Population pharmacokinetics and cerebrospinal fluid penetration of fluconazole in adults with cryptococcal meningitis.

Katharine E Stott[a,b §], Justin Beardsley[c §], Ruwanthi Kolamunnage-Dona[d], Anahi Santoyo Castelazo[a], Freddie Mukasa Kibengo[e], Nguyen Thi Hoang Mai[f], Jeremy Day[c,g], William Hope[a*]

[a] Centre for Antimicrobial Pharmacodynamics, Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, UK

[b] Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

[c] Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

[d] Department of Biostatistics, Institute of Translational Medicine, University of Liverpool, UK

[e] MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda

[f] Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam

[g] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, UK

[§] These authors contributed equally to this work.

* Corresponding author: hopew@liverpool.ac.uk

Keywords: Cryptococcal meningitis, pharmacokinetics, pharmacodynamics, fluconazole, central nervous system pharmacokinetics

ABSTRACT

Robust population pharmacokinetic (PK) data for fluconazole are scarce. The variability of fluconazole penetration into the CNS is not known. A fluconazole PK study was conducted in 43 patients receiving oral fluconazole (usually 800 mg q24h) in combination with amphotericin B deoxycholate (1 mg/kg q24h) for cryptococcal meningitis (CM). A 4-compartment PK model was developed and Monte Carlo simulations performed for a range of fluconazole dosages. A meta-analysis of trials reporting outcomes of CM patients treated with fluconazole monotherapy was performed. Adjusted for bioavailability, the PK parameter means (standard deviation) were: clearance, 0.72 (0.24) litres/hour; volume of the central compartment, 18.07 (6.31) litres; volume of central nervous system (CNS) compartment, 32.07 (17.60) litres; first-order rate constant from central to peripheral compartment, 12.20 (11.17) $hours^{-1}$; from peripheral to central compartment, 18.10 (8.25) $hours^{-1}$; from central to CNS compartment 35.43 (13.74) $hours^{-1}$; from CNS to central compartment 28.63 (10.03) $hours^{-1}$. Simulations of area under concentration-time curve resulted in median (interquartile range) values 1143.2 mg.h/litre (988.4 – 1378.0) in plasma and 982.9 (781.0 – 1185.9) in CSF after a dosage of 1200mg q24h. The mean simulated ratio of AUC_{CSF}:AUC_{plasma} was 0.89 (SD 0.44). The recommended dosage of fluconazole for CM induction therapy fails to attain the PD target in respect to the wild-type MIC distribution of *C. neoformans*. The meta-analysis suggested modest improvements in both CSF sterility and mortality outcomes with escalating dosage. This study provides the pharmacodynamic rationale for the long-recognised fact that fluconazole monotherapy is an inadequate induction regimen for CM.

Antimicrobial Agents and Chemotherapy

INTRODUCTION

Mortality from cryptococcal meningitis remains unacceptably high. More than 90% of the estimated 223,100 annual incident cases of cryptococcal meningitis occur in Sub-Saharan Africa and Asia-Pacific regions (1). The most effective regimen for induction is amphotericin B deoxycholate and flucytosine (2, 3). However, access to these drugs is limited in many regions where the burden of cryptococcal meningitis is greatest (4, 5). In these settings, high-dose fluconazole is used for induction monotherapy, despite consistent evidence of reduced survival in comparison with other agents and combinations (6-8).

Fluconazole was discovered by Pfizer Inc. (Sandwich, UK) in 1978 (9). The objective was to discover an orally bioavailable agent for the treatment of invasive mycoses with a lower propensity to develop resistance than flucytosine (9). Fluconazole inhibits cytochrome P450-dependent demethylation of lanosterol in the ergosterol biosynthetic pathway (10). The ratio of the area under the concentration-time curve (AUC) to the minimum inhibitory concentration (MIC) is the pharmacodynamic (PD) index that best links drug exposure of fluconazole with the observed antifungal effect (11, 12).

Successful antimicrobial therapy within the central nervous system depends on the achievement of effective drug concentrations within relevant subcompartments that include the cerebrum, meninges and CSF (13). Fluconazole has a low molecular weight (approximately 300g/mol), is weakly protein bound and is not known to be a substrate for central nervous system (CNS) efflux pumps (14, 15). Its ability to partition from the endovascular compartment into the CNS has been established in laboratory animal models (16, 17) and clinical studies (18, 19). Brain:plasma penetration ratios up to 1.33 have been reported in humans (19). However, there is a surprising paucity of population

pharmacokinetic (PK) data for fluconazole in all clinical contexts. Furthermore, the extent and variability of penetration into the CNS is not known.

The primary aim of this study was to quantify the extent and variability of CNS penetration of fluconazole in adults with cryptococcal meningitis. We developed a population PK model that quantified the inter-individual variability in drug exposure in plasma and cerebrospinal fluid (CSF). We investigated the impact of a range of clinically relevant covariates on fluconazole PK. Monte Carlo simulation was used to assess the implications of PK variability in terms of achieving fluconazole PD targets. Finally, we conducted a meta-analysis of clinical trials of fluconazole monotherapy to estimate the contribution of dosage to clinical outcome.

RESULTS

Patients

A total of 43 patients (23 from Vietnam and 20 from Uganda) were recruited over an 11-month period between January and November 2016. Twenty-two patients (52%) were female. The overall median (range) age was 33 years (20 – 73 years), weight 48 kg (32 – 68 kg), body mass index 18 kg/m^2 (12 – 25 kg/m^2), creatinine at enrolment 70 µmol/L (37 – 167 µmol/L) and estimated glomerular filtration rate using the Cockcroft Gault equation 84.8 mL/min/1.73m^2 (35.4 – 146.7 mL/min/1.73m^2). The baseline creatinine concentration was significantly lower in Vietnamese patients than in Ugandan patients (median 56 versus 79 µmol/L; p-value 0.02). However, this did not manifest as a significant difference in eGFR due to different age, sex and weight profiles between the two patient populations. There were no statistically significant differences between ethnic groups for other demographic variables. The demographic data are shown by ethnicity and for the study population as a whole in Table 1.

Pharmacokinetic data

The final dataset included 312 plasma observations and 52 CSF observations from the Vietnamese cohort. From the Ugandan cohort, the dataset included 196 plasma observations and 115 CSF observations. A single CSF observation from 1 Ugandan patient was excluded because no fluconazole was detectable in an isolated sample after 13 days of therapy. This was inconsistent with results from other patients and could not be verified. The mean number of plasma samples and CSF samples per patient was 11.8 and 3.9, respectively. Figure 1 shows the raw plasma and CSF concentration-time profiles from study participants.

104

105 **Population pharmacokinetic analysis**

106 The final mathematical model was a linear model comprised of an absorption
107 compartment, central compartment, peripheral compartment and CSF compartment. The
108 fit of the final model to the clinical data was acceptable. The mean parameter estimates
109 better fitted the data than medians, and were used to calculate Bayesian estimates of drug
110 exposure for each individual patient. A linear regression of the observed-versus-predicted
111 fluconazole concentrations in plasma after the Bayesian step was given by: observed
112 fluconazole concentration = 1.03*predicted fluconazole concentration + 0.27; r^2 = 0.80. For
113 the observed-versus-predicted fluconazole concentrations in CSF, the linear regression was
114 given by observed fluconazole concentration = 1.03*predicted fluconazole concentration -
115 0.07; r^2 = 0.81 (Figure 2 and table 3). The mean weighted population bias for fluconazole
116 concentrations in plasma and CSF was 0.20 and -0.30, respectively. The bias-adjusted
117 population imprecision in plasma and CSF was 2.21 and 1.55, respectively. The population
118 PK parameter estimates for the final model are shown in Table 2.

119

120 **Covariate investigation**

121 Multivariate linear regression of each subject's covariates versus the Bayesian
122 posterior parameter values revealed a weak relationship between patient weight and
123 estimated volume of distribution (slope 0.22, 95% confidence interval for the slope -0.06 to
124 0.51, p-value 0.05). Incorporation of weight into the PK model was therefore explored.
125 However, values for log likelihood, Akaike information criterion (AIC) and population bias
126 and imprecision were comparable between the 2 models. The simple base model was

therefore used to describe the data and for the subsequent simulations. The model comparisons and the fit to data are summarized in Table 3.

