Population Pharmacokinetics of Escalating Doses of Caspofungin in a Phase II Study of Patients with Invasive Aspergillosis

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Caspofungin (CAS) is approved for second-line management of proven or probable invasive aspergillosis at a dose of 50 mg once daily (QD). Preclinical and limited clinical data support the concept of the dose-dependent antifungal efficacy of CAS with preservation of its favorable safety profile. Little is known, however, about the pharmacokinetics (PKs) of higher doses of CAS in patients. In a formal multicenter phase II dose-escalation study, CAS was administered as a 2-h infusion at doses ranging from 70 to 200 mg QD. CAS PK sampling ($n = 468$ samples) was performed on day 1 and at peak and trough time points on days 4, 7, 14, and 28 (70 mg, $n = 9$ patients; 100 mg, $n = 8$ patients; 150 mg, $n = 9$ patients; 200 mg, $n = 20$ patients; total, $n = 46$ patients). Drug concentrations in plasma were measured by liquid chromatography tandem mass spectroscopy. Population pharmacokinetic analysis (PopPK) was performed using NONMEM (version 7) software. Model evaluation was performed using bootstrap analysis, prediction-corrected visual predictive check (pcVPC), as well as standardized visual predictive check (SVPC). The four investigated dose levels showed no difference in log-transformed dose-normalized trough levels of CAS (analysis of variance). CAS concentration data fitted best to a two-compartment model with a proportional-error model, interindividual variability (IIV) fitted best on clearance (CL), central and peripheral volume of distribution (V_1 and V_2 , respectively) covariance fitted best on CL and V_1 , interoccasion variability (IOV) fitted best on CL, and body weight fitted best as a covariate on CL and V_1 (CL, $0.411 \text{ liters/h} \pm 29\% \text{ IIV}$; IOV on CL, 16% ; V_1 , $5.785 \text{ liters} \pm 29\% \text{ IIV}$; intercompartmental clearance, 0.843 liters/h ; V_2 , $6.53 \text{ liters} \pm 67\% \text{ IIV}$). None of the other examined covariates (dose level, gender, age, serum bilirubin concentration, creatinine clearance) improved the model further. Bootstrap results showed the robustness of the final PopPK model. pcVPC and SVPC showed the predictability of the model and further confirmed the linear PKs of CAS over the dosage range of 70 to 200 mg QD. On the basis of the final model, geometric mean simulated peak plasma levels at steady state ranged from 13.8 to 39.4 mg/liter (geometric coefficient of variation, 31%), geometric mean trough levels ranged from 4.2 to 12.0 mg/liter (49%), and geometric mean areas under the concentration-time curves ranged from 170 to 487 mg $\cdot$ h/liter (34%) for the dosage range of 70 to 200 mg QD. CAS showed linear PKs across the investigated dosage range of 70 to 200 mg QD. Drug exposure in the present study population was comparable to that in other populations. (This study has been registered with the European Union Drug Regulating Authorities Clinical Trials website under registration no. 2006-001936-30 and at ClinicalTrials.gov under registration no. NCT00404092.)

Invasive aspergillosis remains an important cause of infectious morbidity and mortality in immunocompromised patients. Next to currently approved first-line therapies with liposomal amphotericin B and voriconazole, caspofungin (CAS) is a generally well tolerated alternative with favorable pharmacokinetic (PK) properties at standard maintenance doses of 50 mg once a day (QD) (1, 2). Preclinical and limited clinical data support the concept of the dose-dependent antifungal efficacy of CAS (3–5). Data from healthy volunteers demonstrate that CAS can be safely given at doses of up to 210 mg QD for up to 14 days (10). In patients with invasive *Candida* infections, treatment with CAS at 150 mg QD was as safe as the standard dose of 50 mg QD, and no new toxicities were observed (7). Further clinical data indicate that CAS is generally safe and well tolerated at doses higher than the approved standard dose (8–10).

