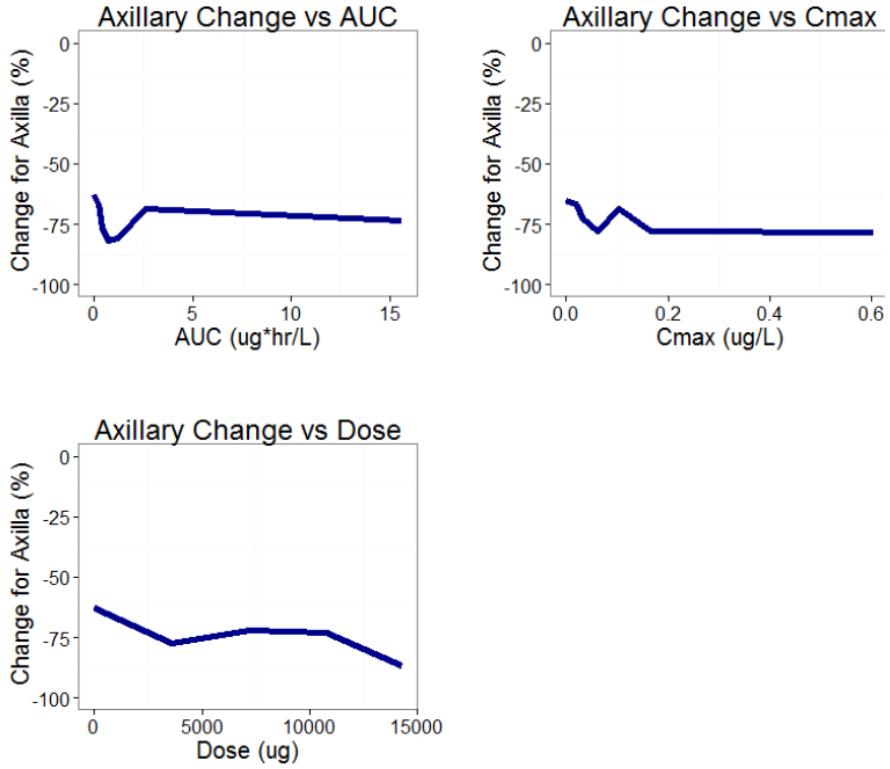
Additional methodological details for the population pharmacokinetic analysis are included at the end of this document.

Supplemental Figure 1. Phase 2 Studies in Adults with Primary Axillary Hyperhidrosis.



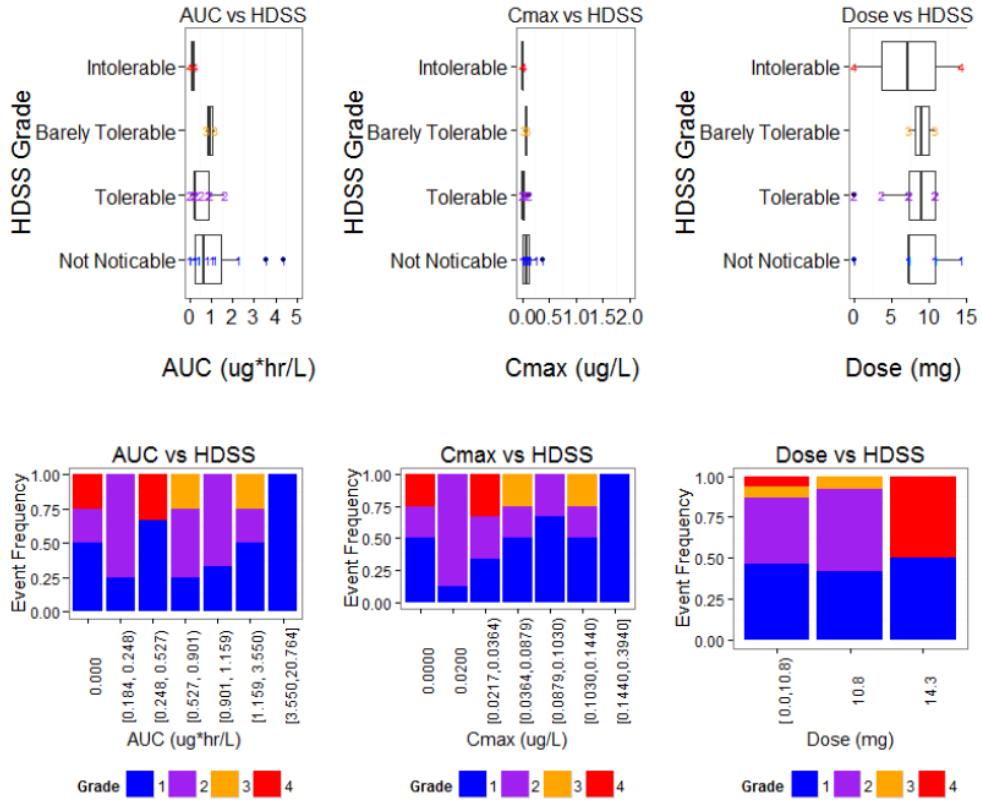
[a]n=32 subjects provided pre-dose samples during the trial; [b]n=32 subjects; [c]n=79 subjects provided only pre-dose samples during the trial; [d]n=10 subjects (these subjects also provided pre-dose samples at Weeks 4, 5 and 6); [e]n =14 subjects (these subjects also provided pre-dose samples at Day 1 and Weeks 4, 5, and 6).
h, hours

