Population Pharmacokinetics and Pharmacodynamics of Itraconazole for Disseminated Infection Caused by *Talaromyces marneffei*

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Abstract (250 words)

First-line treatment of talaromycosis with amphotericin B deoxycholate (DAmB) is labour intensive and toxic. Itraconazole is an appealing alternative antifungal agent. Pharmacokinetic data were obtained from 76 patients who were randomized to itraconazole in the Itraconazole versus Amphotericin B for Talaromycosis (IVAP) trial. Plasma levels of itraconazole and its active metabolite, hydroxyitraconazole, were analysed alongside longitudinal fungal colony forming unit counts in a population model. Itraconazole and hydroxyitraconazole pharmacokinetic variability was considerable, with area under the concentration-time curve over 24 hours (AUC_{24}) mean ± standard deviation 3.34 ± 4.31 mg*h/litre and 3.57 ± 4.46 mg*h/litre, respectively. Levels of both analytes were low; itraconazole minimum concentration (Cmin) 0.11 ± 0.16 mg/liter; hydroxyitraconazole Cmin 0.13 ± 0.17 mg/litre. The mean maximal rates of drug-induced killing were 0.206 and 0.208 $\log_{10}$ CFU/mL/h, respectively. There were no associations between itraconazole Cmin:MIC and time to sterilisation of the bloodstream (HR 1.01, 95% CI 0.99 to 1.03, p=0.43), time to death (HR 0.99, 95% CI 0.96 to 1.02, p=0.77) or early fungicidal activity EFA (coefficient -0.004, 95% CI -0.010 to 0.002, p=0.18). Similarly, there was no relationship between AUC:MIC and time to sterilisation of the bloodstream (HR 1.00, 95% CI 0.99 to 1.00, p=0.50), time to death (HR 1.00, 95% CI 0.99 to 1.00, p=0.91) or EFA (coefficient -0.0001, 95% CI -0.0003 to 0.0001, p = 0.19). This study raises the possibility that the failure of itraconazole to satisfy non-inferiority criteria against DAmB for talaromycosis in the IVAP trial was a pharmacokinetic and pharmacodynamic failure.

52 **Introduction**

53 *Talaromyces marneffei* is a thermally dimorphic fungus with endemicity limited to
54 Southeast Asia (in northern Thailand, Vietnam and Myanmar), South Asia (in northeastern
55 India) and East Asia (in southern China, Hong Kong and Taiwan) (1). In these regions,
56 talaromycosis is the third most common opportunistic infection after tuberculosis and
57 cryptococcal meningoencephalitis and a leading cause of morbidity and mortality among
58 people living with HIV/AIDS (2, 3). Mortality rates are as high as 30% at 6 months, despite
59 modern antifungal chemotherapy and supportive care (3-5). Talaromycosis is also
60 increasingly reported in patients with underlying immunosuppressive conditions other than
61 HIV (6). Disseminated infection is the most common form and manifests as fever, bone
62 marrow involvement (anemia, leukopenia, thrombocytopenia), skin lesions, weight loss,
63 lymphadenopathy, hepatosplenomegaly, respiratory failure and circulatory collapse (3, 7).

64 Itraconazole is an orally bioavailable broad-spectrum antifungal agent with a
65 relatively favorable safety profile in comparison to other systemic antifungal agents (8). It is
66 used for the prevention and treatment of a wide range of fungal diseases including
67 aspergillosis, candidiasis and those caused by dimorphic fungi such as histoplasmosis,
68 blastomycosis and talaromycosis (9-13). Itraconazole is lipophilic, poorly soluble at
69 physiological pH and highly protein bound (14). It partitions into lipid-rich tissues and drug
70 exposure increases at the effect site in the setting of tissue infection and inflammation (9,
71 15). Higher exposures are associated with greater clinical response but also increased
72 likelihood of toxicity (16-23). Itraconazole has recently been shown in the Itraconazole
73 versus Amphotericin B for Talaromycosis (IVAP) trial to be inferior to amphotericin B
74 deoxycholate (DAmB) for the induction phase of treatment for talaromycosis, with risk of
75 death at week 24 of 21.0% compared to 11.3% in the amphotericin B group ($p < 0.001$) (8).

76 This study investigates the population pharmacokinetics (PK) and
77 pharmacodynamics (PD) of itraconazole for patients with talaromycosis. The PK-PD study
78 was performed as a substudy of the IVAP trial (8). The pharmacodynamics of itraconazole
79 were estimated using serial quantification of fungal colony forming units (CFU) in the
80 bloodstream of patients who were fungaemic.