There was no relationship between the Bayesian estimates of clearance and volume, and ethnicity or sex in the base model. The mean (95% CI) clearance was 0.74 liters/hour (0.64 – 0.83) and 0.71 liters / hour (0.59 – 0.82) for Vietnamese and Ugandan patients, respectively; p= 0.51. The mean (95% CI) volume was 16.88 liters (14.33 – 19.44) and 19.44 liters (16.88 – 22.0) for Vietnamese and Ugandan patients, respectively; p= 0.16. In males, the mean (95% CI) clearance was 0.79 liters /hour (0.67 – 0.90). In females, clearance was 0.66 liters / hour (0.57 – 0.75); p= 0.09. In males, the mean (95% CI) volume was 18.07 liters (15.47 – 20.67). In females, volume was 18.07 liters / hour (15.41 – 20.73); p= 0.97.

Fluconazole penetration into the CSF

There was large variability in the AUCs generated from each patient's posterior estimates. The 38 patients who received 800mg fluconazole q24h had a median (IQR) $AUC_{144\text{-}168}$ of 945.4 (799.2 – 1139.8) mg.h/L in plasma and 784.2 mg.h/L (615.9 – 879.4) in CSF. From these posterior estimates, the mean ratio of AUC_{CSF}:AUC_{plasma} was 0.82 (standard deviation 0.22).

Monte Carlo simulation was used to estimate the distribution of drug exposure for dosages of 400mg, 800mg, 1200mg and 2000mg q24h of fluconazole (Figure 3). PK variability was marked, both in plasma and CSF. After administration of a dosage of 1200mg fluconazole q24h, median (IQR) simulated plasma $AUC_{144\text{-}168}$ was 1143.2 mg.h/L (988.4 – 1378.0) and CSF $AUC_{144\text{-}168}$ was 982.9 mg.h/L (781.0 – 1185.9). The mean simulated ratio of AUC_{CSF}:AUC_{plasma} was 0.89 (SD 0.44).

Probability of target attainment analysis

Monte Carlo simulation was used to predict the probability of achieving a total drug AUC:MIC ratio of ≥ 389.3 in plasma. This PD target was shown in a murine model of cryptococcal meningitis to be associated with a stasis endpoint (i.e. no net change in fungal density at the end of the experiment compared with that at treatment initiation) (11). Only 61% of simulated patients receiving 1200mg fluconazole q24h achieved this PD target when the MIC of the infecting strain was 2.0 mg/L. For MICs ≥ 4.0mg/L, < 1% of simulated patients administered 1200mg q24h achieved the PD target (Figure 4).

Meta-analysis of clinical outcome data

A systematic review identified 163 relevant manuscripts, of which 11 were duplicates. After reviewing titles and abstracts, 28 studies were deemed potentially relevant for inclusion in the meta-analysis. Detailed examination of these studies resulted in the ultimate inclusion of 12 papers describing clinical outcomes from cryptococcal meningitis treated with fluconazole monotherapy. In total, 28 patients in 1 study received 200mg fluconazole q24h (20), 19 patients in 2 studies received 400mg fluconazole q24h (7, 21), 97 patients in 3 studies 800mg q24h (22-24), 113 patients in 4 studies 1200mg q24h (8, 23-25), and 1 study described outcomes of 16 patients on 1600mg (24) and 8 patients on 2g fluconazole q24h (24). All included patients were HIV positive. Baseline characteristics and reported clinical outcomes are presented in Table 4.

The final model suggests that the combination of dose and baseline fungal burden explains the total heterogeneity in the estimated proportion of patients with sterile CSF after 10 weeks of treatment (P-value for residual heterogeneity 0.64). However, there was not a significant relationship between dose and CSF sterility at 8-10 weeks (p-value 0.45).

After adjustment for dose, the test for residual heterogeneity in both 2 and 10-week mortality was not significant (p-value 0.70 and 0.22, respectively), indicating that dose alone adequately explained total heterogeneity in mortality outcomes at both time points. For both 2 and 10-week mortality outcomes, there was a non-significant trend towards reduced mortality with escalating dosage (Figure 5).

181 DISCUSSION

182 Fluconazole is the only drug available for induction therapy for cryptococcal
183 meningitis in many regions of the world where the incidence of disease is highest. An
184 accumulating body of evidence suggests that fluconazole is a suboptimal agent for this
185 indication (26). Whilst this has long been recognised, an explanation for the relatively poor
186 efficacy of fluconazole is absent. This study presents a uniquely comprehensive clinical
187 dataset describing the PK of fluconazole. It provides robust estimates of CNS penetration
188 and the variability of those estimates. A high degree of CNS partitioning has been observed
189 in previous clinical studies with fluconazole (19, 27). Distribution into the CNS is facilitated
190 by low molecular weight, low protein binding and moderate lipophilicity (15, 28).
191 Fluconazole has proven activity against *Cryptococcus neoformans*. (29, 30). This study
192 provides a further understanding as to why, despite these attributes, fluconazole is an
193 inferior agent for induction monotherapy for cryptococcal meningitis compared with
194 amphotericin B deoxycholate (6-8).

195 In contrast to previous studies of fluconazole PK (31-33), our data do not suggest a
196 significant relationship between fluconazole clearance and creatinine clearance, nor
197 between patient weight and volume of distribution. The reason for this is not immediately
198 clear but may relate to the relatively narrow range of creatinine clearance in our population,
199 and the fact that the vast majority of patients in our cohort had low body weight, with the
200 range of this covariate also being relatively narrow.

201 The PK model suggests that current regimens of fluconazole are inadequate for
202 induction therapy for cryptococcal meningitis. This has routinely been ascribed to the
203 overly simplistic notion that fluconazole is a fungistatic agent. Our analyses provide further
204 insight into the limitations of this drug. Previous estimates of fluconazole CNS:plasma

partition ratios have ranged from 0.52 to 1.33 (18, 19, 27, 34). We have extended these estimates by rigorously quantifying the marked variability in the CSF PK. This variability has consequences at both microbiological and clinical levels. Suboptimal exposure of fluconazole promotes the expansion of intrinsically resistant cryptococcal subpopulations present at the initiation of therapy (35). In addition, the evolution of *C. neoformans* during therapy to become increasingly triazole resistant has been demonstrated in clinical studies (36, 37). To be clinically effective, adequate concentrations of drug must be present at the site of infection for long enough to exert antimicrobial effect on both susceptible and resistant subpopulations. The present analysis demonstrates the challenges in achieving that aim.

At recommended fluconazole dosages of 1200mg q24h, the probability of PD target attainment (PTA) bisects the MIC distribution of WT *C. neoformans* isolates. This is consistent with the findings of Sudan *et al* (11). Approximately half of patients will fail therapy because they are not able to generate the drug exposure required to prevent progressive fungal growth. Since clinical PK-PD targets are not available for fluconazole in cryptococcal meningitis, we have used a target derived from a murine study (11). This assumes that CNS partitioning is the same in mice and humans. The cerebrum:plasma AUC ratio in the murine study was 46.9% (11). It is conceivable that this is in keeping with our CSF:plasma AUC ratio of 82%, though clearly it would be preferable to have clinical PK-PD targets defined. Nevertheless, our PTA analysis is supported by the 53% 10-week mortality outcomes for patients receiving 1200mg fluconazole q24h, estimated in the meta-analysis. Importantly, such PTA analyses are based on an AUC/MIC of 389.3, which is more than an order of magnitude greater than the AUC/MIC ratio required for *Candida albicans* (12).

Progressive escalation of the dosage of fluconazole is not likely to be an effective strategy for improving cryptococcal meningitis induction therapy. The drug exposure required to reliably treat isolates with MICs ≥ 4.0mg/L is difficult to achieve and potentially toxic. Our meta-analysis suggests that escalating dosages of fluconazole do not increase the proportion of patients with sterile CSF at 10 weeks. Dosages of 2000mg q24h do not appear to significantly improve 10-week mortality outcomes in comparison to 1200mg q24h. The ACTG study (https://clinicaltrials.gov/show/NCT00885703) is investigating the use of higher dosages of fluconazole (1600mg and 2000mg q24h) for the treatment of cryptococcal meningitis in HIV-infected individuals and results are pending. The addition of flucytosine to high-dose fluconazole (≥ 1200mg q24h) for cryptococcal meningitis increases antifungal activity and improves mortality outcomes (8, 24), suggesting that combination therapy is required to optimise antifungal activity in fluconazole-containing regimens.

In summary, this study provides part of the pharmacodynamic rationale for the long-recognised fact that fluconazole monotherapy is an ineffective induction regimen for cryptococcal meningitis. We have developed a fluconazole population PK model that suggests that approximately half of patients with cryptococcal meningitis caused by WT strains of *C. neoformans* will be undertreated by currently recommended dosages of fluconazole for induction therapy. In doing so we have addressed a knowledge gap regarding the reason for the inferiority of this drug for cryptococcal meningitis. There is a pressing need for improved provision of affordable combination treatments and development of more effective drugs.