Supplemental Figure 2. Median Percent Change in Axial Gravimetric Score at Week 4 Versus Exposure



AUC, area under the plasma concentration over time curve; C_{max}, maximum plasma concentration

Supplemental Figure 3. HDSS at Week 4 Versus Exposure



AUC, area under the plasma concentration over time curve; C_{max}, maximum plasma concentration; HDSS, Hyperhidrosis Disease Severity Scale

Supplemental Table 1. Phase 1 Study: Day 1 PK Findings for Topical Gycopyrronium (Adult and Pediatric Patients) and Oral Glycopyrrolate (Adults Only)

PK Parameter	Topical Glycopyrronium Tosylate Adult	Topical Glycopyrronium Tosylate Pediatric	Oral glycopyrrolate[a] Adult
	2.4%	**2.4%**	**1 mg/q 8 h**
	Day 1 of 5	**Day 1 of 5**	**Day 1 of 15**
C_{max} (ng/mL)	n=11	n=19	n=18
Mean ± SD	0.14 ± 0.14	0.05 ± 0.07	0.12 ± 0.05
Median (min, max)	0.09 (0.02, 0.45)	0.03 (0, 0.23)	0.11 (0.04, 0.23)
AUC_{0-6h} (ng h/mL)	n=8	n=16	n= 18
Mean ± SD	0.54 ± 0.43	0.14 ± 0.14	0.46 ± 0.20
Median (min, max)	0.38 (0.08, 1.08)	0.14 (0, 0.37)	0.43 (0.16, 0.88)
AUC_{0-24h} (ng h/mL)	n=5	Not determined[b]	Not determined
Mean ± SD	2.57 ± 1.00	Not determined[b]	Not determined
Median (min, max)	2.90 (1.43, 3.62)	Not determined[b]	Not determined
T_{max} (h)	n=11	n=12	n= 18
Mean ± SD	2.55 ± 2.83	2.33 ± 2.33	2.53 ± 0.61
Median (min, max)	1.5 (0.5, 10.0)	1.0 (0.5, 6.0)	2.8 (1.0, 3.0)
$T_{1/2}$ (h)			n=15
Mean ± SD	Not determined[c]	Not determined[c]	1.94 ± 0.48
Median (min, max)	Not determined[c]	Not determined[c]	1.87 (1.46, 3.31)

[a]Fasting; [b]Pediatric samples were only collected up to 6 hours post-dose per guidelines on safe blood sampling; therefore, AUC_{0-24h} could not be determined; [c]A clear terminal elimination phase was not evident following topical glycopyrronium tosylate administration due to a lack of concentrations above the lower limit of quantitation; thus, no half-life could be determined in adult and pediatric patients.
The PK evaluable population included subjects who received study drug and had ≥1 PK sample collected.