81

82

83 **Results**

84 **Study participants**

85 PK data were obtained for a randomly-selected sub-group of 76 patients in the
86 itraconazole treatment arm; PD data were available for 65 of these. All 76 patients had
87 culture-positive disseminated talaromycosis, with *T. marneffei* isolated from blood, skin
88 lesions, lymph nodes and/or serous fluid.

89 Forty-three percent of patients were female. The median age was 33 years
90 (interquartile range [IQR], 29 – 36), weight 45 kg (IQR, 40 – 50), body mass index 17.1 kg/m^2
91 (IQR, 15.6 – 19.0), and estimated glomerular filtration rate (eGFR) using the abbreviated
92 Modification of Diet in Renal Disease Study (MDRS) calculation 113.9 ml/min/1.73m^2 (IQR,
93 86.7 – 139.1). All patients had advanced HIV disease, with median CD4 cell count of 9 cells
94 per microlitre (IQR, 4 to 20).

95

96 **Pharmacokinetic data**

97 The dataset included 1316 itraconazole observations and 1314 hydroxyitraconazole
98 (OH-itraconazole) observations; an arithmetic mean of 17.3 observations of each analyte
99 per patient. A total of 95 itraconazole observations and 106 OH-itraconazole observations
100 were below the lower limit of quantification (LLQ). The median ratio of OH-itraconazole
101 concentration/itraconazole concentration per time point was 0.905. This ratio did not
102 change significantly over time (ratio = 1 + 0.0003*time, r^2 0.03, p 0.32). Figure 1 shows the
103 raw concentration data for both analytes.

104

105 **Population pharmacokinetic analysis**

106 The PK model was built in two stages. First, the parent drug (itraconazole) was
107 modelled in isolation with a saturable term for drug clearance. The second stage of model
108 building involved adding metabolite (OH-itraconazole) data into the model. The saturable
109 clearance mechanism of the parent drug fed into the central compartment of the
110 metabolite. An acceptable fit of the base model to the data was achieved with first-order
111 clearance of the metabolite from the central compartment.

112 The potential impact of covariates on the PK was assessed. Multivariate linear
113 regression of covariates was performed using the PMstep command in Pmetrics; this did not
114 reveal any significant associations between the Bayesian posterior PK estimates (i.e.
115 clearance, volume) versus age, sex, weight, body mass index (BMI), renal function
116 (creatinine level and eGFR) or CD4 cell count. Hence, further model building was not
117 performed. The final PK model comprised 5 compartments, representing the
118 gastrointestinal tract, the parent drug in the central compartment (circulation), the
119 metabolite in the central compartment, the parent drug in the peripheral compartment and
120 the metabolite in the peripheral compartment (Figure 2).

121 The observed-versus-predicted values for the plasma concentrations of itraconazole
122 and OH-itraconazole are shown in Figure 3 A and plots of weighted residuals against
123 predicted concentrations and time are displayed in Figure 3 B. Parameter values for the
124 final model are summarised in Table 1. Mean predicted parameter values described the
125 observed values better than medians and were used in subsequent modelling and analyses.
126 The mean AUC_{0-24} of itraconazole was 3.34 mg*h/liter (standard deviation (SD) 4.31
127 mg*h/liter; coefficient of variation (CV) 129%), median AUC_{0-24}1.91 mg*h/liter. For
128 hydroxyitraconazole, the mean $AUC_{0-24}$was 3.57 mg*h/litre (SD 4.46 mg*h/litre; CV 125%),
129 median AUC_{0-24}2.27 mg*h/litre. The mean Cmin of itraconazole was 0.11 mg/litre (SD 0.16

130 mg/litre, CV 147%), median Cmin 0.06 mg/litre. For OH-itraconazole, the mean Cmin was
131 0.13 mg/litre (SD 0.17 mg/litre, CV 132%), median Cmin 0.08 mg/litre.
132
133 ***In vitro* susceptibility tests**
134 Minimum inhibitory concentrations (MICs) against itraconazole were determined
135 using Clinical and Laboratory Standards Institute (CLSI) methodology (M38)(24) for isolates
136 from 69 patients. Of these, 70% had an MIC of 0.008 mg/litre, 27% 0.016 mg/litre and 3%
137 0.03 mg/litre.
138
139 **Population pharmacodynamic modelling**
140 PD data (CFUs/ml of blood) were available from 65 patients who received
141 itraconazole. Of those 65 patients, 52 were fungaemic at baseline. In total, 452
142 quantitative cultures were obtained with a mean of 7 observations per patient. There was a
143 large degree of variation in the time-to-sterilisation of blood cultures with a mean of 330
144 hours and a range of 13 – 3306 hours. Early fungicidal activity (EFA) was calculated by
145 performing a linear regression of Log_{10}CFU/mL versus day of blood culture per patient. EFA
146 was defined as the slope of the regression line. The median EFA was -0.3 (range -1.6 to 0.1)
147 Log_{10}CFU/mL/day.
148 Of the 452 quantitative culture results available, 162 were below the LLQ. Handling
149 values below the LLQ as LLQ/2 provided the best model fit to the data, with acceptable
150 levels of bias and imprecision (Figure 4). The parameter estimates for the population PD
151 model are summarised in table 2. Mean parameter values predicted the observed values
152 better than median values. After completion of the 14-day induction phase of treatment,
153 25 of the 52 patients who were fungaemic at baseline remained fungaemic (48%; Figure 5a).

