**Pharmacokinetics and pharmacodynamics of remimazolam (CNS7056) after continuous infusion in healthy male volunteers: Part I. Pharmacokinetics and clinical pharmacodynamics**

**Supplemental Digital Content**

**S1: Methods of pharmacokinetic and pharmacodynamic modeling**

***Pharmacokinetic modeling***

Pharmacokinetics of the parent drug remimazolam and its metabolite CNS 7054 were modeled using linear mammillary two- or three-compartment models linked by a transit compartment to account for the formation of the metabolite. It was assumed that 100% of the eliminated amount of remimazolam entered the transit compartment. Pharmacokinetic models were parametrized using elimination and intercompartmental clearances (CL, Q2, Q3), and volumes of distributions (V1, V2, V3). Pharmacokinetics of remimazolam and CNS 7054 were modeled separately in a sequential manner. Modeling of remimazolam was performed first, and the individual Bayesian parameter estimates of the best remimazolam model were fixed in the subsequent step for modeling of CNS 7054. Interindividual variability of the parameters was modeled using log-normal distributions:

where θi is the parameter value in the ith subject, θTV is the typical value of the parameter in the population, and η is a normally distributed random variable with a mean of zero and a variance of ω2. For the residual intraindividual variability, we tested a combined additive and proportional error model:

and also an exponential error model:

where Cmij is the *jth* measured concentration of the *ith* individual, Cpij is the corresponding predicted concentration, and εij is a random number with mean zero and variance σ2.

Nonlinear mixed-effects modeling was performed using NONMEM 7.4.1 (ICON plc, Dublin, Ireland) The first-order conditional estimation method with interaction (FOCE-I) was used with NONMEM subroutines ADVAN3 and ADVAN11 for remimazolam, and ADVAN6 for CNS 7054, respectively.

***Pharmacodynamic modeling***

For pharmacodynamic modeling, an effect site was assumed as site of action with the effect site concentration CE defined by

where CP is the remimazolam plasma concentration and ke0 assesses the equilibration between CP and CE. The individual Bayesian parameter estimates of the best remimazolam model were used to calculate CP and CE. As the MOAA/S scale is not an interval ratio scale but an ordinal scale, probability models were used for pharmacodynamic modeling. Two types of models were tested: an ordinal logistic regression and a sigmoid probability model. In the ordinal logistic regression model, the probability to achieve a MOAA/S score ≤m was defined as:

with

where the ai define the baseline probabilities when no drug is present (CE=0), and the parameter b assesses the drug effect. Model parameters to be estimated were a0, a1, a2, a3, a4, b, and ke0. As the highest possible MOAA/S score is a value of 5, it follows that P(MOAA/S≤5)=1. The condition P(MOAA/S≤m+1) ≥ P(MOAA/S≤m) was realized by the constraint ai ≥ 0 for i=1 to 4. Log-normal distributions were assumed for the interindividual variability of b and ke0. The intersubject variability of the ai was assessed with the random parameter η.

As an alternative model, a model with sigmoid probability functions was tested:

where EC50,m is the effect site concentrations for a 50% probability of MOAA/S≤m, and γ defines the steepness of the probability functions. From the condition P(MOAA/S≤m+1) ≥ P(MOAA/S≤m) follows the constraint EC50,m ≥EC50,m+1. Therefore, the following incremental parametrization was chosen: EC50,4=θ1, EC50,3=EC50,4+θ2, EC50,2=EC50,3+θ3, EC50,1=EC50,2+θ4, EC50,0=EC50,1+θ5, with θ1,2,3,4,5 ≥0. Further model parameters to be estimated were γ=θ6 and ke0=θ7. Log-normal distributions were assumed for the interindividual variability of all model parameters. Residual intraindividual variability is not defined for probability models. For both pharmacodynamic models, the probability to achieve exactly a particular MOAA/S score was calculated as following:

Parameters were estimated using the Laplacian estimation method and NONMEM subroutine ADVAN6.

#### Model Development

Modeling was performed sequentially: The basic structural model (i.e. two- or three-compartment model for pharmacokinetics, ordinal logistic or sigmoid probability model for pharmacodynamics) was determined first, followed by covariate analysis. Selection of the best structural model was primarily based on the Bayes information criterion (BIC) defined as BIC=OFV+Ln(Nobs)\*Npar, where OFV is the NONMEM objective function value to be minimized, Nobs is the number of observations and Npar is the number of parameters to be estimated. The model with the lowest BIC was selected as best model.