AUC, area under the plasma concentration over time curve; C_{max}, maximum plasma concentration; h, hours; PK, pharmacokinetics; SD, standard deviation; $T_{1/2}$, elimination (terminal) half-life; T_{max}, time to maximum plasma concentration

Supplemental Table 2. Pop PK Study: Number of Patients and Samples

Study	Formulation	Strength	No. Patients Randomized in Study	No. Patients in Study PK Population[a]	No. Patients in Pop PK Database	No. Patients in Pop PK AE & Efficacy Databases	No. Samples in Pop PK Database
DRM04-HH01	Glycopyrronium (bromide)	0.8% 1.6% 2.4% 3.2%	38 40 40 40	6 6 6 7	6 6 6 7	6 6 6 7	64 65 66 75
	Vehicle		40	7	Not applicable[b]	7	Not applicable[b]
DRM04-HH02	Glycopyrronium (bromide)	1.6% 2.4%	21 20	21 20	20 19	21 20	183 172
	Glycopyrronium (tosylate)	1.6% 2.4%	22 20	22 20	21 20	22 20	205 155
	Vehicle		22	20	Not applicable[b]	22	Not applicable[b]
Patients with no concentration records[c]					3		0
Total					108	137	985

[a]PK population in DRM04-HH01 and DRM04-HH02 defined as subjects who had blood collected for PK analysis pre-dose and at least once post-dose; as specified in the DRM04-HH01 protocol, only a subset of randomized patients had PK samples collected; [b]The Pop PK database included only data from patients randomized to receive active treatment; [c]These subjects were included in the database to determine likely exposure for subsequent exposure-response evaluations (dose and covariate data were available).

AE, adverse event; PD, pharmacodynamics; PK, pharmacokinetics; Pop, population

4 METHODS

This section describes procedures used to develop and evaluate the PPK PPKAE and PPKPD models. Methods employed were consistent with the recommendations made by the regulatory agencies [12, 13]. In summary, the structural and stochastic models were developed first. Once a base model was established, covariates were evaluated and a full model was constructed. Afterward, the final models evaluated using unstratified nonparametric bootstrapping, and for the

PPK model, visual predictive check (VPC) and other approaches. The analysis plan is provided in Appendix 1, Section 1.1.

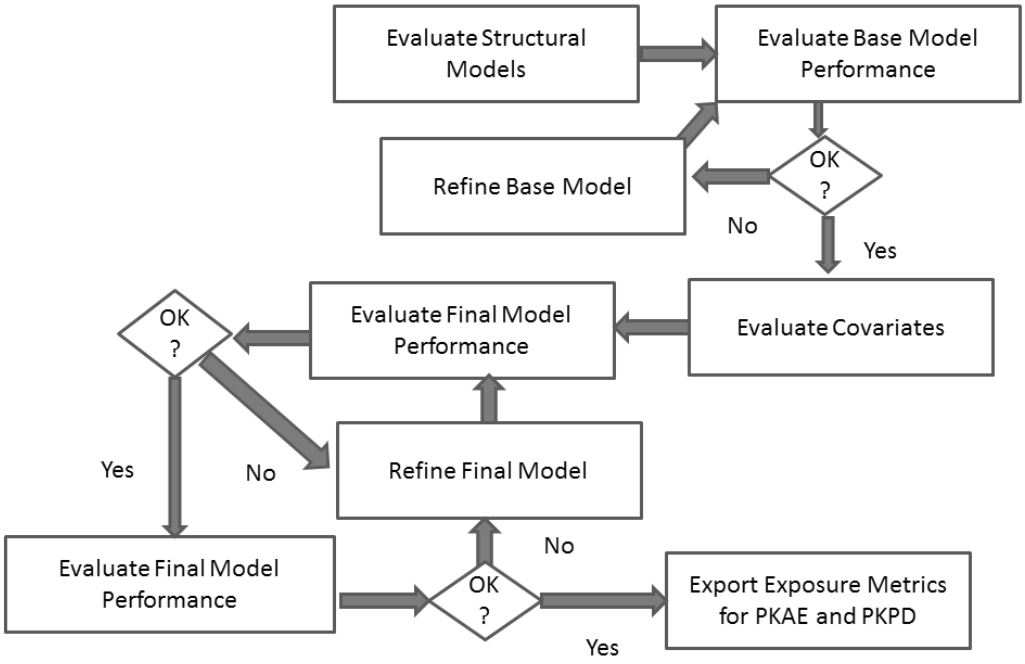
4.1 Deviations from Planned Evaluations

There were no deviations from the planned evaluation.

4.2 Population Pharmacokinetics

The PK of glycopyrrolate were characterized by nonlinear mixed-effects ("population") compartmental models that were developed and evaluated using the methods described in Sections 4.2.1 and 4.2.2. Figure 12 presents the schematic of the PPK analysis.