154 The time-course of the reduction in fungal burden over the first 14 days of itraconazole
155 treatment in all 65 patients for whom PD data were available is displayed in Figure 5b.
156
157 **Pharmacodynamics**
158 We explored both AUC/MIC and Cmin/MIC as measures of drug exposure.
159 Associations between drug exposure and various PD endpoints including time-to-
160 sterilisation, time-to-death and EFA in the first 14 days were evaluated. Higher baseline
161 fungal burden was significantly associated with a longer time-to-sterilisation (Hazard ratio
162 (HR) 0.25; 95% confidence interval (CI) 0.17 to 0.37; $p<0.001$). There was a trend towards
163 an association between higher baseline fungal burden and time-to-death (HR 1.47, 95% CI
164 0.97 to 2.19, $p < 0.1$). All subsequent analyses were adjusted for baseline fungal burden.
165 Cox proportional hazard models revealed that there were no associations between
166 Cmin:MIC and time-to-sterilization (HR 1.01, 95% CI 0.99 to 1.03, p=0.38) or time-to-death
167 (HR 0.99, 95% CI 0.96 to 1.02, p=0.71). Similarly, there were no associations between
168 AUC:MIC and time to sterilisation of the bloodstream (HR 1.00, 95% CI 0.99 to 1.00, p=0.46)
169 or time to death (HR 1.00, 95% CI 0.99 to 1.00, p=0.99) (figure 6). Linear regression
170 following adjustment for baseline fungal burden revealed that Cmin:MIC had no significant
171 impact on EFA (EFA (log_{10} CFU/ml/day) = -0.64 - 0.004 * (Cmin:MIC), p = 0.18). There was
172 also no significant relationship between AUC:MIC and EFA (EFA (log_{10} CFU/ml/day) = -0.64 –
173 0.0001* (AUC:MIC), p = 0.19). These associations are shown in Figure 7. To test whether
174 these negative findings were a function of the fact that a small number of patients achieved
175 rapid sterilisation of the bloodstream, data from individual patients with the greatest EFA
176 values and fastest time to sterilization were examined closely for higher AUC and Cmin
177 values, and for higher AUC:MIC and Cmin:MIC values. No such correlation was found.

178

179 **Discussion**

180 Itraconazole is attractive as a potential agent for the treatment for talaromycosis
181 because of its potent *in vitro* activity against *T. marneffei* (25, 26), oral bioavailability,
182 improved tolerability profile and improved access compared with DAmB. Multiple large
183 case series have demonstrated outcomes from talaromycosis treated with itraconazole that
184 are comparable to treatment with DAmB (3, 27, 28). However, itraconazole induction
185 therapy was shown in the IVAP trial to be associated with excessive mortality from
186 talaromycosis at 6 months and significantly reduced EFA when compared with DAmB (8).
187 Our study raises the possibility that the poor clinical outcomes from itraconazole are related
188 to concentration-dependent therapeutic failure.

189 Our PK parameter estimates for itraconazole are in keeping with those described in
190 previous population PK models (29). Itraconazole is extensively metabolised in the liver
191 with negligible renal clearance. Renal function did not account for any portion of PK
192 variability in the present analysis. There was very modest variability in weight among study
193 participants; this was insufficient to fully explore the potential impact of weight on PK. The
194 rates of kill induced by itraconazole and OH-itraconazole were similar. This *in vivo* finding is
195 consistent with comparable *in vitro* potencies of itraconazole and OH-itraconazole, which
196 are seen against a large range of fungal pathogens (30).