MATERIALS AND METHODS

Clinical pharmacokinetic studies

Patients from whom plasma and CSF samples were obtained for this PK study have been described previously (38). Briefly, adult patients were initially recruited from a multi-centre randomised controlled trial of adjuvant dexamethasone in HIV-associated cryptococcal meningitis. The trial is reported elsewhere (n=3, International Standard Registered Clinical Number 59144167) (39). Following the early cessation of this trial, patients were recruited from a prospective descriptive study at the same sites (n=40). Study sites were The Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam, and Masaka General Hospital, Uganda. The study protocols were approved by the relevant institutional review boards and regulatory authorities at each trial site and by the Oxford University Tropical Research Ethics Committee.

Fluconazole was administered orally. Where conscious level did not enable oral administration, fluconazole was administered via nasogastric tube. The majority of patients received 800mg fluconazole q24h. Two patients received one-off doses of 400mg q24h. Two received one-off doses of 600mg q24h. One patient's regimen of 800mg fluconazole q24h was escalated to 1200mg q24h for 6 days from day 8 of treatment. All patients received combination therapy with amphotericin B deoxycholate 1mg per kg infused over 5-6 hours.

Measurement of fluconazole concentrations

Fluconazole concentrations were measured using a validated LC/MS/MS methodology (1260 Agilent UPLC coupled to an Agilent 6420 Triple Quad mass spectrometer, Agilent Technologies UK Ltd, Cheshire, UK). Briefly, fluconazole was

extracted by protein precipitation; 300 µl of cold methanol containing the internal standard fluconazole-D4 at 0.625 mg/L (TRC, Canada) was added to 10 µl of sample (plasma or CSF). The solution was vortex mixed for 5 seconds and filtered through a Sirocco precipitation plate (Waters Ltd, Cheshire, UK). One hundred fifty µl of supernatant was transferred to a 96-well auto sampler plate, and 3 µL were injected on an Agilent ZORBAX C18 RRHD (2.1 X 50mm, 1.8 µm) (Agilent Technologies UK Ltd, Cheshire, UK).

Chromatographic separation was achieved using a gradient consisting of 70% A:30% B (0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B). The organic phase was increased to 100% over 90 seconds, with additional 90 seconds of equilibration.

The mass spectrometer was operated in multiple reaction monitoring scan mode in positive polarity. The precursor ions were 307.11 *m/z* and 311.1 *m/z* for fluconazole and internal standard, respectively. The product ions for fluconazole were 220.1 *m/z* and 238.1 *m/z*; for the internal standard 223.2 m/z and 242.1 *m/z*. The source parameters were set as follows: capillary voltage 4000 V, gas temperature 300°C and nebulizer gas 15 lb/in^2.
The standard curve for fluconazole encompassed the concentration range 1-120 mg/L and was constructed using blank matrix. The limit of quantitation was 1 mg/L. In plasma, the intra-day coefficient of variation (CV) was <3.4% and the inter-day CV was <6.7%, over the concentration range 1-90 mg/L. In CSF, the intra-day CV was <5.2% and the inter-day CV was <5.3% over the same concentration range.

Population pharmacokinetic modelling

The concentration-time data for fluconazole in plasma and CSF were analysed using the non-parametric adaptive grid (NPAG) algorithm of the program Pmetrics (40) version

1.5.0 for R statistical package 3.1.1. The initial PK mathematical model fitted to the data contained four compartments and took the following form:

1. $\frac{dX(1)}{dt} = -Ka * X(1)$

2. $\frac{dX(2)}{dt} = Ka * X(1) - \left(Kcp + Kcs + \frac{SCL}{V}\right) * X(2) + Ksc * X(3) + Kpc * X(4)$

3. $\frac{dX(3)}{dt} = Kcs * X(2) - Ksc * X(3)$

4. $\frac{dX(4)}{dt} = Kcp * X(2) - Kpc * X(4)$

5. $Y(1) = X(2)/V$

6. $Y(2) = X(3)/Vcns$

Where equations (1), (2), (3) and (4) describe the rate of change in amount of drug in milligrams (mg) in the gut, central, CSF and peripheral compartment, respectively. *Ka* is the absorption rate constant from the gut to the central compartment. *X(1)*, *X(2)*, *X(3)* and *X(4)* are the amount of fluconazole (mg) in the gut, central (c), CSF (s) and peripheral compartments (p), respectively. *Kcp*, *Kpc*, *Kcs* and *Ksc* represent first-order transfer constants connecting the various compartments. *SCL* is the first-order clearance of drug (L/h) from the central compartment. *V* is the volume of the central compartment. The CSF compartment (*X(3)*) has an apparent CSF volume (*Vcns)*, given in litres.

Model error was attributed separately to process noise (including errors in sampling times or dosing) and assay variance. Process noise was modelled using lambda, an additive error term. The data were weighted by the inverse of the estimated assay variance.

The data for some patients indicated that they had taken fluconazole at an undocumented time prior to study enrolment, since there was detectable drug in the first PK sample. To accommodate this, non-zero initial conditions of all four compartments were estimated in the structural model. A switch was coded whereby the parameterised

estimate of each initial condition was multiplied by a binary covariate equal to 1 where fluconazole was detected in the first PK sample, or 0 where no fluconazole was detected in the first PK sample.

Population pharmacokinetic covariate screening

The impact of patent weight, BMI, sex, ethnicity and baseline eGFR on the PK of fluconazole were investigated. Bidirectional stepwise multivariate linear regression was employed to assess the relationship between each covariate and the Bayesian estimates for volume of distribution and clearance from the central compartment from the standard population PK model. Covariates that were retained with significant multivariate p-values (≤0.05) in the regression model were explored individually. The relationship between retained continuous covariates and Bayesian estimates of PK parameters was explored using univariate linear regression. The difference between Bayesian estimates of volume and clearance according to categorical covariates (sex and ethnicity) was compared using the Mann-Whitney test.

Population pharmacokinetic model diagnostics

The fit of the model to the data was assessed by visual inspection of diagnostic scatterplots displaying observed-versus-predicted values before and after the Bayesian step. Linear regression was performed and the coefficient of determination, intercept and regression slope noted for each model. In addition, the log-likelihood value, Akaike Information criterion (AIC), mean weighted error (a measure of bias) and bias-adjusted, mean weighted squared error (a measure of precision) were calculated and compared for each model.

Monte Carlo Simulation and calculation of probability of target attainment

Monte Carlo simulation (n = 5000) was performed in Pmetrics (40). The support points from the final joint density were used. For the simulations, the initial conditions of all compartments were defaulted to zero. Fluconazole was administered at a range of dosages: 400mg q24h, 800mg q24h, 1200mg q24h and 2000mg q24h. The plasma and CSF AUC for fluconazole was calculated using trapezoidal approximation after the sixth dose, from 144 to 168 hours after treatment initiation.

Wild type fluconazole MIC data were obtained from a previously published collection of 5,733 *C. neoformans* isolates estimated using Clinical and Laboratory Standards Institute (CLSI) methodology (41). The modal MIC was 4mg/L (1,629 of 5,733 strains; 28%). Almost half of strains had MICs ≥ 4mg/L (2,834 of 5,733 strains; 49%). The epidemiological cut-off value for *C. neoformans* versus fluconazole was 8mg/L. This collection of strains included molecular types VNI to VNIV and the pattern of MIC distribution was comparable across all molecular types (41). The proportion of simulated patients that would achieve a previously published plasma AUC/MIC target of 389.3 was determined. This target was defined as the magnitude of drug exposure required for fungal stasis (defined as prevention of progressive fungal growth) in a murine study that employed CLSI methodology (11). To our knowledge, no CSF PK/PD target has been defined in preclinical or clinical studies of fluconazole for cryptococcal meningitis. In the present study, the probability of attaining this plasma PK/PD target was examined at each simulated fluconazole dose.

Meta-analysis of clinical outcome data

The AUC/MIC target used in the probability of target attainment analysis was derived from murine studies. To enhance clinical relevance, we sought PD data from humans. The PD data from patients in the present PK study are confounded by the co-administration of amphotericin B deoxycholate. For this reason, a search for clinical trials of fluconazole monotherapy for cryptococcal meningitis was performed. The electronic databases Pubmed and Medline were searched on 31st January 2018 using the terms "fluconazole" and "cryptococcal meningitis". Preclinical studies and case reports were excluded. To reduce potential heterogeneity, only studies of HIV-positive participants were included in the meta-analysis. Baseline variables were chosen a priori for extraction from the studies if they had previously been determined to have a significant impact on clinical outcome. These were mental status, CSF fungal burden and patient age (6, 42). Where it was not reported, baseline CSF fungal burden was extrapolated from CSF cryptococcal antigen titre according to a correlation published by Jarvis et al (6).