Dose escalation of CAS for treatment of invasive aspergillosis has not been formally studied, and the optimal dose for treatment of invasive aspergillosis in general and at specific sites, such as the central nervous system (CNS), has not been defined yet. In order to explore the feasibility of administering

higher doses of CAS in this setting, the safety, tolerability, and PKs of doses of CAS of up to 200 mg QD were investigated in a dose-escalation study in adult patients with proven or probable invasive aspergillosis (11). Here we report the detailed population pharmacokinetic (PopPK) analysis of CAS in patients enrolled in this clinical trial. (This study has been registered with the European Union Drug Regulating Authorities Clinical Trials website under registration no. 2006-001936-30 and at ClinicalTrials.gov under registration no. NCT00404092.)

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(The results of the population-based pharmacokinetic analysis were presented in preliminary form at the 21st Population Approach Group in Europe Meeting, Venice, Italy, 5 to 8 June 2012 [12].)

MATERIALS AND METHODS

Study design overview. The study was designed as a formal phase II dose-escalation study in patients with invasive aspergillosis to define the maximum tolerated dose of CAS in this setting and to determine the pharmacokinetic properties of CAS given over a dosage range of 70 to 200 mg QD. The complete results of this trial have been published previously in this journal (11).

Study endpoints. The safety and tolerability of CAS as primary endpoints as well as descriptive pharmacokinetics and efficacy as secondary endpoints of the study were published by Cornely et al. (11). Here we report the determination of PopPK parameters and the explorative analysis of trough levels at four escalating doses as further secondary endpoints.

Patient eligibility criteria. Adults ages 18 years or older were eligible if they had an immunocompromising condition associated with development of invasive fungal disease and evidence of proven or probable invasive aspergillosis defined by modified European Organisation for Research and Treatment of Cancer (EORTC) criteria as described previously (13).

Study-specific exclusion criteria were pregnancy or breast-feeding; elevated liver function tests as defined in detail by the study protocol (serum bilirubin levels >3 times the upper limit of the age-adjusted normal range, serum glutamic oxalacetic or serum glutamic pyruvic transaminase levels >5 times the upper limit of the age-adjusted normal range, alkaline phosphatase levels >5 times the upper limit of the age-adjusted normal range); clinical or laboratory evidence of active veno-occlusive disease; hemodynamic instability or an expected survival time of <5 days, previous enrollment in this study; concurrent receipt of efavirenz, nevirapine, rifampin, systemic dexamethasone, phenytoin, carbamazepine, phenobarbital, or cyclosporine; a documented history of intolerance to echinocandin antifungals; concomitant receipt of other systemic antifungal agents; chronic invasive fungal disease; prior systemic therapy of ≥ 4 days with any polyene antifungal agent within 14 days of study enrollment; and prior systemic therapy of ≥ 4 days with nonpolyenes for the current, documented invasive fungal disease. Before enrollment, written informed consent was obtained from each patient or her/his legal guardian. The study was approved by the institutional review board or ethics committee at each participating center (14).

Study drug treatment. CAS was administered once daily as an intravenous infusion over 120 min at 70 mg, 100 mg, 150 mg, or 200 mg. In the absence of dose-limiting toxicity, treatment with CAS was continued until at least a partial response was achieved or a switch to sequential oral antifungal therapy was considered feasible. The maximum duration of treatment with CAS study medication was limited to 28 days.

The study was based on a minimum cohort size of eight patients per dose group. Escalation to the next-higher dose level was carried out only after the external data safety monitoring board and the trial committee agreed that the safety and tolerability criteria were met (14). All patients who had received at least one dose of study drug were included in the PK analysis.

Pharmacokinetic sampling and recording of covariates. CAS PK sampling was performed on day 1 (immediately prior to dosing and 2 h [peak level], 3 h, 5 to 7 h, and 24 h [trough level] after the start of infusion) and at peak and trough time points on days 4, 7, 14, and 28. No PK sampling at a time after the last dose of greater than 24 h was planned. Blood specimens (5 ml) were collected in heparinized tubes and immediately centrifuged for 10 min at $1,500 \times g$. Separated plasma was stored at -80°C until assay. Concentrations of CAS were measured by a liquid chromatography tandem mass spectroscopy method as previously described in detail (15). Due to the expected high drug levels, the method

was modified to allow quantitation of CAS over the range from 84 $\mu\text{g/liter}$ (lower limit of quantification) to 84,000 $\mu\text{g/liter}$. Accuracies were within $\pm 11.9\%$, and intraday variability (precision) was $\pm 8.1\%$.