Figure 12 Chart of PPK Model Development



4.2.1 PPK Model Development

The PPK model was developed in the following steps. First, a base model was developed without consideration of covariate effects. Additional evaluations of stochastic models were conducted. Then, a full-covariate model was developed by testing pre-specified covariate-parameter relationships graphically and as single covariate models. Covariates that showed a trend or were known to influence glycopyrrolate PK were tested as single covariate models. Covariates that met the criteria for selection were incorporated into a full model. Lastly, the final PPK model was chosen using backwards elimination by retaining only the statistically significant covariate effects.

The PPK model is specified in terms of fixed- and random-effect parameters that were estimated by the NONMEM software program (version 7 level 2) [14]. The first-order conditional estimation (FOCE) method was used to estimate glycopyrrolate PK parameters. The INTERACTION option was not necessary as the PK data were fit using a log transform both

sides (LTBS) approach with an additive residual error model (proportional when data are back-transformed).

4.2.1.1 Base PPK Model

The base PPK model consisted of the following components: a structural model that described serum concentrations of glycopyrrolate as a function of time for the structural model, an interindividual variability (IIV) model that described random variability among individuals in the study population, and a residual error model that characterized the random variability in observed data within an individual.

The criteria used for model selection are described below, followed by descriptions of the structural, residual error, and IIV model development.

Model Selection Criteria

Model development was based on the following criteria, but not limited to:

- Successful achievement of NONMEM minimization and covariance steps indicates that the parameters are all identifiable.
- Assessment of standard goodness-of-fit plots.
- Reduction in NONMEM objective function value (OFV) for hierarchical models.
- Reductions in IIV and residual variability.

In addition, the stability of the models throughout the model development process was given close attention. To avoid ill-conditioning, inspection of the covariance matrix of estimates at every stage of model development was performed to verify that extreme pairwise correlations (p greater than 0.95) of the parameters were not encountered. The condition number of the correlation matrix of the parameter estimates (e.g., the square root of the ratio of the largest to smallest eigenvalues) was also assessed to ensure values $\leq$ 20. Values greater than 40 are indicative of an ill-conditioned model [15]. If during the course of model development convergence or covariance estimation problems occurred, ad hoc NONMEM runs were performed to evaluate the nature of the ill-conditioning. Skewness and kurtosis of the distributions of individual eta values were also periodically checked. Shrinkage was also tested for all models to determine the appropriateness of covariate evaluations [16] as were the normalized prediction distribution errors (NPDE) [17].

Diagnostic plots were used to assess model assumptions and goodness-of-fit. Lowess smoothing lines or smoothing regression was included in these plots to help visualize trends in the data [18]. The following diagnostic plots were used in base model selection:

- Population prediction (PRED) and individual prediction (IPRED) versus observations (OBS)
- CWRES versus PRED and Individual WRES(IWRES) vs. IPRED
- CWRES versus time
- Histograms and Quantile-Quantile (QQ) plots of CWRES and IWRES to compare it to standard normal distribution

- QQ plots and histograms of IIV.

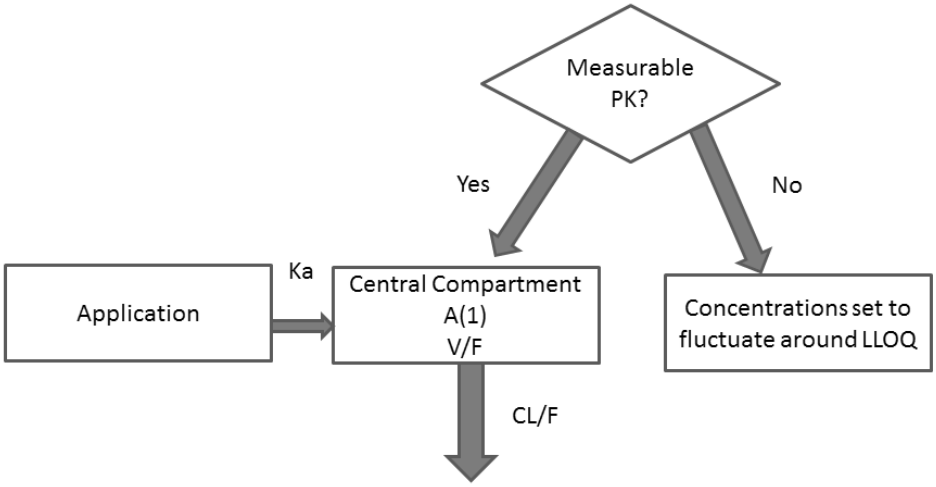
Population prediction (PRED) refers to model predictions made with estimates of typical values of structural model parameters (defined in Section 4.2.1), and individual prediction (IPRED) refers to model predictions made with estimates of individual random effects.

