Supplementary Information S1: Model development and qualification

The qualification of a PBPK modeling system for the prediction of drug interactions encompasses in general the qualification of the software platform, the PK model for the new drug, and validation versus clinical interaction data. The following sections address these topics.

Qualification of Simcyp as PBPK framework for DDI

The Simcyp population based human PBPK simulator was used for all PBPK simulations. Key information on the qualification of this software tool is published by Jamei et al. (2013) on "The Simcyp Population Based simulator: Architecture, Implementation, and Quality Assurance".

Several aspects of the Simcyp population-based human simulator platform were developed and qualified specifically as a framework to describe DDI mechanistically as presented by Jamei 2016. The PBPK compound models for the index probe drugs were developed and qualified by the software vendor Simcyp/Certara for this specific purpose, i.e. "Sim-Midazolam" as CYP3A substrate, "SV-Bupropion" as CYP2B6 substrate, and "SV-Dextromethorphan" as CYP2D6 substrate. They were used as provided without modification, thus their suitability was maintained.

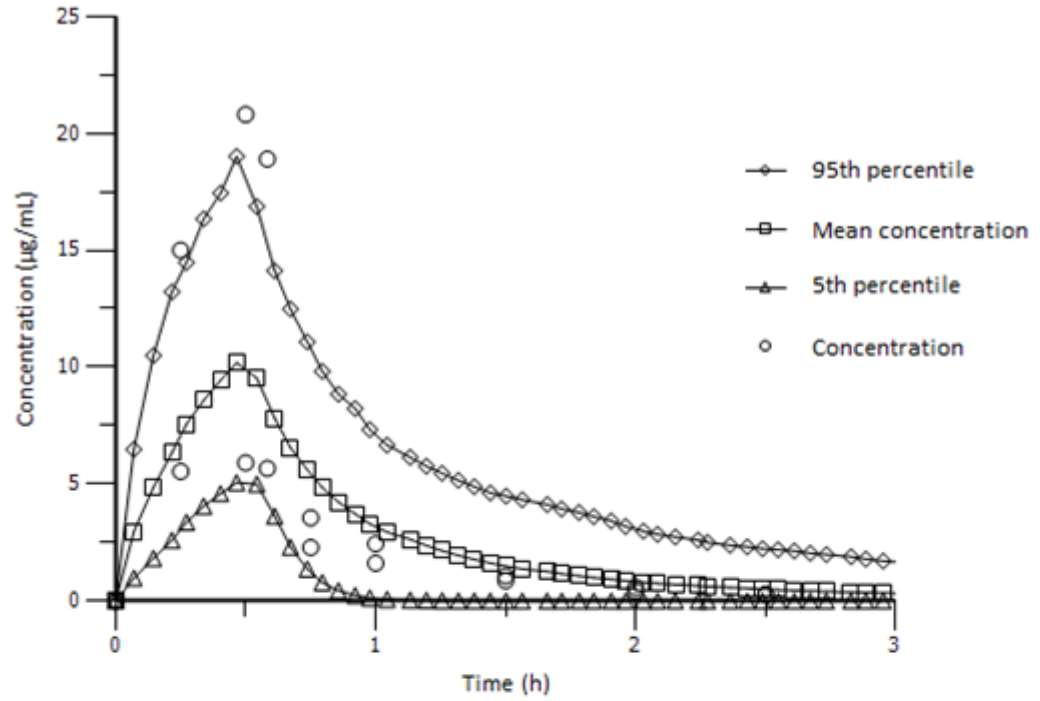
Evofosfamide PK model qualification

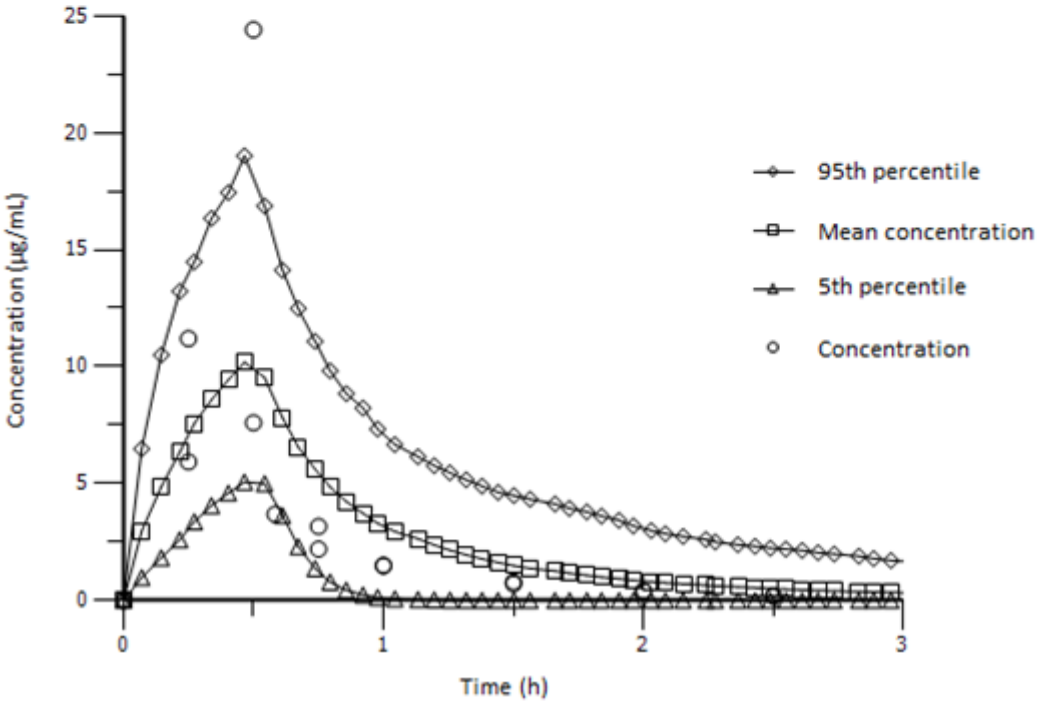
The PBPK model describing the PK of evofosfamide was developed using data from a Phase I monotherapy dose-escalation study in patients with solid tumors (NCT00495144). Concentration time data were grouped according to infusion duration. Dose levels closest to the target dose levels of 300 mg/m² and 340 mg/m² were used, as follows:

Dose level (mg/m²)	Planned infusion duration (min)	Number of concentration - time profiles
240	30	4
480	30	49
480	60	25
575	30	26
575	60	61