197 Drug exposure targets for itraconazole have been established for oropharyngeal
198 candidiasis, invasive aspergillosis, cryptococcosis and histoplasmosis based on observations
199 that patients tend to have better clinical outcomes with trough concentrations of at least
200 0.5-1 mg/litre (11, 13, 20, 31, 32). The appropriate PD target for patients with talaromycosis
201 is not known. Our study did not provide further insight into this issue because an

202 association between drug exposure and microbiological and/or clinical response was not
203 evident, despite exploration of multiple different PK and PD measures and indices. There
204 were too few patients with drug exposures high enough to elicit maximal antifungal activity
205 and thereby separate the population into groups with a high and low probability of
206 therapeutic success. We were also unable to explore models of multi-exponential decline in
207 fungal burden, despite this rich dataset, because so few patients mounted an appreciable
208 PD response. At the end of the 14-day induction period, approximately 50% of patients
209 were still fungaemic. There was extensive variability in the PK, such that one would expect
210 an association between the PK and the PD to be apparent if it exists. The MICs against
211 itraconazole were uniformly low and cannot be implicated in the poor PD response
212 observed. Only 3 of 76 patients (4%) achieved a Cmin of 0.5 mg/litre. Therefore, almost all
213 patients were at the lower end of the exposure-response curve as defined for other fungal
214 pathogens. Our data are subject to a potential limitation - due to biosafety regulations in
215 handling *T. marneffei*, drug was extracted from samples in Vietnam prior to shipment to the
216 UK. Extraction of calibration curves and quality control assays were then performed at the
217 University of Liverpool. The potential for data discrepancies induced by these
218 methodologies was limited by freezing the calibration curve and quality control samples
219 following extraction, so that they were subject to the same conditions as patient samples. It
220 is nevertheless reassuring that our estimates for the population PK are consistent with those
221 described by others (29). A potential limitation in our PK-PD model is that we fixed the
222 volume of OH-itraconazole as a scalar of itraconazole volume. This approach fails to
223 account for the molecular masses of each compound, which are nonidentical.

224 The concept of concentration-dependent therapeutic failure is well understood for
225 the triazoles. This results from a number of issues common to this class of antifungals.

226 Firstly, oral bioavailability is frequently suboptimal. In the case of itraconazole, dissolution
227 and absorption depend on an acidic environment (pK_a value 3.7). In healthy volunteers,
228 itraconazole absorption from capsule formulations has been improved by 80% by co-
229 administration of cola (pH 2.5) (33) and Cmax increased by approximately 70% after a meal
230 (34). Patients in the IVAP trial were given itraconazole after a meal or a cola drink.
231 However, since the basis for these recommendations were data collected from studies of
232 healthy volunteers, it is possible that these are insufficient or ineffective approaches to
233 gastric acidification in the presence of HIV-associated achlorhydria or gastrointestinal
234 disease (35). The second contributor to concentration-dependent therapeutic failure in
235 triazoles is significant PK variability, principally related to variation in oxidative metabolism
236 (36). Itraconazole is extensively metabolised by cytochrome (CY)P450 3A4 isoenzymes, the
237 phenotype of which varies significantly between individuals. In our model, estimates of
238 AUC_{0-24} were highly variable, with CV values of 129% and 125% for the parent drug and the
239 metabolite, respectively. Similarly, CV values for estimates of Cmin were 147% and 132%
240 for the parent and the metabolite, respectively. Thirdly, drug-drug interactions are common
241 among the triazoles and we were unable to account for these in this analysis. First-line
242 antiretroviral treatment in Vietnam at the time of the trial consisted of tenofovir,
243 lamivudine and efavirenz. Efavirenz in an inducer of numerous hepatic enzymes, including
244 CYP3A4. Coadministration of itraconazole (200 mg twice daily) and efavirenz (600 mg once
245 daily) decreases itraconazole Cmax, AUC and Cmin by 37%, 39% and 44%, respectively and
246 decreases hydroxyitraconazole Cmax, AUC and Cmin by 37%, 35% and 43%, respectively
247 (37). Finally, the formulation of itraconazole is known to have significant impact on serum
248 drug concentrations, the oral bioavailability of capsule formulations being approximately
249 30% that of oral solutions (19, 34). Patients in the described cohort were administered a

250 capsule formulation of itraconazole from Stada (now Stellapharm), Vietnam. To the best of
251 our knowledge, there are no published data on the bioequivalence of this formulation
252 versus other formulations of itraconazole.

253 The large PK variability of itraconazole and the capacity for drug interactions mean
254 that TDM is widely advocated in clinical practice to achieve therapeutic levels. This study
255 represents an opportunity to define target levels for TDM, yet it is unable to do so due to
256 the universally low levels of drug exposure achieved and consequent lack of PD effect
257 produced in the study population. Moreover, treatment guidelines for talaromycosis were
258 recently updated as a result of the IVAP trial, to state that all patients with talaromycosis
259 should receive amphotericin B induction therapy regardless of disease severity (38). The
260 evidence for this has been graded as the highest possible (AI), since data demonstrating the
261 inferiority of itraconazole were obtained from a large randomised controlled trial. This
262 could deprioritise the future question of the role of itraconazole for talaromycosis.