For consistency with the literature, we collected data on clinical outcomes commonly presented in cryptococcal meningitis trials: CSF sterility at 8-10 weeks, 2-week mortality and 10-week mortality. Mixed-effects meta-analysis adjusted for fluconazole dosage was performed. Fungal burden in CSF, CD4 count and proportion of patients with reduced Glasgow Coma Score (GCS) at baseline were explored to assess the degree to which these modifiers accounted for inter-study heterogeneity in clinical outcome. The mixed-effects model took the form:

$$\theta_i = \beta_0 + \beta_1 Z_{i1} + \cdots + \beta_1 Z_{ij} + u_i$$

where θ_i is the corresponding (unknown) true effect of the ith study, Z_{ij} is the value of the jth moderator variable for the ith study and u_i are study-specific random effects such that $u_i \sim N(0, \tau^2)$. Here, τ^2 denotes the amount of residual heterogeneity, estimated using the DerSimonian-Laird estimator (43). Additional model parameters were estimated via weighted least squares with weights relative to the estimated τ^2. The null hypothesis $H_0: \tau^2 = 0$ was tested using Cochran's Q-test, and model parameters were tested with the Wald-type test statistic.

Conflicts of Interest

William Hope holds or has recently held research grants with F2G, AiCuris, Astellas Pharma, Spero Therapeutics, Matinas Biosciences, Antabio, Amplyx, Allecra and Pfizer. He holds awards from the National Institutes of Health, Medical Research Council, National Institute of Health Research, and the European Commission (FP7 and IMI). WH has received personal fees in his capacity as a consultant for F2G, Amplyx, Ausperix, Spero Therapeutics, Medicines Company, Gilead and Basilea. WH is Medical Guideline Director for the European Society of Clinical Microbiology and Infectious Diseases, and an Ordinary Council Member for the British Society of Antimicrobial Chemotherapy.

Jeremy Day holds or has recently held research awards from The Wellcome Trust, The Medical Research Council UK, The UK Department for International Development, The National Institutes for Health, The Li Ka Shing Foundation, The British Infection Society and The British Medical Association. He has received personal fees in his capacity as a consultant to Viamet Pharmaceuticals.

Funding statement

Supported by the United Kingdom Department for International Development, the Wellcome Trust, and the Medical Research Council through a grant (G1100684/1) from the Joint Global Health Trials program, part of the European and Developing Countries Clinical Trials Partnership, supported by the European Union.

Katharine Stott is a Wellcome Trust Clinical PhD Fellow [203919/Z/16/Z].

Jeremy Day is a Wellcome Trust Intermediate Fellow [WT097147MA].

REFERENCES

1. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis.
2. Day JN, Chau TT, Wolbers M, Mai PP, Dung NT, Mai NH, Phu NH, Nghia HD, Phong ND, Thai CQ, Thai le H, Chuong LV, Sinh DX, Duong VA, Hoang TN, Diep PT, Campbell JI, Sieu TP, Baker SG, Chau NV, Hien TT, Lalloo DG, Farrar JJ. 2013. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med 368:1291-302.
3. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, Mfinanga S, Temfack E, Lakhi S, Lesikari S, Chan AK, Stone N, Kalata N, Karunaharan N, Gaskell K, Peirse M, Ellis J, Chawinga C, Lontsi S, Ndong JG, Bright P, Lupiya D, Chen T, Bradley J, Adams J, van der Horst C, van Oosterhout JJ, Sini V, Mapoure YN, Mwaba P, Bicanic T, Lalloo DG, Wang D, Hosseinipour MC, Lortholary O, Jaffar S, Harrison TS, Team ATS. 2018. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med 378:1004-1017.
4. Bicanic T, Wood R, Bekker LG, Darder M, Meintjes G, Harrison TS. 2005. Antiretroviral roll-out, antifungal roll-back: access to treatment for cryptococcal meningitis. The Lancet Infectious Diseases 5:530-1.
5. Loyse A, Thangaraj H, Easterbrook P, Ford N, Roy M, Chiller T, Govender N, Harrison TS, Bicanic T. 2013. Cryptococcal meningitis: improving access to essential antifungal medicines in resource-poor countries. Lancet Infect Dis 13:629-37.
6. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, Longley N, Muzoora C, Phulusa J, Taseera K, Kanyembe C, Wilson D, Hosseinipour MC, Brouwer AE, Limmathurotsakul D, White N, van der Horst C, Wood R, Meintjes G, Bradley J, Jaffar S, Harrison T. 2014. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clin Infect Dis 58:736-45.
7. Bicanic T, Meintjes G, Wood R, Hayes M, Rebe K, Bekker L-G, Harrison T. 2007. Fungal Burden, Early Fungicidal Activity, and Outcome in Cryptococcal Meningitis in Antiretroviral-Naive or Antiretroviral-Experienced Patients Treated with Amphotericin B or Fluconazole. Clinical Infectious Diseases 45:76-80.
8. Nussbaum JC, Jackson A, Namarika D, Phulusa J, Kenala J, Kanyemba C, Jarvis JN, Jaffar S, Hosseinipour MC, Kamwendo D, van der Horst CM, Harrison TS. 2010. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. Clin Infect Dis 50:338-44.
9. Richardson K, Cooper K, Marriott MS, Tarbit MH, Troke PF, Whittle PJ. 1990. Discovery of Fluconazole, a Novel Antifungal Agent. Reviews of Infectious Diseases 12:S267-S271.
10. Vanden Bossche H, Koymans L, Moereels H. 1995. P450 inhibitors of use in medical treatment: focus on mechanisms of action. Pharmacol Ther 67:79-100.
11. Sudan A, Livermore J, Howard SJ, Al-Nakeeb Z, Sharp A, Goodwin J, Gregson L, Warn PA, Felton TW, Perfect JR, Harrison TS, Hope WW. 2013. Pharmacokinetics and pharmacodynamics of fluconazole for cryptococcal meningoencephalitis: implications for antifungal therapy and in vitro susceptibility breakpoints. Antimicrob Agents Chemother 57:2793-800.

12. Andes D, van Ogtrop M. 1999. Characterization and quantitation of the pharmacodynamics of fluconazole in a neutropenic murine disseminated candidiasis infection model. Antimicrob Agents Chemother 43:2116-20.
13. Felton TW, McCalman K, Malagon I, Isalska B, Whalley S, Goodwin J, Bentley AM, Hope WW. 2014. Pulmonary penetration of piperacillin and tazobactam in critically ill patients. Clin Pharmacol Ther 96:438-48.
14. Kethireddy S, Andes D. 2007. CNS pharmacokinetics of antifungal agents. Expert opinion on drug metabolism & toxicology 3:573-581.
15. Gubbins PA, EJ. 2009. Antifungal therapy, p 161 - 196. *In* Anaissie EM, MR; Pfaller, MA (ed), Clinical Mycology, 2nd ed. Churchill Livingstone, Elsevier.
16. Arndt CA, Walsh TJ, McCully CL, Balis FM, Pizzo PA, Poplack DG. 1988. Fluconazole penetration into cerebrospinal fluid: implications for treating fungal infections of the central nervous system. Journal of Infectious Diseases 157:178-80.
17. Madu A, Cioffe C, Mian U, Burroughs M, Tuomanen E, Mayers M, Schwartz E, Miller M. 1994. Pharmacokinetics of fluconazole in cerebrospinal fluid and serum of rabbits: validation of an animal model used to measure drug concentrations in cerebrospinal fluid. Antimicrob Agents Chemother 38:2111-5.
18. Tucker RM, Williams PL, Arathoon EG, Levine BE, Hartstein AI, Hanson LH, Stevens DA. 1988. Pharmacokinetics of fluconazole in cerebrospinal fluid and serum in human coccidioidal meningitis. Antimicrobial Agents and Chemotherapy 32:369-373.
19. Thaler F, Bernard B, Tod M, Jedynak CP, Petitjean O, Derome P, Loirat P. 1995. Fluconazole penetration in cerebral parenchyma in humans at steady state. Antimicrobial Agents and Chemotherapy 39:1154-1156.
20. Mayanja-Kizza H, Oishi K, Mitarai S, Yamashita H, Nalongo K, Watanabe K, Izumi T, Ococi J, Augustine K, Mugerwa R, Nagatake T, Matsumoto K. 1998. Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. Clin Infect Dis 26:1362-6.
21. Larsen RA, Leal MA, Chan LS. 1990. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS. A randomized trial. Ann Intern Med 113:183-7.
22. Rothe C, Sloan DJ, Goodson P, Chikafa J, Mukaka M, Denis B, Harrison T, van Oosterhout JJ, Heyderman RS, Lalloo DG, Allain T, Feasey NA. 2013. A prospective longitudinal study of the clinical outcomes from cryptococcal meningitis following treatment induction with 800 mg oral fluconazole in Blantyre, Malawi. PLoS One 8:e67311.
23. Longley N, Muzoora C, Taseera K, Mwesigye J, Rwebembera J, Chakera A, Wall E, Andia I, Jaffar S, Harrison TS. 2008. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clin Infect Dis 47:1556-61.
24. Milefchik E, Leal MA, Haubrich R, Bozzette SA, Tilles JG, Leedom JM, McCutchan JA, Larsen RA. 2008. Fluconazole alone or combined with flucytosine for the treatment of AIDS-associated cryptococcal meningitis. Medical Mycology 46:393-5.
25. Gaskell KM, Rothe C, Gnanadurai R, Goodson P, Jassi C, Heyderman RS, Allain TJ, Harrison TS, Lalloo DG, Sloan DJ, Feasey NA. 2014. A prospective study of mortality from cryptococcal meningitis following treatment induction with 1200 mg oral fluconazole in Blantyre, Malawi. PLoS One 9:e110285.