For analysis of covariates, the gender, age, and body weight of each patient were recorded on day 1; serum bilirubin and serum creatinine levels were recorded on days 1, 4, 7, 14, and 28. Creatinine clearance was calculated using the formula by Cockcroft and Gault (14). Missing covariates (20 serum bilirubin and 2 serum creatinine values) were replaced as follows: for day 1 and day 28, the values of day 4 and day 14, respectively, were used; for all other days, the mean of the value before and the value after was used.

Assessment of trough levels. To define an acceptable deviation from nominal time for trough levels, a sampling window from 0 to 1 h before the next drug administration was used. The dose dependency of trough levels of CAS were analyzed exploratively (analysis of variance [ANOVA]). On the basis of trough levels of day 1 and day 28—or, alternatively, day 14, if values for day 28 were missing—the accumulation ratio was evaluated by calculating the geometric mean ratio (last day/day 1) and the geometric coefficient of variation (GCV).

Population pharmacokinetic analysis. Nonlinear mixed-effect modeling by extended least-squares regression using the first-order conditional estimation (FOCE) method with interaction was implemented with the NONMEM (version 7.1.0, level 1.0; ICON Development Solutions, Ellicott City, MD). The objective function value (OFV), a measurement of goodness of fit estimated by NONMEM (equal to the -2 log likelihood value of the data), as well as graphical model diagnostics within the Xpose (version 4.3.2) (16) and Pirana (version 2.4.0) (6) programs, were used to search for appropriate models. For hierarchical models, an OFV drop of 6.62 units, designating an improved fit at the P equal to 0.01 level for 1 degree of freedom, was considered to be statistically significant.

In the first step, the structural PopPK model was developed. One-, two-, and three-compartment models as well as a two-compartment model with Michaelis-Menten elimination were tested. Inclusion of interindividual variability (IIV) was tested by exponential models on all PopPK parameters. The distribution of the interindividual random effects and the correlation between them were examined graphically to evaluate the normality and the independency assumption, respectively. The random effects with the highest correlation were tested by including the corresponding off-diagonal elements in the matrix of random effects. If implementing a correlation significantly improved the fit, the off-diagonal element of the random-effects matrix was kept in the model. Interoccasion variability (IOV) was tested by exponential models on clearance (CL) and central volume of distribution (V_1) on five occasions (days 1, 4, 7, 14, and 28). For assessing the residual unexplained variability, additive-, proportional-, and combined-error models were examined.

Thereafter, covariates such as dose level, gender, body weight (linear, allometric scaling [17] and in groups with body weights of ≤ 80 kg versus body weights of >80 kg), age (linear and in groups of ≤ 65 years of age versus >65 years of age), baseline serum bilirubin level, and baseline creatinine clearance were explored to explain some of the IIV in CAS PKs. On the basis of the structural PopPK model, empirical Bayesian estimates (EBEs) of interindividual random effects were computed, and the shrinkage was evaluated. The covariate screening was based on scientific plausibility and was guided by graphical inspection of the relationships between EBEs and the covariates. Stepwise inclusion of covariates was tested at a significance level of 0.05. On the basis of the full model, backward exclusion of covariates from the full model was tested at a significance level of 0.01. For continuous covariates, the following equation was tested by taking body weight as the covariate on CL as an example: $\text{CL}_i = \theta_1 \cdot [1 + \theta_2 \cdot (\text{BW} - \text{BW}_{\text{median}})]$, where CL_i is the individual value of CL, θ_1 is the typical value of CL in the population, θ_2 is the fractional change in the CL for each unit change in body weight from the median value of body weight, BW is body weight, and $\text{BW}_{\text{median}}$ is the median body weight of the total population. Categorical covariates were tested according to the following equation: $\text{CL}_i = \theta_1 \cdot (1 + \theta_2)$ for group = 2.