PPK Structural Model

Owing to the high number of patients who had no measurable concentration data, a mixture of models approach [10, 11] was used to separate patients into two subgroups, "absorbers" who had measurable concentrations and "non-absorbers" who had no measurable concentrations. For absorbers, a 1-compartment model with first-order absorption and elimination with terms describing IIV on clearance (CL/F), absorption rate constant (Ka) and relative bioavailability (F) was identified as an appropriate base model (Model 12 in Table 12).

For the absorber population, the 1-compartment model for the base structural model was defined in terms of the following parameters: CL/F, volume of distribution (V/F) absorption rate constant (Ka), relative bioavailability (F) and the baseline LLOQ value (C0). Figure 13 provides a schematic for the PK structural model as implemented in NONMEM.

Figure 13 Schematic for Structural PPK Model



Interindividual Variability Model (IIV Model)

The IIV model describes random variability among individuals in structural model parameters.

Individual values of structural model parameters that were constrained to positive values followed were assumed to follow a lognormal distribution. The lognormal IIV model for structural model parameter *P* is given by:

$$P_i \sim P_{TV} \cdot \exp(\eta_{P,i})$$

where P_i is the value of parameter P for the i^{th} individual, P_{TV} is the typical value of parameter P, and $\eta_{P,i} \sim N(0, \omega_P^2)$ is a realization of a normally distributed random variable with zero mean and variance ω_P^2.

During PPK model development with the model building database, the distribution of individual eta values for all parameters were checked for skewness and kurtosis, which would have necessitated the use of the Manly transform [19] to ensure normality.

A several models were tested to describe correlation between parameters. However these models failed to converge and thus a diagonal OMEGA matrix was used.

Residual Error Model

Residual variability is a composite measure of assay error, dose/sample time collection errors, model misspecification, and any other unexplained variability within a subject. For the PPK model, residual variability was modeled using the LTBS approach with an additive error model:

$ln(Y_{ij}) = ln(C_{ij}) + \varepsilon_{ij}$

where Y_{ij} denotes the observed concentration for the i^{th} individual at time t_j, C_{ij} denotes the corresponding predicted concentration based on the PPK model, and ε_{ij} denotes the intraindividual (residual) random effect, which is assumed to have a normal distribution with a zero mean and variance σ^2.

4.2.1.2 Full PPK Model

The covariate model development strategy was designed to assess the relationship between covariates of interest and structural model parameter values, while acknowledging that the range of available values for particular covariates may not always be sufficient to provide meaningful assessments of their effect.

Clinical judgment and mechanistic plausibility were used to determine which covariates should be tested on which parameters. Table 10 provides a list of the covariates that were tested in the PPK model and the structural parameters that they were tested on. Covariates were evaluated graphically first and only those that showed a trend or were known to be influential for glycopyrrolate PK were tested via single covariate models.

Table 10 Covariates Included in the PPK Analysis

Parameter	Covariates
CL/F	AGE, WT, BMI, SEX, RACE, BILI, ALB, ALT, AST
V/F	WT, BSA, BMI, SEX, RACE
Ka	AGE, WT, BMI, SEX, RACE, FORM
F	AGE, WT, BMI, SEX, RACE, FORM

For most covariates, the relationship between the typical value of a parameter (P_{TV}) and a continuous valued covariate (R) was tested using the following relationship.

$$P_{TV} = P_1\left(\frac{R}{R_{ref}}\right)^{P_2}$$

where P_1 and P_2 are fixed effect parameters, and R_{ref} is a reference value of the covariate. Time varying continuous valued covariates were incorporated by allowing the value of R to vary with time.