After the structural model had been selected, the individual Bayesian estimates of the model parameters were used for the detection of covariate effects. The informativeness of the individual Bayesian estimates was assessed by the η‑shrinkage of the model parameters, defined as 1‑SD(ηi)/ω where ηi are the individual Bayesian estimates of the interindividual random variable η and ω is the population model estimate of the corresponding standard deviation. For detection of covariate effects, the individual Bayesian estimates of the model parameters were plotted independently against age and weight. Linear regression analysis was used as a first test for covariate effects. Subsequently, selected covariates were incorporated into the basic structural model using linear relationships with centering on the median value of the covariate (COV) within the population:

where θTV is the typical value of the parameter in the population and θCOV quantifies the covariate effect. For the effect of body weight, we tested linear relationships as well as power models:

with the exponent θBW fixed to 1 (proportional weight scaling) or estimated from the data. An allometric model with the exponent θBW fixed to1 for volumes and fixed to 0.75 for clearances was also tested.

Covariate analysis was also primarily based on the BIC. A model with additional covariates was favored if the BIC was lower than for the model without covariates. The likelihood ratio test (LRT) was also performed: the difference in the OFV was tested for significance by the chi-square test with the degree of freedom being equal to the difference in the number of model parameters. If the covariate model has one additional parameter more than the basic model, a decrease of OFV by more than 3.84 is significant with p<0.05. If a covariate model included additional parameters, it was also tested whether the 95% confidence interval of the additional parameters included the null hypothesis value. Covariates were included step-by-step starting from the basic structural model. Subsequently, backward deletion analysis was performed, testing each covariate effect for signiﬁcance by ﬁxing the corresponding parameter at *θCOV*=0.

#### Model Evaluation and Validation

For pharmacokinetic modeling, diagnostic plots (measured concentrations vs. population predictions and vs. individual predictions, conditional weighted residuals vs. time and vs. population predictions) were used to assess goodness of fit. Additionally, the prediction error (PE*ij*) and the absolute prediction error (APE*ij*) were calculated for each plasma concentration:

where Cmij is the *jth* measured concentration of the *ith* individual, and Cpij is the corresponding predicted concentration. Prediction errors were calculated for individual and population predictions, and goodness of fit was assessed by the median values of PEij (MDPE) and APEij (MDAPE).

Models were further validated by visual predictive check with 1000 simulated datasets using the doses and sampling times of the original data set, and model parameters with distributions as determined in the final pharmacokinetic model. Bootstrap analysis was performed to analyze the stability of the model and to obtain nonparametric confidence intervals of the final population model parameters. From the observed data, 1000 new data sets with the same number of individuals as the original data set were generated by resampling with replacement, and the final model was fitted to these new data sets. Median and confidence intervals of the bootstrap estimates were determined from successful bootstrap runs only, omitting runs with estimates near a boundary and runs which did not converge. The reliability of the parameter estimates was further assessed by log-likelihood profiling (LLP). For this purpose, the parameter to be assessed was fixed at particular values around its final population estimate, and the corresponding OFV values were plotted vs. the parameter values. This profile should have a clear minimum at the final estimate and its shape should not be too flat. As the change of the OFV follows approximately a chi-square distribution with one degree of freedom, nonparametric 95% and 99% confidence intervals of the parameter are defined by those areas of the likelihood profile where ΔOFV< 3.84 and <6.63, respectively.

For pharmacodynamic modeling, the diagnostic plots, visual predictive check and prediction errors used in pharmacokinetic modeling were not available, as the model prediction was not a MOAA/S score but probabilities to observe the different MOAA/S scores. Therefore, concentration-effect curves were generated by plotting the predicted probabilities P(MOOA/S≤m) vs. the effect site concentrations. The corresponding observed probabilities were determined as the incidence of MOOA/S≤m within discrete concentration ranges. Bootstrap analysis and log-likelihood profiling were also performed for the pharmacodynamic models.