TABLE 1 Demographic data and covariates in the 46 patients included in the pharmacokinetic analyses

Parameter	Result for the following dose:				
	70 mg	100 mg	150 mg	200 mg	total
Gender (no. of males/no. of females)	2/7	5/3	4/5	10/10	21/25
Median (range) age (yr)	56 (33–71)	63 (18–71)	61 (38–73)	63 (24–74)	61 (18–74)
No. ≤65 yr of age/no. >65 yr of age					28/18
Median (range) body wt (kg)	75 (54–94)	78 (61–89)	79 (62–90)	76 (43–104)	76 (43–104)
No. with body wt of ≤80 kg, no with body weight of >80 kg					32/14
Median (range) bilirubin concn (mg/dl) on day 1	0.85 (0.4–2.0)	0.8 (0.4–2.3)	0.6 (0.3–1.3)	0.8 (0.3–1.4)	0.8 (0.3–2.3)
Median (range) Crea.CL ^a (ml/min) on day 1	89 (56–174)	117 (82–200)	93 (42–134)	89 (39–260)	102 (39–260)
Number of PK samples	96	80	94	198	468

^a Crea.CL, creatinine clearance, determined according to the formula of Cockcroft and Gault (14).

The fit of the PopPK models was assessed by visual inspection using goodness-of-fit plots (observations versus population or individual predictions) and separate plots of the conditional weighted residual versus population predictions or time after dose. The percentage of η and ϵ shrinkage (18) was calculated to obtain an indication of the reliability of the standard goodness-of-fit plots (those based on the quality of the EBEs).

A nonparametric bootstrap method was generated in the Pearl-speaks-NONMEM (PsN) tool kit (19) to assess the accuracy and robustness of the final population model and to provide information on parameter uncertainty. If the final parameter estimates fell into the 95% confidence interval obtained from the bootstrap analysis, the model was considered unbiased.

In order to verify that the model predicted both the central tendency and the variability in the observed data, a prediction-corrected visual predictive check (pcVPC) was performed using the PsN tool kit (19) and the Xpose aid (16). In addition, a standardized visual predictive check (SVPC) was also conducted (20). This test was performed by plotting the percentile of each observation in the data set in relation to its 1,000 simulated observations derived from the final model as a function of time after dose or dose level. If the model and PopPK estimates are adequate, these percentiles should be uniformly distributed between 0 and 1.

External model evaluation was carried out with an evaluation data set of 458 CAS samples from 36 patients under standard CAS doses (50 mg QD with a loading dose of 70 mg on day 1, 1-h infusion) collected in a previous study (CASLAMB trial) (21). Plasma CAS concentrations were predicted by applying the final model without reestimation of the parameters. The predicted values were compared with the corresponding observed values. Bias (mean prediction error [MPE]) and precision (mean absolute prediction error [MAPE]) were calculated according to equations 1 and 2:

$$\text{MPE} = \frac{\sum_{i=1}^N pe_i}{N} \quad (1)$$

$$\text{MAPE} = \frac{\sum_{i=1}^N |pe_i|}{N} \quad (2)$$

where N denotes the total number of observations, i is the number of the actual observation, and pe_i is the prediction error, expressed as a percentage. pe_i is calculated for each observation according to $100 \times [(C_{\text{pred}} - C_{\text{obs}})/C_{\text{obs}}]$ or $100 \times [(C_{\text{ipred}} - C_{\text{obs}})/C_{\text{obs}}]$, where C_{pred} , C_{obs} , and C_{ipred} are the predicted, observed, and individual predicted concentrations, respectively. The model parameters were also reestimated for the combined data set.

Statistical comparisons were performed in SAS (version 9.2, TS level 2M2; SAS, Heidelberg, Germany).

RESULTS

Patients. From September 2006 until July 2009, a total of 46 patients with proven or probable invasive aspergillosis were enrolled at three German university hospitals. All patients were included in the PK analysis. Nine patients were enrolled in the 70-mg-QD dose group, 8 patients were enrolled in the 150-mg-QD dose group, 9 patients were enrolled in the 150-mg-QD dose group, and 20 patients were enrolled in the 200-mg-QD dose group. The median duration of study drug treatment was 24.5 days. Demographic data (age, gender, body weight; Table 1), as well as baseline characteristics of all 46 patients, including fever and/or neutropenia, underlying disease (27 had acute leukemia, and 31 were neutropenic), and the EORTC/National Institute of Allergy and Infectious Diseases Mycoses Study Group classification of invasive aspergillosis, were comparable among the dose groups (11).