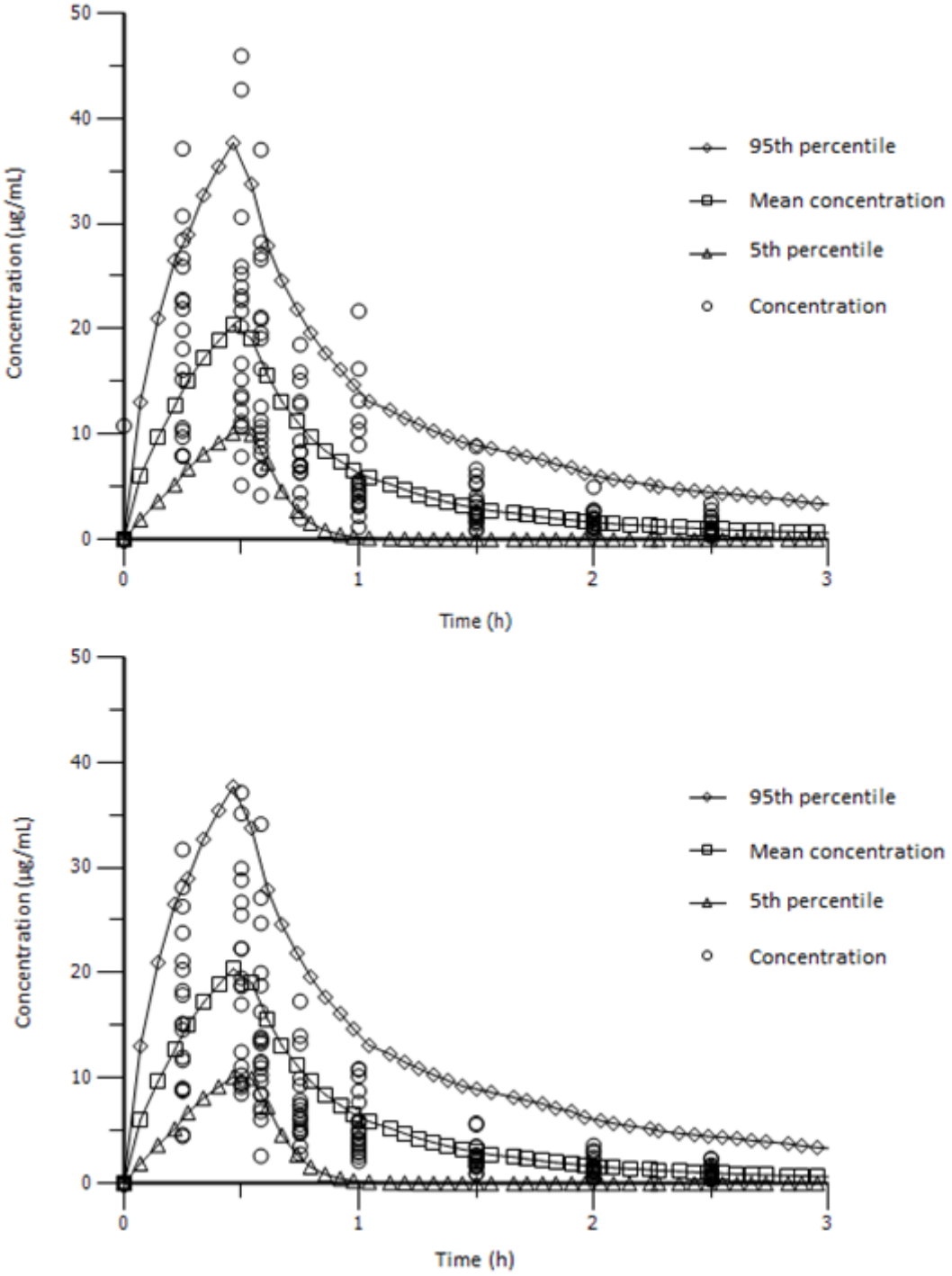
After refinement of the model, the data from across the above dose levels, and from both week 1 (first infusion) and week 3 (third infusion), were properly predicted by the PBPK model. That is, the final model predictions fitted the observed data well, or were slightly higher than the clinically observed concentrations. Some overprediction is acceptable since it fits with the conservative approach to estimate the interaction potential of evofosfamide as perpetrator. The overall purpose of the model is not to provide a precise estimate of the quantitative impact of the interaction, but rather to use a worst-case model to exclude the interaction potentials. The data per dose level as indicated in the table are shown in the respective figures below.

Simulated and observed individual plasma concentration-time profiles of evofosfamide given as intravenous infusion of **240 mg/m² over 30 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of the phase I monotherapy study (NCT00495144) are plotted as a function of time (circles). They derive from 2 patients, and refer to week 1 (top) and week 3 (bottom).