In addition because the data were from adult patients with a wide range in body sizes, an allometric function [20] was also tested, with the allometric coefficient of 0.75 for clearance terms (CL and Q) and a coefficient of 1 for volume terms (Vc and Vp).

$$P_{TVClearance} = P_1 * \left(\frac{Weight(kg)}{WTstd}\right)^{0.75}$$

$$P_{TVVolume} = P_2 * \left(\frac{Weight(kg)}{WTstd}\right)$$

The relationship between the typical value of a parameter (P_{TV}) and a categorical covariate (R) was tested using the following relationship.

$$P_{TV} = P_1(1 + RP_2)$$

Where P_1 and P_2 are fixed effect parameters. Alternatively this relationship was tested using the following relationship.

$$P_{TV} = P_1 R^{P2}$$

The effect of formulation on PK parameters was tested using the following relationship (clearance shown as a reference example).

$$P_{TVClearance} = P_1$$

$$If\ (Form = 2)\ P_{TVClearance} = P_2$$

$$If\ (Form = 3)\ P_{TVClearance} = P_3$$

Covariates listed in Table 10 were evaluated first graphically, then tested as single covariate models.

4.2.1.3 Final PPK Model

Once all important covariates were identified, a full model including all relevant covariates was tested. A stepwise backward elimination from the full model was implemented.

The same diagnostic plots discussed for the base model (see Section 4.2.1.1) were generated for the final PPK model. In addition, η plots versus each covariate were compared to similar plots

[redacted]

for the base model to verify that the final PPK model account for trends observed with the base model. A comparison of the objective function value (OFV) and parameter estimates for the base, full, and final models were used to assess the degree of parsimony of the final models.

Covariate-parameter relationships in the full-covariate model were retained in the final model provided they were statically significant (p less than 0.001). A continuous covariate was considered clinically relevant if its inclusion resulted in more than 20% change in point estimates for low (5%) and high (95%) values of the covariate and the 95% confidence interval (CI) was outside the range of 80%-120% of the typical value of the PK parameter without this covariate (but including all other significant covariates in the model). For a categorical covariate, the clinical relevance was defined as 20% change in point estimates compared to the typical parameter values of the reference population and the 95% CI was outside the range of 80%-120% of the typical value without this covariate. For both continuous and categorical covariates, covariates that resulted in less than ± 20% change in point estimates and the 95% CI fell within 20% of the reference value were determined to be not clinically important. If the point estimates of a covariate effect were within 80%-120% of the reference value, but the 95% CIs exceeded the range of 80%-120%, it was concluded that there was insufficient information in the present database to include the parameter as a covariate.

4.2.2 PPK Model Evaluation

VPCs were performed on the final PPK model and the model evaluation databases [21]. The parameter estimates were assumed to have a multivariate normal distribution with the mean vector set to the population parameter estimates and the covariance matrix set to the covariance matrix of the estimates from the final model. The final model was used to simulate 1,000 databases replicating the design, dose regimen, and covariates of the final model. Relevant summary measures were generated for both the observed and simulated data. The observed summary measure was then compared to selected percentiles (5^{th}, 50^{th}, and 95^{th}) of the 1,000 simulated summary measures.

A nonparametric stratified bootstrap [22] was conducted on the PPK model to determine the confidence intervals of the parameters for the final models. Parameters were evaluated to ensure the 95% confidence intervals did not include zero.