26. Beyene T, Zewde AG, Balcha A, Hirpo B, Yitbarik T, Gebissa T, Rajasingham R, Boulware DR. 2017. Inadequacy of High-Dose Fluconazole Monotherapy Among Cerebrospinal Fluid Cryptococcal Antigen (CrAg)-Positive Human Immunodeficiency Virus-Infected Persons in an Ethiopian CrAg Screening Program. Clin Infect Dis 65:2126-2129.
27. Fischman AJ, Alpert NM, Livni E, Ray S, Sinclair I, Callahan RJ, Correia JA, Webb D, Strauss HW, Rubin RH. 1993. Pharmacokinetics of 18F-labeled fluconazole in healthy human subjects by positron emission tomography. Antimicrob Agents Chemother 37:1270-7.
28. Pasko MT, Piscitelli SC, Van Slooten AD. 1990. Fluconazole: a new triazole antifungal agent. Dicp 24:860-7.
29. Palou de Fernandez E, Patino MM, Graybill JR, Tarbit MH. 1986. Treatment of cryptococcal meningitis in mice with fluconazole. J Antimicrob Chemother 18:261-70.
30. Kartalija M, Kaye K, Tureen JH, Liu Q, Tauber MG, Elliott BR, Sande MA. 1996. Treatment of experimental cryptococcal meningitis with fluconazole: impact of dose and addition of flucytosine on mycologic and pathophysiologic outcome. Journal of Infectious Diseases 173:1216-21.
31. Aoyama T, Hirata K, Hirata R, Yamazaki H, Yamamoto Y, Hayashi H, Matsumoto Y. 2012. Population pharmacokinetics of fluconazole after administration of fosfluconazole and fluconazole in critically ill patients. J Clin Pharm Ther 37:356-63.
32. Alobaid AS, Wallis SC, Jarrett P, Starr T, Stuart J, Lassig-Smith M, Mejia JL, Roberts MS, Sinnollareddy MG, Roger C, Lipman J, Roberts JA. 2016. Effect of Obesity on the Population Pharmacokinetics of Fluconazole in Critically Ill Patients. Antimicrob Agents Chemother 60:6550-6557.
33. McLachlan AJ, Tett SE. 1996. Pharmacokinetics of fluconazole in people with HIV infection: a population analysis. Br J Clin Pharmacol 41:291-8.
34. Brammer KW, Farrow PR, Faulkner JK. 1990. Pharmacokinetics and tissue penetration of fluconazole in humans. Rev Infect Dis 12 Suppl 3:S318-26.
35. Sanglard D. 2002. Resistance of human fungal pathogens to antifungal drugs. Current Opinion in Microbiology 5:379-385.
36. Chen Y, Farrer RA, Giamberardino C, Sakthikumar S, Jones A, Yang T, Tenor JL, Wagih O, Van Wyk M, Govender NP, Mitchell TG, Litvintseva AP, Cuomo CA, Perfect JR. 2017. Microevolution of Serial Clinical Isolates of Cryptococcus neoformans var. grubii and C. gattii. MBio 8.
37. Bicanic T, Harrison T, Niepieklo A, Dyakopu N, Meintjes G. 2006. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. Clin Infect Dis 43:1069-73.
38. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, Binh TQ, Chau NV, Farrar J, Merson L, Phuong L, Thwaites G, Van Kinh N, Thuy PT, Chierakul W, Siriboon S, Thiansukhon E, Onsanit S, Supphamongkholchaikul W, Chan AK, Heyderman R, Mwinjiwa E, van Oosterhout JJ, Imran D, Basri H, Mayxay M, Dance D, Phimmasone P, Rattanavong S, Lalloo DG, Day JN, CryptoDex I. 2016. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. New England Journal of Medicine 374:542-54.

39. Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, Binh TQ, Chau NV, Farrar J, Merson L, Phuong L, Thwaites G, Van Kinh N, Thuy PT, Chierakul W, Siriboon S, Thiansukhon E, Onsanit S, Supphamongkholchaikul W, Chan AK, Heyderman R, Mwinjiwa E, van Oosterhout JJ, Imran D, Basri H, Mayxay M, Dance D, Phimmasone P, Rattanavong S, Lalloo DG, Day JN. 2016. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. N Engl J Med 374:542-54.

40. Neely M, van	Guilder M, Yamada W, Schumitzky A, Jelliffe	RW. 2012. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacokinetic modeling and simulation package for R. Ther	Drug	Monit 34:467-476.

41. Espinel-Ingroff A, Aller AI, Canton E, Castañón-Olivares LR, Chowdhary A, Cordoba S, Cuenca-Estrella M, Fothergill A, Fuller J, Govender N, Hagen F, Illnait-Zaragozi MT, Johnson E, Kidd S, Lass-Flörl C, Lockhart SR, Martins MA, Meis JF, Melhem MSC, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, St-Germain G, Trilles L, Turnidge J. 2012. Cryptococcus neoformans-Cryptococcus gattii Species Complex: an International Study of Wild-Type Susceptibility Endpoint Distributions and Epidemiological Cutoff Values for Fluconazole, Itraconazole, Posaconazole, and Voriconazole. Antimicrobial Agents and Chemotherapy 56:5898-5906.

42. Montezuma-Rusca JM, Powers JH, Follmann D, Wang J, Sullivan B, Williamson PR. 2016. Early Fungicidal Activity as a Candidate Surrogate Endpoint for All-Cause Mortality in Cryptococcal Meningitis: A Systematic Review of the Evidence. PLOS ONE 11:e0159727.

43. DerSimonian R, Laird N. 1986. Meta-analysis in clinical trials. Control Clin Trials 7:177-88.

Table 1: Patient demographics

Demographic or clinical characteristic	Vietnam	Uganda	Combined	p-value†
Sex [a] (Male:Female)	13:10	8:12	23:20	
Age (years) [b]				
Mean	38	33	35	0.75
Median	33	33	33	
Range	20 - 73	24 - 50	20 - 73	
Weight (kg) [c]				
Mean	46	49	48	0.23
Median	45	49	48	
Range	32 - 68	35 - 60	32 – 68	
BMI (kg/m^2) [d]				
Mean	18	18	18	0.73
Median	18	18	18	
Range	12 - 25	15 - 22	12 - 25	
Creatinine (µmol/L) [a]				
Mean	67	81	74	0.02
Median	56	79	70	
Range	37 – 167	43 - 145	37 - 167	
eGFR (ml/min/1.73m^2) [e]				
Mean	88.3	80.7	84.7	0.10
Median	84.8	81.4	84.8	
Range	35.4 – 136.1	49.8 – 146.7	35.4 – 146.7	

[a] *n* = 43

[b] *n* = 31

[c] *n* = 41

[d] *n* = 35

[e] *n* = 33

† p-value for difference between Vietnam and Uganda by Mann-Whitney test of significance.

BMI: Body Mass Index; eGFR: estimated Glomerular Filtration Rate, by Cockcroft-Gault equation.