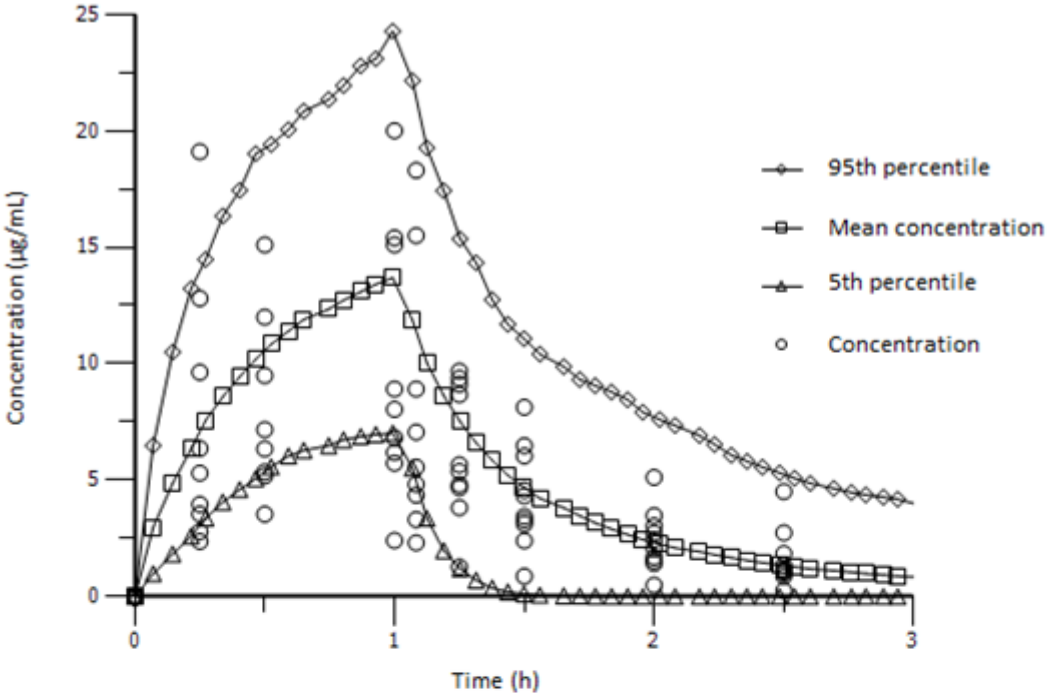


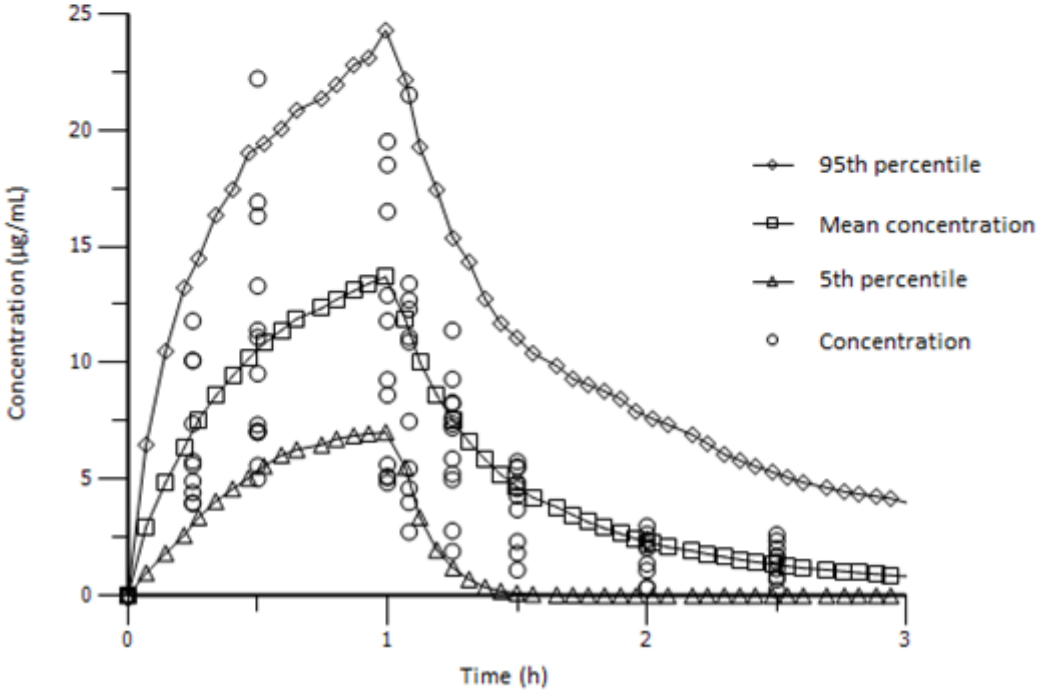


Simulated and observed individual plasma concentration-time profiles of evofosfamide given as intravenous infusion of **480 mg/m² over 30 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of the phase I monotherapy study (NCT00495144) are plotted as a function of time (circles), and refer to week 1 (top) and week 3 (bottom).