Assessment of trough levels. On the basis of visual inspection, some patients appeared to have already reached steady-state trough levels on day 4 (70-mg dose, $n = 8$ patients; 100-mg dose, $n = 3$; 150-mg dose, $n = 3$; 200-mg dose, $n = 7$). However, few patients might not have reached the steady-state phase until day 28 of drug administration (70-mg dose, $n = 1$; 100-mg dose, $n = 0$; 150-mg dose, $n = 1$; 200-mg dose, $n = 2$). These findings were independent with regard to the dose group. To test for the dose dependency of trough levels, we therefore used the last observed trough level as well as the time-averaged trough level from day 4 to day 28 for each patient for comparisons. For both data sets, there was no difference in dose-normalized, log-transformed trough levels over the four dose levels (ANOVA; for last trough level per patient, $P = 0.5627$ [Fig. 1]; for averaged trough level per patient, $P = 0.9030$).

Geometric means of the accumulation ratios (day 28/day 1 or day 14/day 1) of the trough levels were 2.12 ($n = 7$, GCV = 33%), 2.32 ($n = 3$, GCV = 21%), 3.0 ($n = 5$, GCV = 26%), and 2.25 ($n = 15$, GCV = 36%) for dose levels of 70, 100, 150, and 200 mg, respectively; some data for the 150-mg group were slightly offset. The combined overall accumulation ratio was 2.32 ($n = 30$, GCV = 36%).

Population pharmacokinetics of CAS. The number of patients and PK samples obtained at each dose level are listed in Table 1. Thirty-four percent ($n = 159$) of the 468 plasma samples were obtained on day 1, and 309 samples were obtained on days 4 to 28. One sample was excluded due to uncertainty regarding the exact sampling time, and five data points were considered errone-

FIG 1 Log-transformed dose-normalized (norm.; 1 mg) trough levels of caspofungin (last observed value per patient) versus caspofungin dose.

ous due to implausibly high or low trough or peak levels. Thus, the final data set consisted of 462 plasma samples from 46 patients.

As a structural PopPK model of CAS, a linear two-compartment model with a proportional-error model and IIV included exponentially on CL and V_1 was found to be superior to a linear one- or three-compartment model. Using a nonlinear model (two-compartment model with capacity-limited elimination using the Michaelis-Menten elimination from central compartment), the maximum rate of metabolism (V_{max}) and the concentration with a half-maximal elimination rate (K_m) were estimated to be 118 mg/h and 289 mg/liter, respectively. K_m is much higher relative to the CAS concentration. In this extreme region of the Michaelis-Menten curve, the metabolism rate is proportional to the drug concentration and can be described as a first-order process (22). However, in the nonlinear model, the covariance step was aborted; neither OFV nor goodness-of-fit plots were improved compared to those for the standard two-compartment model. Therefore, the linear two-compartment model was applied for further model testing.

Inclusion of IIV exponentially on the peripheral volume of distribution (V_2) further improved the model (reduction of OFV by -43.996). On the basis of graphical assessment (quantil-quantil plots), one patient with an extremely high value of V_2 of 43.8 liters was identified (highest value of the residual collective, 22.7 liters) and was therefore excluded from further analysis. The covariance between CL and V_1 was estimated, resulting in a reduction of the OFV by 36.155. The inclusion of covariance between other random effects did not improve the fit. Exponential modeling of IOV was included for CL ($P < 0.01$).

During initial covariate selection, the following were found to be statistically significant: gender as a covariate on V_1 , age as a covariate on CL and V_1 , and creatinine clearance (negatively correlated with age) as a covariate on CL. However, the relative standard error (RSE) of the parameter estimates was unacceptably high, as follows: for gender on V_1 , -0.151 (RSE, 40%); for age on CL, -0.0051 (RSE, 46%); for age on V_1 , 0.0046 (RSE, 37%); for creatinine clearance on CL, 0.0024 (RSE, 34%). IIVs were not reduced, and the trend of EBE-versus-covariate plots did not improve (η shrinkages of base model without covariates, 2.6% for IIV on CL and 2.9% for IIV on V_1). Therefore, these covariates were not included in further covariate testing.