597 Table 2: Population parameter estimates from the final 4-compartment pharmacokinetic

598 model

Parameter	Mean	Median	Standard deviation
Ka (h^{-1})	8.78	1.73	11.98
SCL/F (L/h)	0.72	0.65	0.24
$Volume_c$ /F(L)	18.07	17.41	6.31
Kcp (h^{-1})	12.20	8.36	11.17
Kpc (h^{-1})	18.10	18.34	8.25
IC_{gut} (mg)	34.67	49.99	22.74
$IC_{central}$ (mg)	35.86	49.98	19.67
IC_{CNS} (mg)	31.06	49.96	23.47
$IC_{peripheral}$ (mg)	34.29	49.96	13.21
Kcs (h^{-1})	35.43	42.55	13.74
Ksc (h^{-1})	28.63	29.04	10.03
$Volume_{cns}$ /F(L)	32.07	30.49	17.60

599

600 SCL: clearance; $Volume_c$: volume of distribution in central compartment; F: bioavailability;

601 Kcp: first-order rate constant from the central to peripheral compartment; Kpc, first-order

602 rate constant from peripheral to central compartment; IC: initial conditions in respective

603 compartments; Kcs: first-order rate constant from the central to CNS compartment; Ksc,

604 first-order rate constant from CNS to central compartment; $Volume_{cns}$: volume of

605 distribution in CNS compartment.

606

607 Table 3: Evaluation of the predictive performance of the considered and final models

Model	Measured compartment	Log likelihood	AIC	Population bias	Population imprecision	Linear regression of observed-predicted values for each patient			p-value†
						$R^{2,a}$	Intercept	Slope	
Model 1	Plasma	-2451	4928	0.20	2.21	0.80	0.27	1.03	
	CSF			-0.30	1.55	0.81	-0.07	1.03	
Model 2	Plasma	-2413	4854	0.36	2.38	0.80	0.01	1.03	0.56
	CSF			-0.41	1.81	0.80	0.89	1.01	

608

609 Model 1 did not include any covariates. Model 2 incorporated a function to scale the volume of distribution in central compartment to patient

610 weight.

611 AIC: Akaike Information criterion.

612 [a] Relative to the regression line fitted for the observed versus predicted values after the Bayesian step.

613 † Comparison of the joint distribution of population parameter values for each model.

614

615 Table 4: Baseline characteristics and clinical outcomes from trial data of fluconazole monotherapy, by dosing regimen

Fluconazole dosage (mg)	Country	Number of patients	Age*	GCS < 15, %	CD4 cell count per mm^3 *	CSF burden, log10 CFU/mL	CSF sterility, fraction (%) of patients	Time CSF sterility charted	2 week mortality (%)	10 week mortality (%)	Reference
200	Uganda	28	33 (range 23-50)	43	Mean 73	.	4/8 (50)	2 months	10/25 (40)	16/25 (64)	Mayanja-Kizza 1998 (20)
400	USA	14	mean 38(SE 2)	0	Mean 44 (SE 13)	4[§]	6/14 (43)	10 weeks	NR	4/14 (29)	Larsen 1990 (21)
400	South Africa	5	39 (37-51)	60	41	5.53	NR	NR	NR	3/4 (75)	Bicanic 2007 (7)
800	Malawi	58	32 (29-39)	24	37 (11-58)	.	NR	NR	17/58 (29)	33/58 (57)	Rothe 2013 (22)
800	Uganda	30	35 (30-38)	33	7 (3-17)	5.7	NR	NR	11/30 (37)	18/30 (60)	Longley 2008 (23)
800	USA	9	35	100	8	4.8[§]	1/9 (11)	10 weeks	NR	8/9 (89)	Milefchik 2008 (24)
1200	Malawi	47	35 (32-40)	24	36 (17-62)	.	NR	NR	16/47 (34)	26/47 (55)	Gaskell 2014 (24)
1200	Uganda	30	33 (28-42)	60	14 (4-33)	5.9	NR	NR	6/27 (22)	13/27 (48)	Longley 2008 (23)
1200	USA	16	40	100	36	3.5[§]	6/16 (37.5)	10 weeks	NR	10/16 (62.5)	Milefchik 2008 (24)
1200	Malawi	20	36.5 (range 27-71)	40	25 (range 1-66)	5.30	1/20 (5)	2 weeks	7/19 (37)	11/19 (58)	Nussbaum 2010 (8)
1600	USA	16	35	100	33	3[§]	10/16 (62.5)	10 weeks	NR	6/16 (37.5)	Milefchik 2008 (24)
2000	USA	8	36	100	35	2.4[§]	5/8 (62.5)	10 weeks	NR	3/8 (37.5)	Milefchik 2008 (24)

616

617 *Median (interquartile rage) unless otherwise specified. [§]: Extrapolated from cryptococcal antigen titre. CSF: Cerebrospinal fluid. SE:

618 Standard error. CFU: Colony-forming units.

619 Figure 1: Fluconazole concentrations in 43 patients

620

621 Black diamonds represent plasma concentrations. White triangles represent CSF

622 concentrations.

Figure 2: Scatter plots showing observed versus predicted values for the chosen population pharmacokinetic model after the Bayesian step.

A: Population predicted concentration of fluconazole in plasma. R2 = 0.49; intercept = 2.89 (95% CI 0.51 – 5.27), slope = 0.89 (95% CI 0.82 – 0.97)

B: Individual posterior predicted concentration of fluconazole in plasma. R2 = 0.80; intercept = 0.27 (95% CI -1.08 – 1.62); slope = 1.03 (95% CI 0.98 – 1.07)

C: Population predicted concentration of fluconazole in CSF. R2 = 0.46; intercept = 3.39 (95% CI -0.09– 6.87), slope = 1.03 (95% CI 0.87 – 1.2)

D: Individual posterior predicted concentration of fluconazole in CSF. R2 = 0.81; intercept = -0.07 (95% CI -1.97 – 1.84); slope = 1.03 (95% CI 0.95 – 1.10)

Circles, dashed lines, and solid lines represent individual observed-predicted data points, line of identity, and the linear regression of observed-predicted values, respectively. All observed and predicted fluconazole concentrations in mg/L. FLC: fluconazole; CI: Confidence Interval.

641 Figure 3: AUC distributions in 5,000 simulated patients at escalating fluconazole dosages

642

643 Light grey bars indicate simulated plasma $AUC_{144\text{-}168}$. Dark grey bars indicate simulated CSF

644 $AUC_{144\text{-}168}$.

645 Figure 4: Probability of pharmacodynamic target attainment in plasma as a function of
646 isolate MIC and fluconazole dosage.
647
648
649
650 Each line represents the proportion of 5000 simulated patients that achieve the PD target at
651 the respective dosage of fluconazole. The PD target was a plasma AUC/MIC ratio ≥ 389.3.
652 Bars show the proportion of WT strains of *C.neoformans* at the indicated MIC.
653

654 Figure 5: Meta-analysis of clinical trials of fluconazole monotherapy showing dose-adjusted
655 effects on A) 2-week mortality and B) 10-week mortality.
656
657 A)
658
659 Right hand column provides observed and estimated proportions of patients dead at 2
660 weeks.
661
662
663