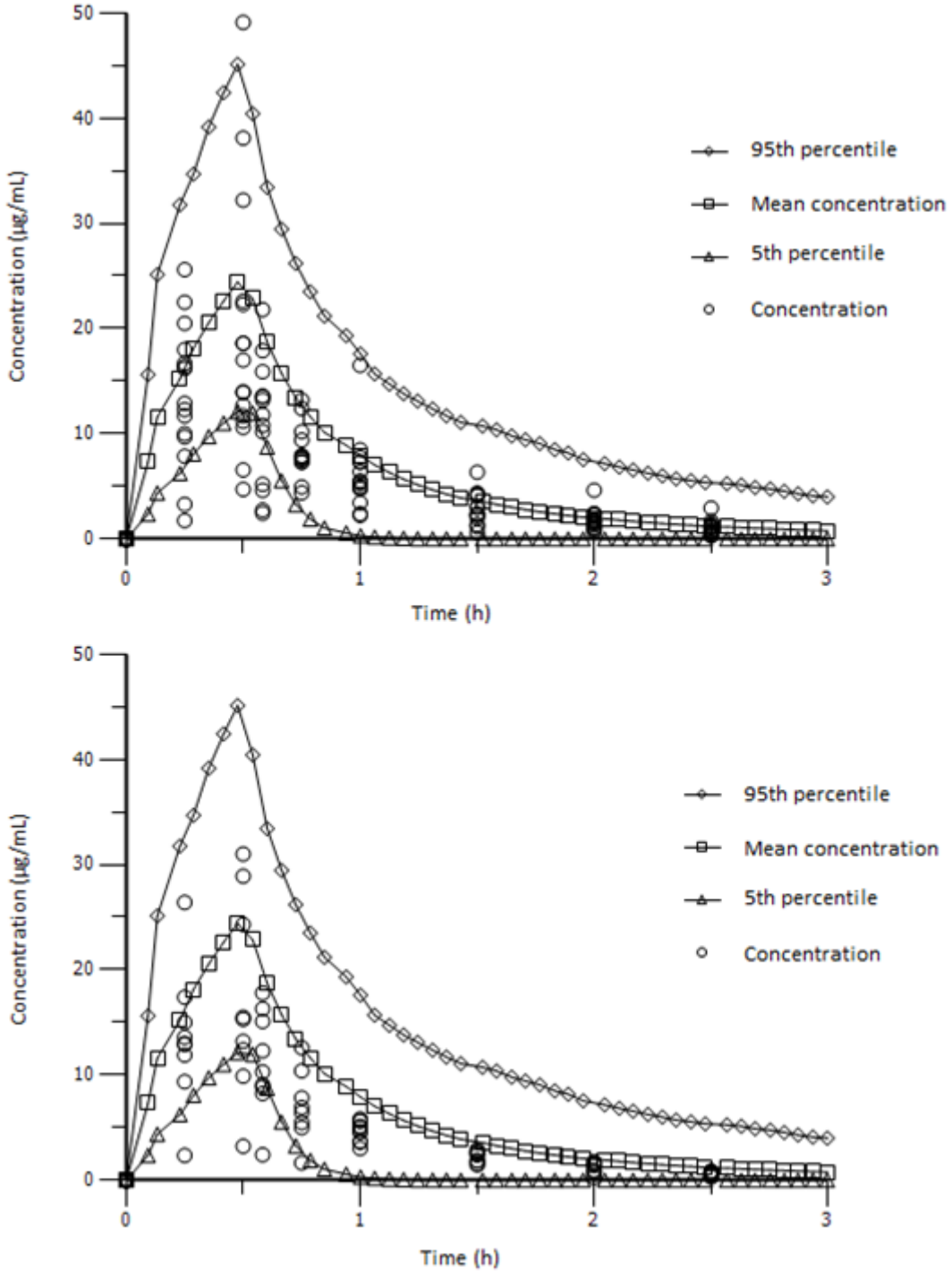


Simulated and observed individual plasma concentration-time profiles of evofosfamide given as intravenous infusion of **480 mg/m² over 60 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of the phase I monotherapy study (NCT00495144) are plotted as a function of time (circles). Data presented refer to week 1 (top) and week 3 (bottom).