**S2: Methods of ECG analysis**

The 12-lead digital continuous ECGs were assessed by a central cardiologist blinded to the trial treatment. A 10-second, 12-lead digital ECG was extracted at each time point specified in the protocol. If targeted ECG time points were artifactual and of poor quality, analyzable 10-second ECGs were captured as close as possible to the targeted time points. Interval duration measurements were collected using computer assisted caliper placements on three consecutive beats. Trained analysts reviewed all ECGs for correct lead and beat placement and adjudicated the pre-placed algorithm calipers as necessary using the proprietary validated electronic caliper system applied on a computer screen. A cardiologist then verified the interval durations and performed the morphology analysis, noting any T-U wave complex that was compatible with an effect on cardiac repolarization. The ECG analysis was conducted in Lead II, or in Lead V5 if Lead II was not analyzable. If Lead V5 was not analyzable then Lead V2 was used, followed by the most appropriate lead if necessary. ECG readers were blinded to subject identifiers, treatment, and visit. All ECGs for a given subject were analyzed by the same reader. On-screen measurements of the RR, PR, QRS, and QT interval durations were performed, and heart rate was determined as HR=60/(RR interval).

The physiologically inverse relationship between HR and QT interval required an adjustment process to ‘correct’, or ‘normalize’, the QT interval to the HR. This corrected QT interval (QTc) allows comparisons of QTc intervals across a range of HRs. There exist different approaches for the QT interval correction which can be generally written as QTc = QT / (RR)β or log(QTc) = log(QT) ‑ β × log(RR). The Fridericia correction (QTcF) uses β=1/3, whereas the Bazett correction (QTcB) uses β=1/2. However, in cases where there is a substantial change in heart rate, the individual QT correction method (QTcI) is preferred. For individual QT correction, β is estimated for each individual such that QTcI is a constant. If QTcI is a constant, one can rewrite the equation log(QTcI) = log(QT) ‑ β × log(RR) as log(QT) = α + β × log(RR). Thus, the exponent β can be determined for each subject by regression analysis on log transformed data of the QT and RR intervals collected on the control visit. Using these individual values of β, the QTcI values were calculated for all extracted data. Subsequently, the control-corrected change of QTcI from baseline (ΔΔQTcI) was calculated for each of the defined time points as the change of QTcI from baseline after remimazolam minus the change of QTcI from baseline during the control visit. The mean and the two-sided 90% CI (i.e. the 5% to 95% CI) of the ΔΔQTcI were determined to assess the change of the QTcI interval after remimazolam in the study population.

**S3: Results of pharmacokinetic modeling of remimazolam**

A three-compartment model was selected as structural model, as it revealed a much smaller BIC than a two-compartment model (4313 vs. 4746). For the assessment of the residual intraindividual error the exponential error model was selected, as the combined proportional and additive intraindividual error model resulted in an underprediction of the late concentrations. The shrinkage of the individual empirical Bayesian estimates was 1.4% for CL, 15.2% for Q2, 13.6% for Q3, 6.5% for V1, 16.6% for V2 and 4.9% for V3, respectively. This indicated that the individual parameter estimates were sufficiently informative for covariate analysis by linear regression. Regression analysis revealed no influence of age (regression coefficient r2<0.05 for all parameters). Concerning the influence of body weight, the regression analysis indicated only an increase of the central volume of distribution V1 with weight, whereas the other parameters did not show any effect of weight (fig. 1S). The influence of body weight on V1 was therefore further investigated. A covariate model with a linear relationship between V1 and weight decreased the OFV but this effect was not significant (ΔOFV= ‑2.1, p=0.15), as well as a for a power model with the exponent to be estimated (ΔOFV= ‑2.0, p=0.16). The allometric power model with fixed exponents of 1 for all volumes and 0.75 for all clearances was worse than the basic model as the OFV increased markedly (ΔOFV= 23.6). The best fit was yielded when V1 was scaled proportional to weight: V1,i=V1,TV\*(BW/75).When compared with the basic model, the OFV and BIC decreased by 1.8 for this covariate model. As this model has the same degrees of freedom as the basic model (i.e. no additional parameter to be estimated), one can, however, not give a significance level for this improvement. The interindividual variability of V1 decreased slightly from 26% without the body weight effect to 25% with weight as covariate. The diagnostic plots revealed a high quality of fit with low prediction errors and homogenously distributed residuals (fig. 2S). The LLP proved that the parameters were estimated reliably (fig. 3S). The visual predictive check demonstrated that the model predictions described the measured concentrations appropriately (fig. 4S). This three-compartment model with V1 scaled proportional to body weight was chosen as final pharmacokinetic model for remimazolam.