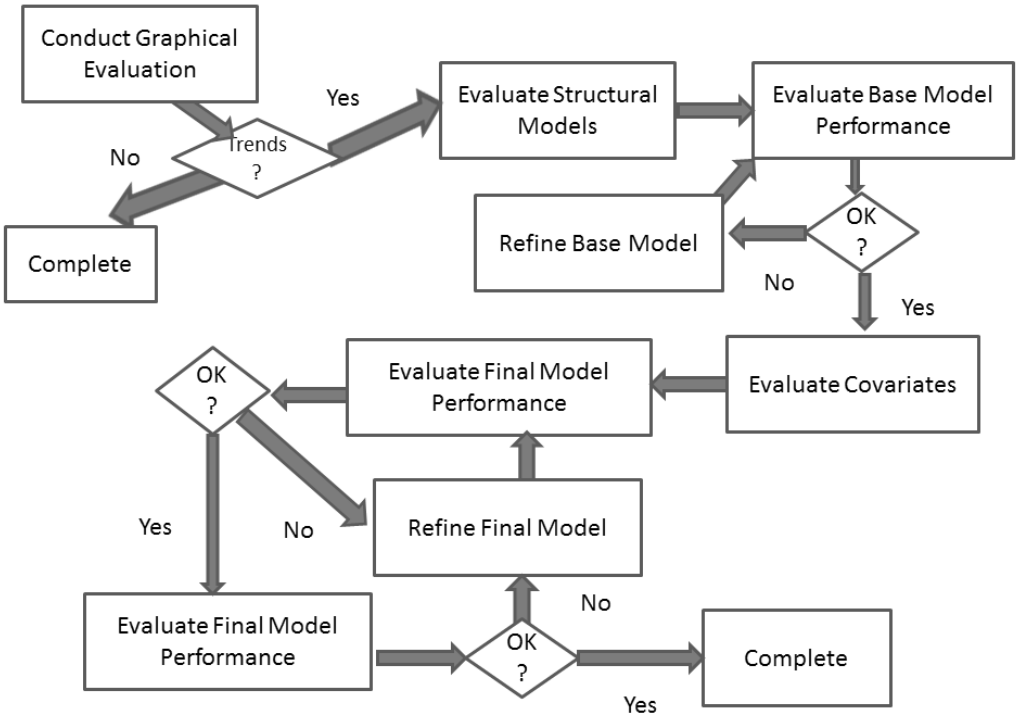
4.2.3 PPK Model Application

After model development and qualification was complete, final PPK model was used to generate metrics of exposure (AUC) for patients who had been randomized to receive active treatment. For subjects in the “absorber” subpopulation, peak concentration values (Cmax) were taken directly from the observed data and AUC was calculated as administered dose divided by clearance. For the “non-absorber” subpopulation, Cmax was set to the LLOQ and the AUC was computed assuming concentrations over the dose interval were at LLOQ. These exposure metrics were then merged with the AE data and the PD data to form the PPKAE and PPKPD databases.

4.3 Exposure-Response Evaluation

The exposure-response relationships for glycopyrrolate were initially evaluated graphically. Plots of glycopyrrolate exposure versus frequency and severity of adverse events (AEs) were generated. Plots of gravimetric score versus time by glycopyrrolate were also generated. When visible trends were evident, the relationship was further characterized by nonlinear mixed-effects ("population") compartmental models that were developed and evaluated using the methods described in Sections 4.2.1 and 4.2.2. Figure 14 presents the schematic of the exposure-response analysis.

Figure 14 Chart of Exposure-Response Evaluation



4.3.1 Graphical Exposure-Response

AE and PD data were graphically evaluated for trends with dose, AUC and Cmax. For the AE data, stacked histograms by exposure and AE severity as well as box and whisker plots by exposure and AE severity were generated. For all categories of AE, visual trends were noted with higher frequency of more severe grades of event noted as exposure increased.

For the PD assessment, median trends of gravimetric response by dose during the active treatment interval were generated. In addition, stacked histograms and box and whisker plots by grade of HDSS and exposure were generated. Although there was a clear trend towards improvements in both gravimetric and HDSS in patients randomized to active treatment arms compared to the responses seen in patients randomized to receive placebo, no clear trend was found with regard to individual exposure or dose. Thus PPKPD model building was not conducted.

4.3.2 PPKAE Model Development

The PPKAE models were developed in the following steps. First, a base model was developed without consideration of covariate effects. Because the PPKAE database contained only one observation for each subject, the stochastic model elements were not estimated and were fixed to zero. A binomial logit model describing the probability of any grade of adverse event was developed as well as an ordered categorical logistic regression model describing the probability of adverse events by grade of severity. Then, a full-covariate model was developed by testing pre-specified covariate-parameter relationships graphically and as single covariate models. Covariates that showed a trend were tested as single covariate models. Covariates that met the criteria for selection were incorporated into a full model. Lastly, the final PPKAE model was chosen using backwards elimination by retaining only the statistically significant covariate effects.

The PPKAE model is specified in terms of fixed-effect parameters that were estimated by the NONMEM software program (version 7 level 2) [14]. The first-order conditional estimation (FOCE) method with the LAPLACE option was used.

4.3.2.1 Base PPKAE Model

The base PPKAE model consisted only of a structural model that described the probability of an adverse event. The criteria used for model selection are described below.

Model Selection Criteria

Model development was based on the following criteria, but not limited to:

- Successful achievement of NONMEM minimization and covariance steps indicates that the parameters are all identifiable.
- Reduction in NONMEM objective function value (OFV) for hierarchical models.

