Supplemental File 1:

Pharmacokinetic Modelling Methodology

Base model

It was assumed that all 13-CRA that is absorbed is metabolized to 4-oxo-13-CRA before being excreted.

The base model was parameterized using volumes and clearances. The difference of individual parameters P_i from the population mean ϑ (inter-individual variability, IIV) was parameterized as η and described using one of the following equations:

$$P_i = \theta + \eta \qquad \text{Constant (additive) variance model}$$

$$P_i = \theta \cdot \exp(\eta) \qquad \text{Exponential (lognormal) variance model}$$

The residual variability, which is the result of assay error, data error and model misspecification was modelled using one of the following equations:

$$C_{ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{Additive error model}$$

$$C_{ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \qquad \text{Proportional error model}$$

Where C_{ij} is the j^{th} observation of the i^{th} individual, $C_{pred,i,j}$ is the model-predicted value and ε_{ij} is the residual error for the current observation. All variability parameters were characterized by assuming normal distributions with a mean of 0 and an estimated variance of ω^2 for IIV and σ^2 for residual error.

The strategy was to first develop a popPK model for 13-CRA only, the incorporation of absorption, transit compartments and further parameters for different formulations were explored. Once a satisfactory model was identified, a second model for 4-oxo-13-CRA was developed. The two models were then combined and inferred onto the 13-CRA and 4-OIT data as a whole. Covariance between parameters for the 13-CRA part and for the 4-OIT part of the model were investigated and further refinements were made until a combined base model was identified that satisfied all model selection criteria.

Covariate model

Age, sex, various scalars of body size (weight, height, BSA, BMI) and formulation sequence were investigated on all structural PK parameters with IIV in the popPK base model.

The covariate effects were implemented in the base model as follows:

- Allometric scaling was done for bodyweight:

$$P_i = \theta_{TV} \cdot \left(\frac{BW_i}{BW_{med}}\right)^{\theta_{cov}} \cdot \exp(\eta)$$

- Continuous covariates

$$P_i = \theta_{TV} \cdot \exp\left(\theta_{cov} \cdot (COV_i - COV_{med})\right) \cdot \exp(\eta)$$

- Categorical covariates

$$P_i = \theta_{TV} \cdot (1 + N1 \cdot \theta_{cov1} + N2 \cdot \theta_{cov2} + \cdots) \cdot \exp(\eta)$$

Where P_i denoted the individual structural parameter value, θ_{TV} the estimated typical parameter value, BW_i the individual's bodyweight, BW_{med} the population median bodyweight. COV_i and COV_{med} are the individual and population median covariate values, respectively. θ_{cov} the estimated parameter for the covariate effect. N1=N2=0 for the most prevalent covariate category, N1=1 and N2=0 for the next most prevalent category, and N1=0 and N2=1 for the next category etc. The number of terms in the equation depends on the number of covariate categories.

The covariate analysis was performed with a full stepwise forward inclusion/backward elimination procedure. During forward inclusion, a reduction in the objective function value (OFV) corresponding to $p < 0.01$ ($\Delta OFV < -6.67$ for one estimated parameter, with adjustment for greater than one parameter according to Chi-squared distribution) was required for the declaration of a significant covariate effect. In backward elimination, the requirement was increased to $p < 0.001$ ($\Delta OFV > +10.828$ for one estimated parameter). The covariate model after the forward inclusion and backward elimination was called the selected popPK model.

The influence of the maturation of renal and enzymatic function on the clearance on 13-CRA and 4-OIT was not investigated due to only 10% of patients being ≤ 2 years old.

Terminal elimination half-life was not calculated due to lack of data during the terminal phase making predictions unreliable.

Model qualification

The main tool used for model qualification was the prediction-corrected VPC. These were generated from 1000 repeat simulations of the entire dataset with all random variables (inter-individual and residual) being sampled. The prediction-correction afforded an effective diagnostic display across a wide variety of doses and covariates (ref). Within each time bin, the 5^{th}, 50^{th}, and 95^{th} percentiles of the prediction-corrected observed and simulated data are calculated. From the 1000 replicates, a non-parametric 90% confidence interval for each of the three percentiles of the simulated data is obtained.

Prediction of individual exposure variables

Individual exposure variables at steady state for 13-CRA ($Tmax_{ss}$, $Cmax_{ss}$, $AUC_{(0\text{-}12)ss}$) and 4-oxo-13-CRA ($Cmax_{ss}$, $AUC_{(0\text{-}12)ss}$) were calculated after seven simulated doses using numerical integration of the popPK model.