Different models were used to test body weight as a potential covariate: by the linear relationship on all four PK parameters, by allometric scaling, and in groups with body weights of ≤ 80 kg

TABLE 2 Population-based pharmacokinetic parameter estimates for CL, Q, V_1 , and V_2 of CAS and nonparametric bootstrap analysis

Model parameter	Estimate (% RSE)		Bootstrap analysis	
	Base model	Final model	Mean (% RSE)	95% confidence interval
Fixed effects				
CL ^a (liter/h)	0.403 (5)	0.411 (5)	0.413 (5)	0.372–0.457
V_1 ^a (liters)	5.73 (5)	5.85 (4)	5.78 (5)	5.19–6.35
Q (liter/h)	0.844 (14)	0.843 (13)	0.917 (21)	0.683–1.27
V_2 (liters)	6.53 (15)	6.53 (15)	6.48 (15)	4.66–8.59
Factor body wt on CL, V_1		0.0102 (20)	0.0102 (25)	0.0049–0.0147
IIV and IOV				
IIV for CL (%)	31.4 (10)	28.5 (11)	27.8 (11)	21.1–33.9
IIV for V_1 (%)	32.2 (9)	28.8 (10)	28.3 (10)	22.3–33.7
IIV for V_2 (%)	66.7 (20)	66.8 (20)	67.5 (21)	34.2–94.7
IOV for CL (%)	16.1 (12)	16.0 (13)	15.9 (13)	11.2–19.5
Proportional residual error (%)	14.3 (10)	14.3 (10)	14.1 (11)	11.3–17.1
Correlation, OFV				
Correlation for CL- V_1	0.842	0.802	0.806	0.767–0.844
OFV	1,469.934	1,460.157		

^a Assuming a median body weight of 76 kg.

versus >80 kg. Body weight group as a potential covariate on CL or V_1 did not improve the model, whereas the linear and allometric model improved the PK model ($P < 0.01$). Evaluation of the accuracy of the scaling factor 0.75 on CL in the allometric model was done by estimating the allometric factor on body weight. Since the parameter was estimated to be 0.952, a linear relationship between body weight and PK parameters was superior to allometric scaling for the present patient population. The linear modeling of different fractional changes for CL and V_1 due to the covariate body weight compared to the model using the same fractional change for CL and V_1 showed no difference (difference in OFV, -0.109). This may be explained in part by the high correlation between CL and V_1 . Therefore, body weight with the same fractional change for CL and V_1 was the only covariate retained in the final PopPK model. Inclusion of body weight on CL and V_1 improved EBE-versus-covariate plots (see Fig. S1 in the supplemental material). However, adding body as a covariate on CL and V_1 explained only 2.9% of the IIV on CL and 3.4% of the IIV on V_1 (Table 2). Of note, the dose level as a potential covariate on CL or V_1 did not influence the PKs of CAS (decrease in OFV with dose level as a covariate on CL, -1.898 ; decrease in OFV with dose level as a covariate on V_1 , -2.415).

The PopPK parameter estimates of the final model for a patient with a median body weight of 76 kg together with results from bootstrapping are listed in Table 2. All model parameters were estimated with high precision ($RSE \leq 20\%$). IIVs on CL and V_1 as well as IOVs on CL were below 30%, whereas a high IIV on V_2 of 67% was found. The correlation coefficient between CL and V_1 was 0.802. The estimates of shrinkage for IIVs on CL and V_1 were low (4.9% and 4%, respectively). The estimates of shrinkage for IIV on V_2 and IOV on CL were moderate (18.2% and 44.3%, respectively).

FIG 2 Final population pharmacokinetic model of caspofungin. (Left) Observations versus population (a) or individual (b) predictions. The line of identity is shown. (Right) Conditional weighted residuals versus population predictions (c) or time after dose (d). The line where conditional weighted residuals are equal to 0 (black line) as well as the spline curve (gray dashed line) is shown.

Goodness-of-fit plots in Fig. 2 show that the model predictions were in reasonable agreement with the observed plasma concentrations. No trend in the conditional weighted residuals versus population predictions or versus time-after-dose plots was observed, indicating no model misspecification (ϵ shrinkage, 22%) (Fig. 2c and d). The robustness of the final model was shown by the bootstrap results (Table 2). The population estimates for the final model were very similar to the mean of the bootstrap replicates and within the 95% confidence interval obtained from the bootstrap analysis.