In addition, the stability of the models throughout the model development process was given close attention. To avoid ill-conditioning, inspection of the covariance matrix of estimates at every stage of model development was performed to verify that extreme pairwise correlations (p greater than 0.95) of the parameters were not encountered. The condition number of the correlation matrix of the parameter estimates (e.g., the square root of the ratio of the largest to smallest eigenvalues) was also assessed to ensure values ≤ 20. Values greater than 40 are indicative of an ill-conditioned model [15]. If during the course of model development convergence or covariance estimation problems occurred, ad hoc NONMEM runs were performed to evaluate the nature of the ill-conditioning. Diagnostic plots were used to assess model assumptions and goodness-of-fit. The following diagnostic plots were used in base model selection:

- Population prediction (PRED) probability overlaid with binned observations (OBS)

PPKAE Structural Model

Owing to the fact that only one observation was available for each subject terms for residual error and between-subject variability (IIV) were not considered. The first PPKAE model developed was a binomial logit model that described the probability of any grade AE occurring based on glycopyrrolate exposure. The second PPKAE model developed evaluated the probability of specific grades of AEs based on glycopyrrolate exposure. Because the number of severe AEs was low, the combined probability of moderate and severe AEs was estimated.

4.3.2.2 Full PPKAE Model

The covariate model development strategy was designed to assess the relationship between covariates of interest and structural model parameter values, while acknowledging that the range of available values for particular covariates may not always be sufficient to provide meaningful assessments of their effect.

Clinical judgment and mechanistic plausibility were used to determine which covariates should be tested on which parameters. Table 11 provides a list of the covariates that were tested in the PPK model and the structural parameters that they were tested on. Covariates were evaluated via single covariate models that included the covariate factor on the overall probability of an event. Covariate factors were added to the probability functions.

Table 11 Covariates Included in the PPKAE Analysis

Parameter	Covariates
P{AE|covariate}	EXPOSURE, AGE, WT, BMI, SEX, RACE, BILI, ALB, ALT, AST

The relationship between the typical value of a parameter (P_{TV}) and a continuous valued covariate (R) was tested using the following relationship.

$$P_{TV} = P_1\left(\frac{R}{R_{ref}}\right)^{P_2}$$

where P_1 and P_2 are fixed effect parameters, and R_{ref} is a reference value of the covariate.

The relationship between the typical value of a parameter (P_{TV}) and a categorical covariate (R) was tested using the following relationship.

$$P_{TV} = P_1(1 + RP_2)$$

Where P_1 and P_2 are fixed effect parameters.

The effect of formulation was tested using the following relationship (clearance shown as a reference example).

[redacted]

$$P_{TVform} = P_1$$

$$If \ \left(Form \ = 2\right) \ P_{TVForm} = P_2$$

$$If \ \left(Form \ = 3\right) \ P_{TVForm} = P_3$$

Covariates listed in Table 11 were evaluated as single covariate models.

4.3.2.3 Final PPKAE Model

Once all important covariates were identified, a full model including all relevant covariates was tested. A stepwise backward elimination from the full model was implemented.

The same diagnostic plots discussed for the base model (see Section 4.3.2.1) were generated for the final PPKAE model. A comparison of the objective function value (OFV) and parameter estimates for the base, full, and final models were used to assess the degree of parsimony of the final models.

Covariate-parameter relationships in the full-covariate model were retained in the final model provided they were statically significant (p less than 0.001).

4.3.3 PPKAE Model Evaluation

A nonparametric stratified bootstrap [22] was conducted on the PPKAE model to determine the confidence intervals of the parameters for the final models. Parameters were evaluated to ensure the 95% confidence intervals did not include zero.

4.4 Data Analysis Platform

4.4.1 Hardware

The analysis was performed on a 2.33 GHz multi quad-pro-quad (Core Intel Xeon E7340) Dell PowerEdge R900 workstation with a NAS drive and multiple CPU packs, running on the 64 bit Microsoft Windows Server Windows SAL Enterprise Edition operating system.

4.4.2 Software

All model fitting was performed using FOCE as implemented in the NONMEM version 7 level 2 software and compiled using Intel Fortran Parallel Studio 2011, installed on a grid server system running Windows Server 2008 x64-bit. Diagnostic graphics, exploratory analyses, and post-processing of NONMEM output were performed using R version 2.15.0 or later. Microsoft Excel 2010 was used for viewing data (which was stored as a comma separated variable, CSV format).