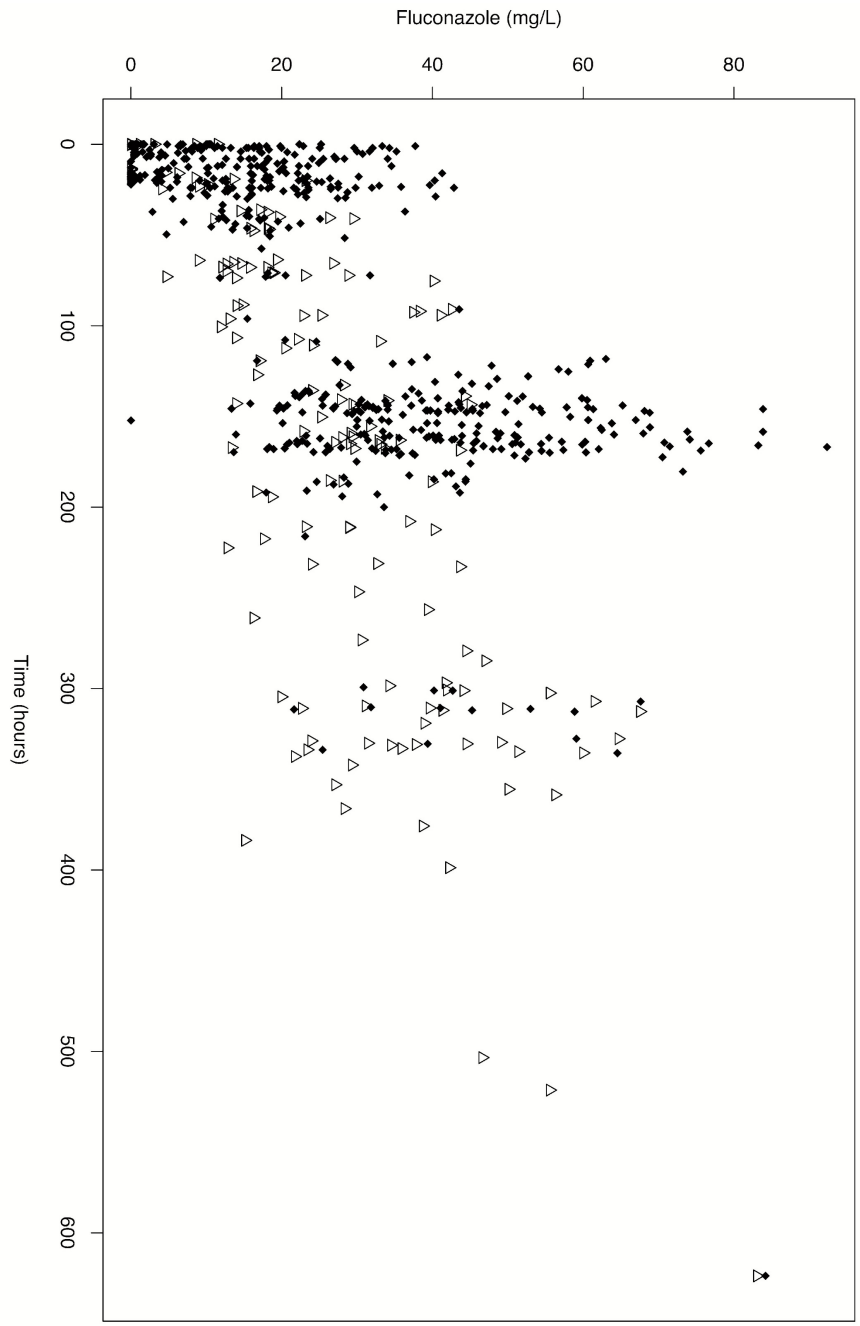
664 B)

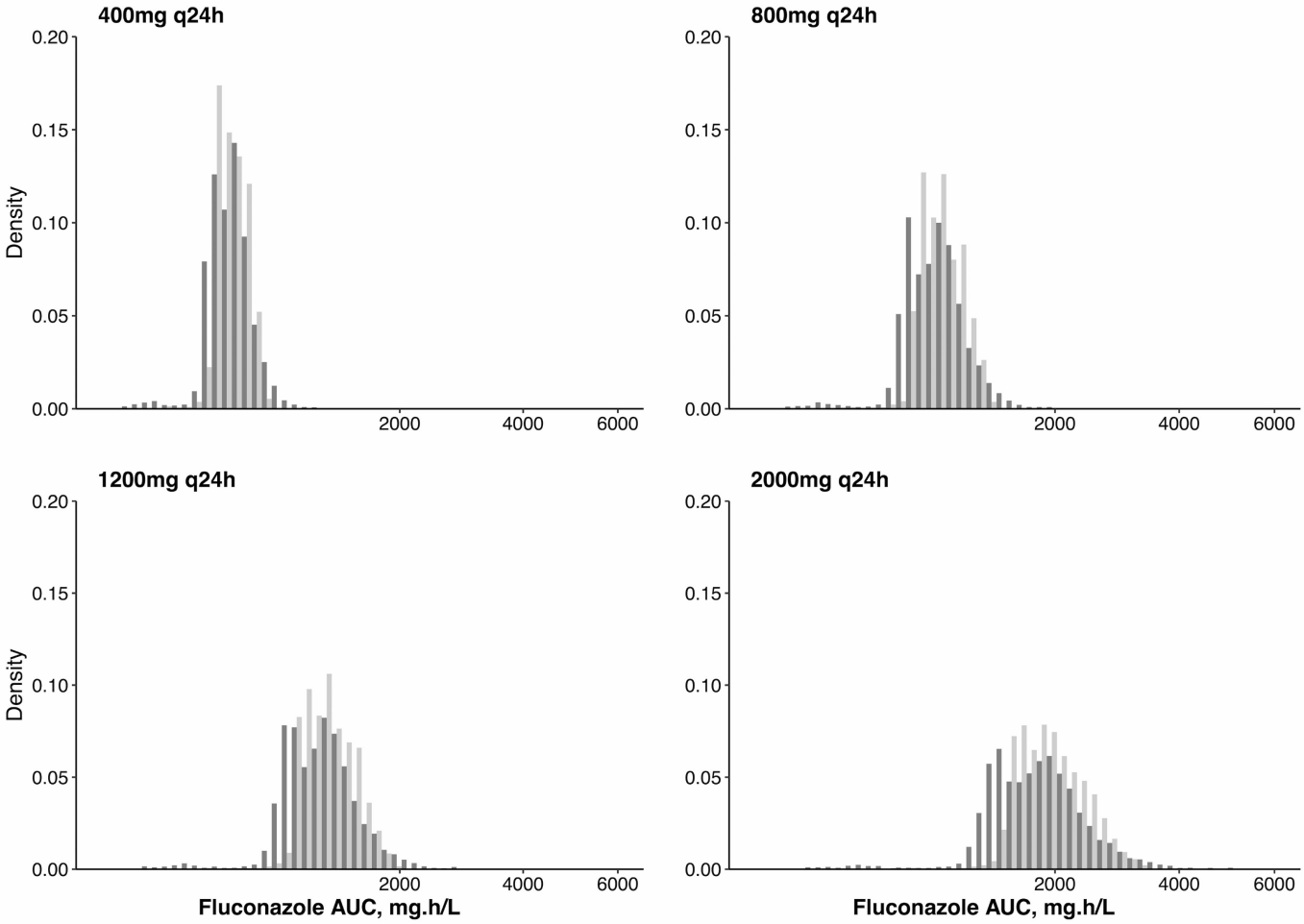
665

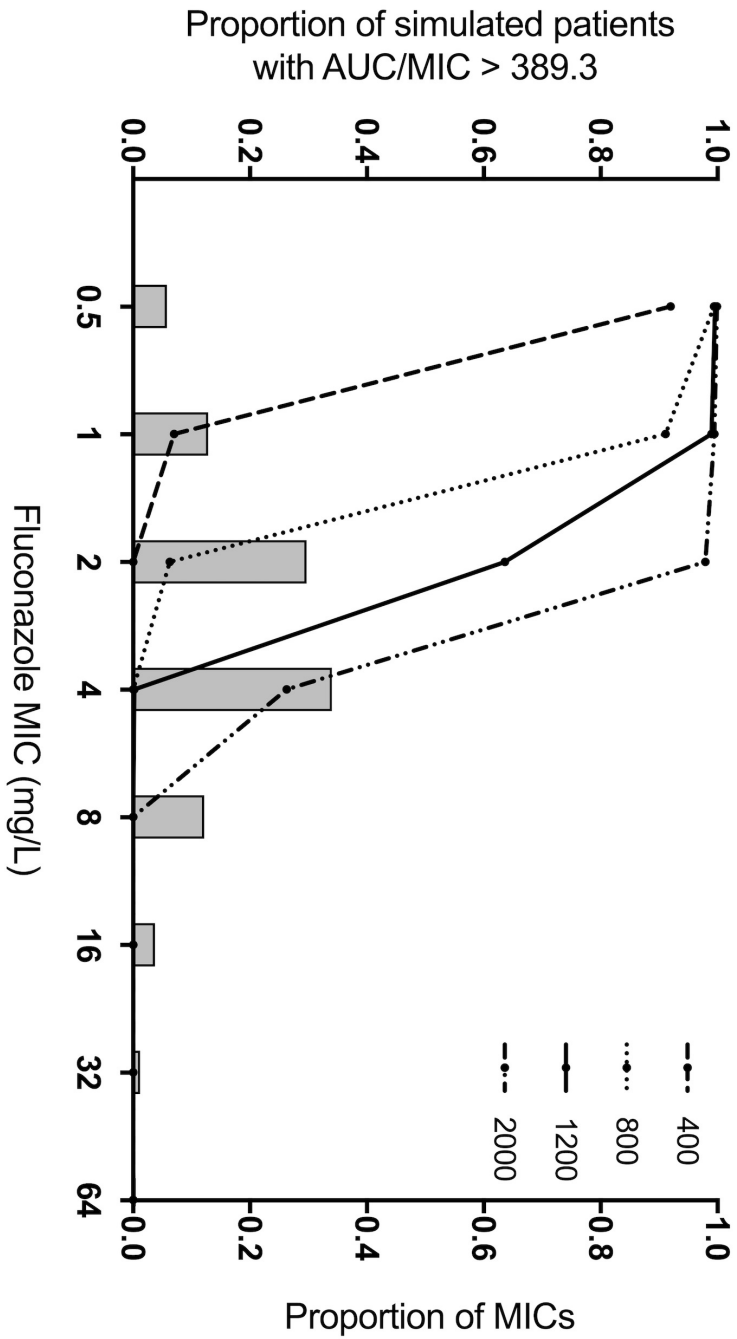
666 Right hand column provides observed and estimated proportions of patients dead at 10