**Figure 1S:** Plots of the individual parameter estimates vs. body weight for the basic three-compartment pharmacokinetic model of remimazolam. The blue lines represent linear regressions through the data. R2 is the regression coefficient, and p is the significance level of the linear regression. CL: elimination clearance; Q2,3: intercompartmental clearances; V1: central volume of distribution; V2,3: peripheral volumes of distribution



**Figure 2S:** Goodness-of-fit plots for the final pharmacokinetic model of remimazolam. Measured concentrations vs. population (A) and individual (B) predictions; ratio of measured and predicted concentrations for the population (C) and individual (D) predictions; conditional weighted residuals vs. time (E) and vs. population predicted concentrations (F). The red lines represent lines of identity (measured = predicted), the blue lines are smoothing lines. MDPE: median prediction error; MDAPE: median absolute prediction error.



**Figure 3S:** Log-likelihood profiles for the final pharmacokinetic model of remimazolam. Critical values of the objective function value are shown as red and blue dotted line for p<0.05 and p<0.01, respectively. The vertical red line marks the population estimate of the parameter. CL: elimination clearance; Q2,3: intercompartmental clearances; V1: central volume of distribution; V2,3: peripheral volumes of distribution



**Figure 4S:** Visual predictive check for the final pharmacokinetic model of remimazolam. Measured concentrations are plotted as dots and the median of the measured concentrations as black line. The blue lines show the 5%, 50% and 95% quantiles of the model predictions.



**S4: Results of pharmacokinetic modeling of CNS 7054**

A combined three- and two-compartment model with a transit compartment was used for pharmacokinetic modeling of CNS 7054. The parameters for remimazolam were fixed to the individual estimates of the best pharmacokinetic model. During development of the basic model, it was found that the model was significantly improved (ΔOFV= ‑55.4, p<0.001) if one assumed an additional lag-time for the formation of CNS 7054. Without this lag-time, the concentrations in the first 5 min were overpredicted. The basic model showed already a good quality of fit without bias and with small prediction errors (MDPE= ­-2.42%, MDAPE=11.6% for the population predictions). The shrinkage of the individual empirical Bayesian estimates was 20.0% for Km, 0.13% for CLM, 26.0% for Q2M, 6.2% for V1M, and 3.7% for V2M , respectively. The interindividual variability of the lag-time was fixed to zero as it showed a relative standard error >200%. Regression plots of the individual estimates vs. weight and age indicated that there was no effect of age (regression coefficient r2<0.1 for all parameters), but an effect of body weight on KM, Q2M and V2M (fig. 5S). The inclusion of body weight as covariate was tested in a sequential process using a general linear relationship:

Body weight as covariate for V2M improved the model significantly (ΔOFV= ‑21.3, p<0.001). When weight was added as covariate for Q2M, the model was further improved significantly (ΔOFV= ‑10.7, p<0.01), whereas a further effect of weight on KM was not significant (ΔOFV= ‑0.24, p=0.62). A simplified linear relationship with proportional body weight scaling for both V2m and Q2m showed a slightly higher OFV (ΔOFV=0.32) with less degrees of freedom and correspondingly a lower BIC (5320 for the proportional weight scaling vs. 5326 for the general linear body weight effect, respectively). The model with a proportional weight scaling of b V2M and Q2M was therefore selected as final pharmacokinetic model for CNS 7054. The interindividual variability of V2M and Q2M decreased from 13.3% and 15.5% for the basic model to 7.2% and 10.4% for the final model, respectively.

The diagnostic plots revealed a high quality of fit with low prediction errors and homogenously distributed residuals (fig. 6S). The LLP proved that the parameters were estimated reliably (fig. 7S). The visual predictive check demonstrated that the predictions of the final model described the measured concentrations appropriately (fig. 8S).