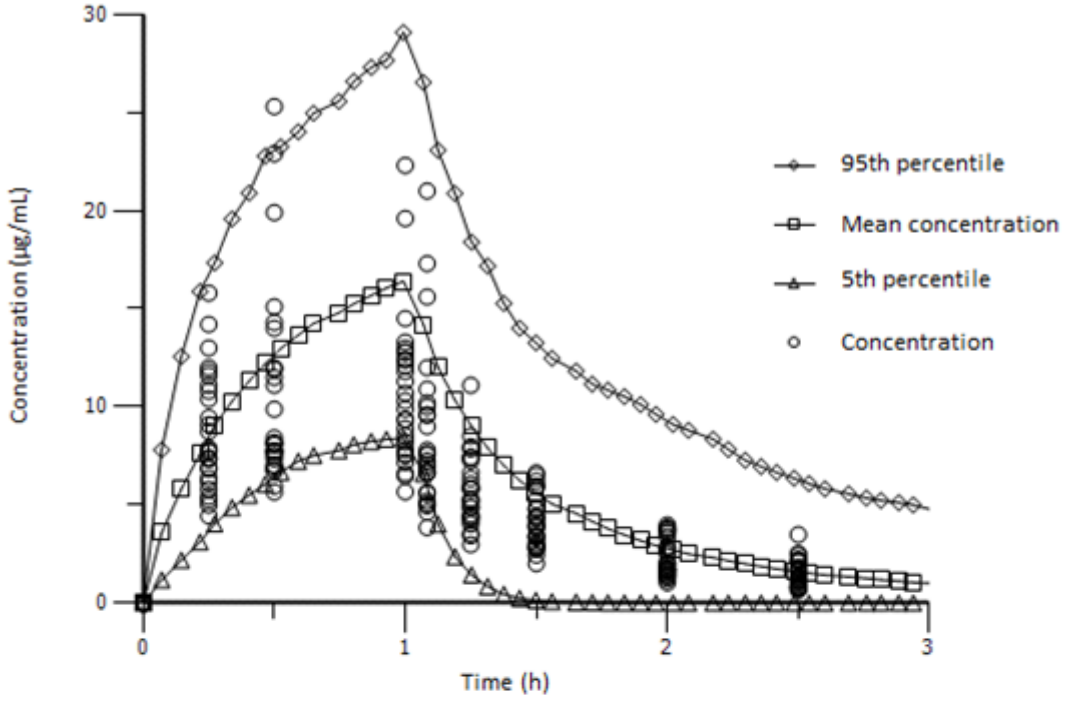


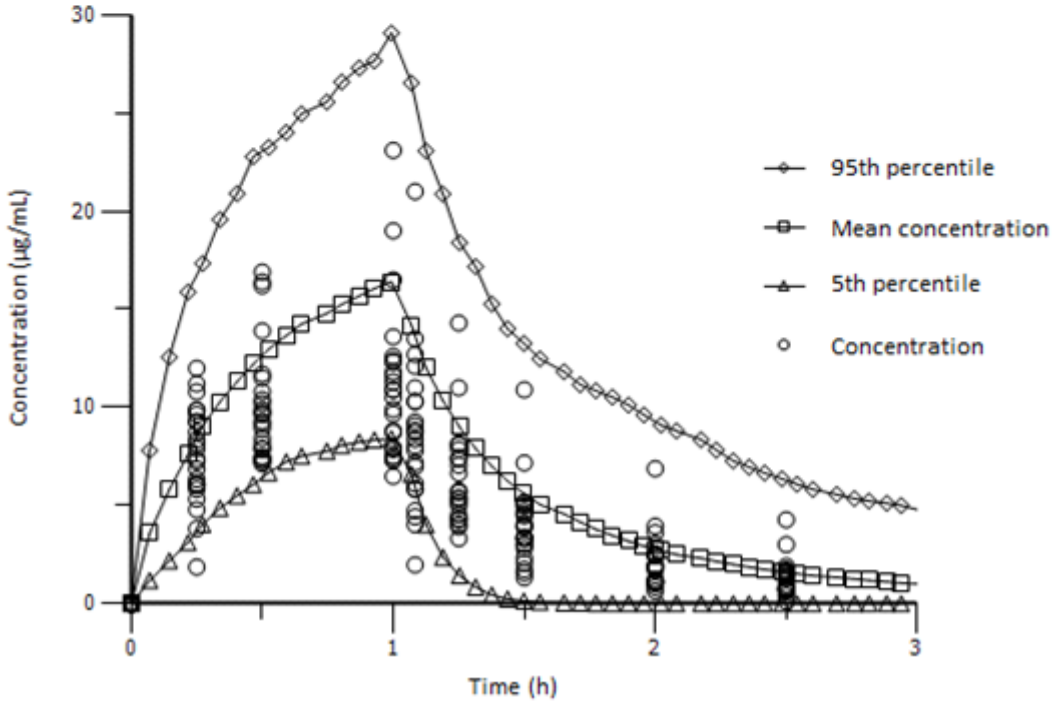


Simulated and observed individual plasma concentration-time profiles of evofosfamide given as intravenous infusion of **575 mg/m² over 30 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of the phase I monotherapy study (NCT00495144) are plotted as a function of time (circles), and refer to week 1 (top) and week 3 (bottom).



Simulated and observed individual plasma concentration-time profiles of evofosfamide given as intravenous infusion of **575 mg/m² over 60 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of phase I monotherapy study (NCT00495144) are plotted as a function of time (circles), and refer to week 1 (top) and week 3 (bottom).