The results of the pcVPC are presented in Fig. 3. Figure 3 provides evidence that the model developed is appropriate to describe the time course of plasma CAS concentrations in the present patient collective.

FIG 3 Prediction-corrected visual predictive check. The median (solid black line) and 5th and 95th percentiles (dashed black lines) for the observed data with 95% confidence intervals for the median (dark gray field) and 5% and 95% percentiles (light gray fields) based on simulations are shown. Individual points represent observed data.

The results of the SVPC show that the percentiles (P_{ij}) of the j th observation for the i th patient versus time after dose are in general uniformly distributed between 0 and 1 and confirm the adequacy of the model to describe the data (see Fig. S2 in the supplemental material). The SPVC was used to filter out the effect of dose on PK predictions: the even distribution of P_{ij} at the different dose levels (see Fig. S2 in the supplemental material) further confirmed the linear PKs of CAS within the examined dose range.

As indicated above, one patient was excluded during model building due to an extremely high value of V_2 . Reevaluation of the final model with all patients showed comparable parameter estimates; only IIV on V_2 increased from 67% to 77%, thus confirming the exclusion of this patient during model building.

When applying the final model without reestimation to the evaluation data set, the population predicted concentrations were slightly biased, with an MPE of 25% and a precision of 36%. For individual predictions, the bias was 20% with a precision of 42%. Reestimation of the model parameters for the combined data set (original plus external) showed a slightly higher CL value (8% difference); the largest differences were seen for V_2 and intercompartmental clearance (Q) (29% decrease and increase, respectively).

On the basis of the final model, simulated peak plasma levels ranged from 13.8 to 39.4 mg/liter, trough levels ranged from 4.2 to 12.0 mg/liter, and the area under the concentration-time curve (AUC) ranged from 170 to 487 mg $\cdot$ h/liter for the dosage range of 70 to 200 mg QD and a median body weight of 76 kg (geometric mean) (Table 3).

DISCUSSION

In order to provide a basis for further clinical investigations of antifungal therapy, the maximum tolerated dose of CAS was studied in a clinical phase II dose-escalation trial in patients with in-

TABLE 3 Estimated steady-state pharmacokinetic parameters of AUC and peak and trough plasma levels

Dose (mg QD)	Geometric mean (GCV [%]) ^a		
	AUC (mg · h/liter)	C _{max} (mg/liter)	C _{min} (mg/liter)
70	170 (34)	13.8 (31)	4.2 (49)
100	243 (34)	19.7 (31)	6.0 (49)
150	365 (34)	29.6 (31)	9.0 (49)
200	487 (34)	39.4 (31)	12.0 (49)

^a C_{max} and C_{min}, peak (maximum) and trough (minimum) plasma levels, respectively.

vative aspergillosis (11). Apart from the assessment of safety and tolerance and observation of treatment success (11), the study protocol also allowed a more detailed PK modeling of CAS to further explore dose linearity and the impact of covariates in this population.

Concentration-time data for CAS were best described by a two-compartment PK model. The typical value of CL in a 76-kg patient was 0.411 liters/h, with moderate between-subject (28.5%) and relatively smaller within-subject (16%) variability. While IIVs on V_1 (28.8%) were low, a high IIV on V_2 was found (67.5%).

Body weight (median, 76 kg; range, 43 to 104 kg; 14 of 46 patients had body weights over 80 kg) was the only covariate included in the final PopPK model. A fractional change in CL of 0.0102 per kg of body weight predicts a 20% decrease in AUC for a patient weighing 80 kg compared to that for a patient weighing 60 kg. These data are in good agreement with results found in subjects receiving standard doses of CAS (AUC decrease, 23% [23]). It is noteworthy that for standard doses of CAS, these findings are interpreted differently: in contrast to the FDA label information (24), a European public assessment report (23) recommends dose adjustment for patients with body weights of >80 kg. Assuming dose-dependent antifungal efficacy and considering the excellent tolerability of CAS at doses of up to 200 mg QD, the effect of body weight on drug exposure is too small to mandate dose adjustments.