**Figure 5S:** Plots of the individual parameter estimates vs. body weight for the basic pharmacokinetic model of CNS 7054. The blue lines represent linear regressions through the data. R2 is the regression coefficient, and p is the significance level of the linear regression. KM: transit rate constant; CLM: elimination clearance; Q2M: intercompartmental clearance; V1M: central volume of distribution; V2M: peripheral volume of distribution



**Figure 6S:** Goodness-of-fit plots for the final pharmacokinetic model of CNS 7054. Measured concentrations vs. population (A) and individual (B) predictions; ratio of measured and predicted concentrations for the population (C) and individual (D) predictions; conditional weighted residuals vs. time (E) and vs. population predicted concentrations (F). The red lines represent lines of identity (measured = predicted), the blue lines are smoothing lines. MDPE: median prediction error; MDAPE: median absolute prediction error.



**Figure 7S:** Log-likelihood profiles for the final pharmacokinetic model of CNS 7054. Critical values of the objective function value are shown as red and blue dotted line for p<0.05 and p<0.01, respectively. The vertical red line marks the population estimate of the parameter. KM: transit rate constant; CLM: elimination clearance; Q2M: intercompartmental clearance; V1M: central volume of distribution; V2M: peripheral volume of distribution; TLag: lag-time



**Figure 8S:** Visual predictive check for the final pharmacokinetic model of CNS 7054. Measured concentrations are plotted as dots, and the median of the measured concentrations as black line. The blue lines show the 5%, 50% and 95% quantiles of the model predictions.



**S5: Results of pharmacodynamic modeling of MOAA/S score**

For modeling of the MOAA/S scores, a logistic regression model and a model with sigmoid probability functions were investigated. The model with sigmoid probability functions achieved a smaller BIC than the logistic regression model (1323 vs. 1490) and was therefore selected as basic pharmacodynamic model. There was a considerable shrinkage for the parameters θ2 (51%) and θ3 (60%), probably due to the fact that MOAA/S scores of 2 and 3 were observed quite seldom. The shrinkage of the other model parameters was below 40%. Regression plots of the individual estimates vs. weight and age indicated that there was no significant effect of body weight (regression coefficient r2<0.15 for all parameters), but a possible effect of age on EC50,0 (fig. 9S). A model with a linear effect of age on the parameter θ5 which described the increment from EC50,1 to EC50,0 was therefore tested:

EC50,0 = EC50,1 +θ5 and θ5= θ5TV+ θ8\*(age-23)

This model, however, could not be estimated reliably. The covariate parameter θ8 reached high negative values so that θ5 became negative if age was greater than 35 years. This means that the EC50 for MOAA/S=0 would be smaller than the EC50 for MOAA/S≤1 if age>35 yrs., which seemed unreasonable. Age was therefore not included as covariate and the basic model was selected as the final pharmacodynamic model.

The LLP proved that the parameters were estimated reliably (fig. 10S). Figure 11S shows the observed and predicted cumulative probabilities for the pharmacodynamic model. The observed and predicted probabilities for MOAA/S≤1,2,3,4 were in good agreement, whereas the observed probability for MOAA/S=0 was not as well predicted by the model.

**Figure 9S:** Plots of the individual parameter estimates vs. age for the basic pharmacodynamic model of MOAA/S score. The blue lines represent linear regressions through the data. R2 is the regression coefficient, and p is the significance level of the linear regression. EC50,0,1,2,3,4: half maximum effect site concentration for a MOAA/S score≤0,1,2,3,4, respectively; γ: steepness of the concentration-effect curve; ke0: effect site equilibration rate constant.



**Figure 10S:** Log-likelihood profiles for the final pharmacodynamic model of MOAA/S score. Critical values of the objective function value are shown as red and blue dotted line for p<0.05 and p<0.01, respectively. The vertical red line marks the population estimate of the parameter. θ1-5 :model parameters defining the half maximum effect site concentrations for a MOAA/S score≤0,1,2,3,4: EC50,4=θ1, EC50,3=EC50,4+θ2, EC50,2=EC50,3+θ3, EC50,1=EC50,2+θ4, EC50,0=EC50,1+θ5; γ: steepness of the concentration-effect curve; ke0: effect site equilibration rate constant



**Figure 11S:** Observed and predicted probabilities for the different MOAA/S scores, as obtained by the final pharmacodynamic model. The grey bars show the observed probabilities in the concentration ranges. The blue lines show the predicted probabilities as obtained for the population parameter estimates. CE: effect site concentration of remimazolam.