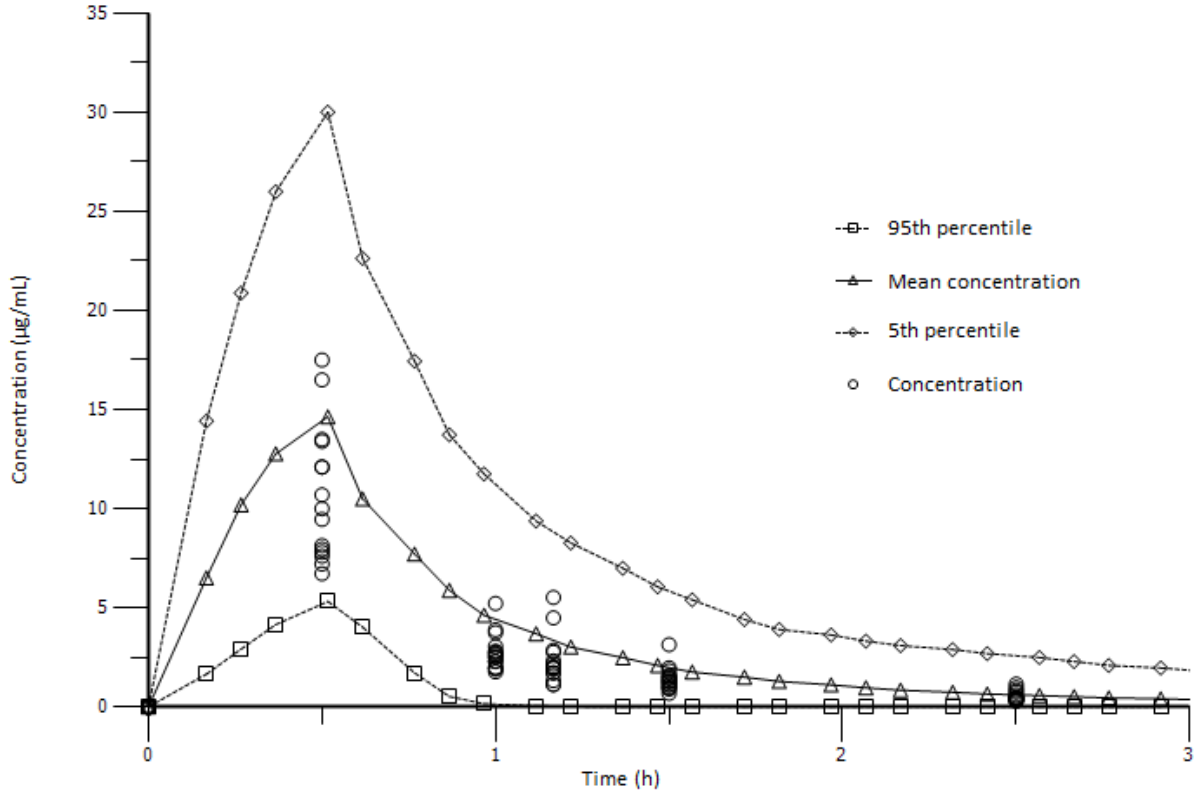


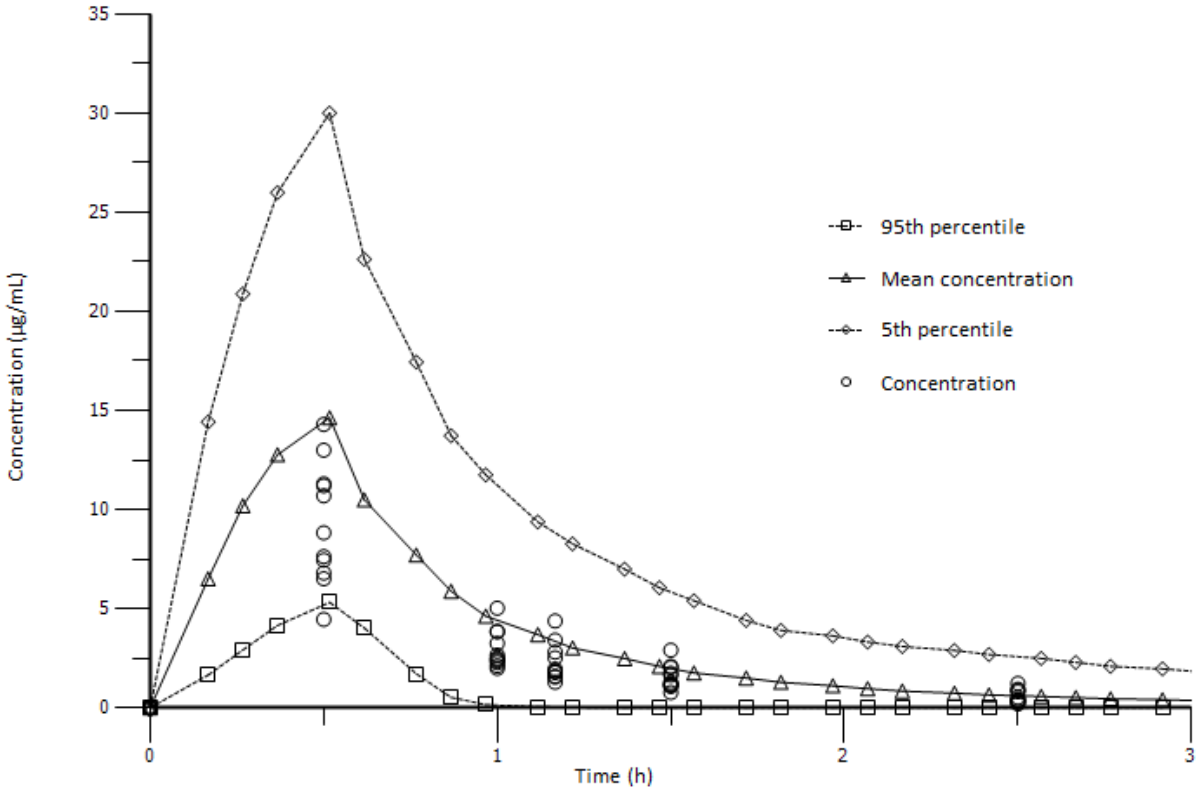
The results show that the evofosfamide PK model reasonably well predicts the PK observed clinically at dose levels of 240 mg/m², 480 mg/m², and 575 mg/m². This applies to both patients with 30 min as well as those with 60 min scheduled infusion duration, and for data obtained in week 1 as well as week 3. Due to the short half-life of evofosfamide, there is no accumulation after weekly administration, and the concentration time profiles of the two weeks are in the same concentration range, respectively.

The evofosfamide PK model was also validated against data from a second, independent clinical study. Due to the toxicity of evofosfamide, no study in healthy volunteers could be conducted. The predictions of the final evofosfamide PK model at the phase III dose of 340 mg/m² were compared with the concentrations of evofosfamide observed in a combination study (NCT00743379) after administration 340 mg/m² evofosfamide in combination with gemcitabine both in weeks 1 and 3. The results are shown in the figures below.

The mean predicted concentrations are high compared to the majority of observed individual concentrations in combination study (NCT00743379), in agreement with the conservative approach chosen during model construction. This confirms that the final evofosfamide PK model is suitable for a conservative estimate of the interaction potential.