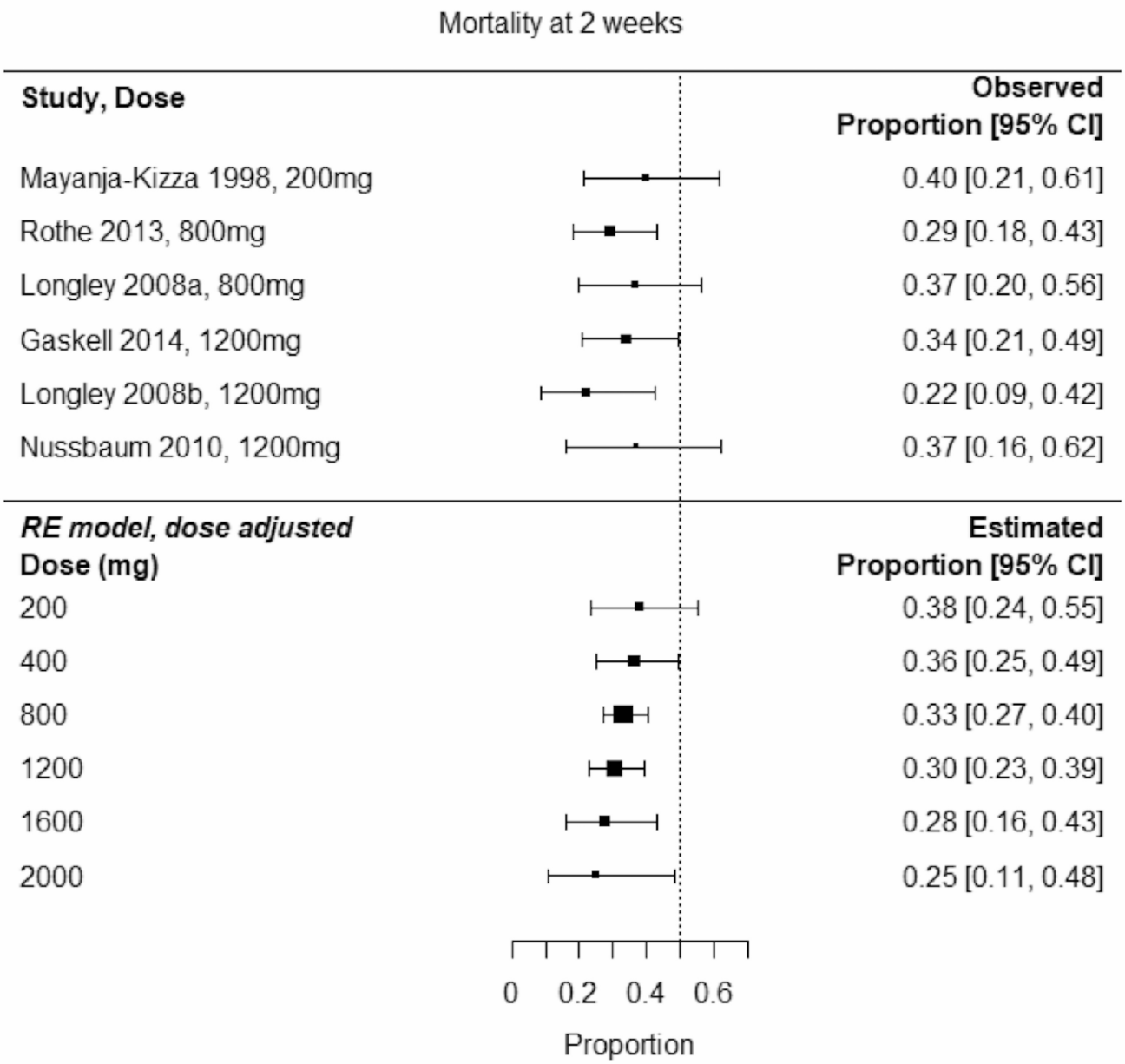
667 weeks.

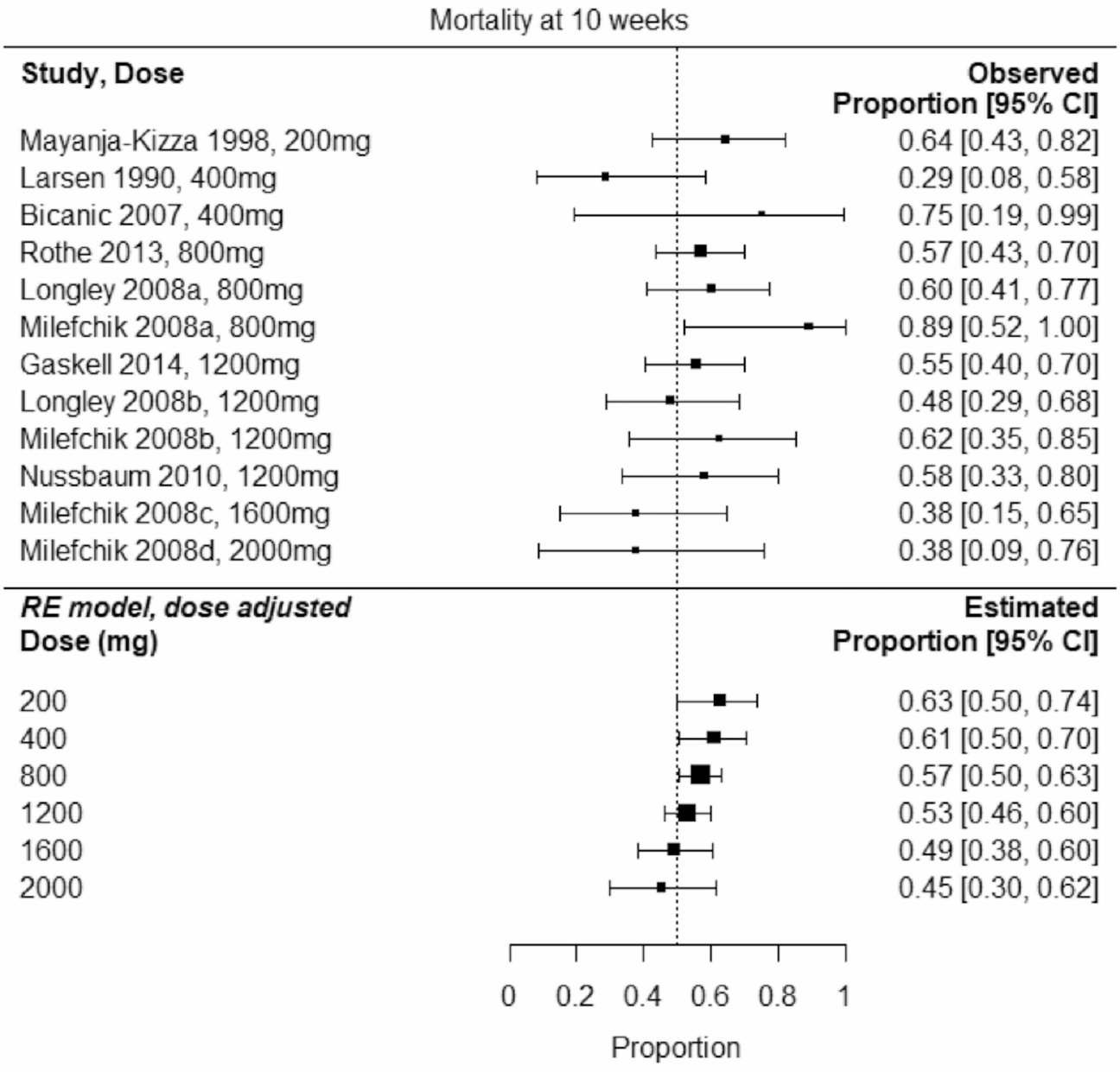
668











Antimicrobial Agents and Chemotherapy