In accordance with published data (21, 23–28), the present PopPK analysis demonstrates that no dose adjustments of CAS are necessary on the basis of gender, age, or renal impairment as assessed by the creatinine clearance. As hepatic dysfunction impacts CL for adult patients with moderate hepatic impairment (Child-Pugh score, 7 to 9), adjustment of the CAS dose on the basis of PK data is recommended (23, 27–30). In the present study, the Child-Pugh score itself was not documented. However, serum bilirubin—one important parameter for calculation of this score—was included in the covariate selection. As patients with pathological laboratory findings were excluded, serum bilirubin is not found to be a significant covariate for CAS CL.

As described by Nguyen et al. (26), body weight and albumin concentration influenced CAS trough levels in patients in a surgical intensive care unit and should therefore be considered prognostic factors. As albumin was not documented in the present study, the influence of this potentially significant covariate could not be evaluated.

Potential limitations of this study may be based on the fact that a large fraction of the PK samples (76.5%) were taken as peak (175 samples) or trough (183 samples) levels; only 110 samples were taken at other time points up to 7.5 h after the start of the infusion

(71 on day 1, 39 after later administrations). Only two PK samples were taken during infusion; no samples were taken between 7.5 and 22.0 h or later than 25 h after the last dose.

There are pronounced preanalytical problems especially linked to peak levels: differences in infusion and rinsing techniques at the end of infusion, the precise definition of the end of infusion, and the different techniques used to take blood samples are factors that substantially influence the measured peak levels. All these factors might contribute to the inter- and intraindividual variabilities found in the present study.

This sampling pattern may explain the rather poor prediction of V_2 (IIV, 66.8%). On the basis of the present two-compartment model, half-lives were estimated to be 2.2 h (distribution phase) and 24 h (elimination phase). In the literature, where CAS plasma concentrations remained above the limit of quantification for more than 3 days, an additional γ phase was observed (24, 31). As there were no PK samples collected later than 25 h after the last dose in the present study, the data did not allow characterization of this additional γ phase.

On the basis of data from a previous study (CASLAMB trial), a PopPK model under standard CAS doses was published (21). In the CASLAMB trial as well as in the present study, a standard two-compartment model was found to describe best the PopPKs of CAS. In both studies, there were no data to evaluate the terminal γ phase of CAS for time after dose of greater than 48 h. However, CL was slightly different (CASLAMB trial, 0.462 liter/h; present study, 0.411 liter/h), and greater differences were found for V_1 , V_2 , and Q (CASLAMB trial, 8.33 liters, 3.59 liters, and 1.25 liters/h, respectively; present study, 5.85 liters, 6.53 liters, and 0.843 liters/h, respectively). In contrast to the model for the CASLAMB trial, in the present study, body weight as a covariate on CL and V_1 further improved the PopPK model. External validation of the present model with data from the CASLAMB trial confirmed these differences, indicating a small bias toward overestimated CAS concentrations in the validation data set. We suggest that these findings are the result of a combination of different factors, such as differences in the patient population, underlying diseases, supportive treatment, surgical and medical interventions, and the study design (the CASLAMB trial included neutropenic patients whose status was determined after bone marrow transplantation, up to 5 to 7 liters of intravenous hydration per day and polychemotherapy were used, the majority of PK samples were collected on day 1 and day 4, and PK samples were collected over the whole time range from 0 to 24 h after the last dose; the present study included patients with acute lymphoblastic leukemia and HIV infection, individuals of older age, and patients with differences in creatinine clearance and serum bilirubin levels; performed PK sampling on day 1; determined peak and trough levels after later dose administrations; and did not collect samples in the time window from 7 to 22 h after dosing).

After single-dose administration of 5 to 210 mg CAS, Stone et al. (31) and Migoya et al. (10) reported an approximately dose-proportional increase of AUC and peak and trough levels. These findings are consistent with linear PKs. However, after administration of multiple doses of 15 to 100 mg CAS, the authors observed a slightly greater than dose-proportional increase in AUC and peak and trough levels. Time to steady state and accumulation ratios of AUC and peak and trough levels were dose dependent. These findings indicated a modest PK nonlinearity after multiple doses. The mechanism of PK nonlinearity is unclear; the γ phase,

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