Simulated and observed plasma concentration time profiles of evofosfamide given as intravenous infusion of **340 mg/m² over 30 min**. 100 subjects were simulated, the 5th and 95th percentile as well as the mean concentration-time profiles are shown (symbols joined by lines). The individual observed clinical data of the combination study (NCT00743379) are plotted as a function of time (circles). Overlay plots are shown for week 1 (top) and week 3 (bottom).





Precise quantification versus “lower-bound” approach

In general, for a precise quantification of an actual interaction, a qualification of an interaction model by comparison of the predicted interaction with results of a clinical interaction study adds confidence. However, this is not necessary and not possible for the questions discussed here. It is not necessary, because the worst-case approach taken gives only a lower bound. However, this is sufficient to exclude the interaction potential in the cases discussed in the main text body. A clinical interaction study in healthy volunteers was not possible due to the known genotoxicity and the side effect profile of evofosfamide.

References

Jamei, M., *et al.* The Simcyp Population Based Simulator: Architecture, Implementation, and Quality Assurance. *In Silico Pharmacology*, **1**:9 (2013)

Jamei, M. Qualification of the Simcyp platform for the intended purposes. Presentation at the EMA workshop on Qualification and Reporting of Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation, London, UK. <http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2016/12/WC500217574.pdf> (21st November 2016)