263 It remains possible that a different formulation, dosage and/or mode of
264 administration of itraconazole may have provided higher systemic drug exposure and led to
265 better mycological and clinical outcomes. This PKPD sub-study illustrates the importance of
266 a deep understanding of dose-exposure-response relationships for any drug-pathogen
267 combination to adequately interpret the conclusions of late phase clinical trials.

Materials and methods

Clinical study

The PK and PD data were collected during a substudy of a multicentre prospective randomised clinical trial (Itraconazole versus Amphotericin B for Talaromycosis (IVAP) trial, ISRCTN59144167), which compared clinical response and mortality following treatment with itraconazole (300mg q12h for 3 days followed by 200mg q12h for 11 days) to DAmB (0.7 mg/kg/day) for induction therapy for HIV-associated talaromycosis (8). Patients were recruited between October 2012 and December 2015 from the 5 hospitals across Vietnam. Patients in the itraconazole arm were asked to take a small meal or drink or cola prior to drug administration, which was directly observed during the 14-day induction period of treatment. Patients over 18 years of age with culture-confirmed talaromycosis and HIV infection were eligible for the trial. Exclusion criteria included infection of the central nervous system, pregnancy, liver transaminase level > 400 U/litre, absolute neutrophil count < 500 cells/mm^3, creatinine clearance < 30 ml/min, or existing prescription of any antifungal therapy for more than 48 hours. IVAP trial participants at the Hospital for Tropical Diseases in Ho Chi Minh City were invited to participate in the PK-PD substudy. Ethical approval was granted by the Hospital for Tropical Diseases, the Oxford University Tropical Research Ethics Committee, and the Vietnam Ministry of Health.

Pharmacokinetic and pharmacodynamic sampling

A total of 76 patients randomised to receive itraconazole agreed to participate in the PK-PD substudy. For the PK, 15 patients underwent intensive sampling, with samples at 0, 0.5 and 2 hours post-dose on day 1; 1, 3, 4, and 12 hour samples on day 2; and 0, 0.5, 1, 2, 3,

292 4, 6 and 12 hour samples on day 8. The remaining 61 patients underwent sparse sampling
293 at 0 hours on day 1, followed by 1 sample on each of day 1 to 4, days 8 to 10, day 12 and at
294 each of their follow-up visits during weeks 4, 8, 12 and 24 of the study. For each PK sample,
295 2 mL of blood was collected in heparinised collection tubes and placed immediately on ice.
296 Within 30 minutes of collection, samples were centrifuged at 2000 rpm for 15 minutes and
297 the plasma stored at -80 °C until analysis. Itraconazole and OH-itraconazole were extracted
298 on site in Ho Chi Minh City (extraction procedure described below). Samples containing
299 acetonitrile as internal standard were plated onto Sabouraud dextrose agar in three
300 independent experiments to confirm sterility. Samples containing extracted drug were
301 stored at -80°C until shipment to the University of Liverpool for analysis.
302 For the PD analysis, blood was collected for quantitative culture on a daily basis for
303 the first 4 days of treatment and then on alternate days for the remainder of the first 14
304 days of treatment, until there was no microbial growth. Quantitative culture was
305 performed by serially diluting 100 μL of blood 10-fold and plating onto Sabouraud dextrose
306 agar. Plates were incubated at 37°C for quantification of fungal burden.
307

308 **Bioanalysis of PK samples**

309 Itraconazole and OH-itraconazole concentrations in plasma were measured using LC-
310 MS/MS methodology (1260 Agilent UPLC coupled to an Agilent 6420 Triple Quad mass
311 spectrometer, Agilent Technologies UK Ltd, Cheshire, UK). Itraconazole was extracted in
312 Vietnam by protein precipitation. In total, 300 µL of acetonitrile containing 6,7-Dimethyl-
313 2,3-Di-(2-Pyridyl)-Quinoxaline 10 ng/mL was added to 100 µL of matrix. Samples were
314 vortexed thoroughly and then centrifuged at 13600 rpm for 3 minutes. Three hundred µl of
315 supernatant was removed and placed in a 500 µL Eppendorf tube for storage at -80 degrees

316 Celsius prior to shipping to the University of Liverpool. Samples were thawed and vortexed
317 before 150 µL supernatant was transferred to a 96-well autosampler plate. Thirty µL was
318 injected on an Agilent ZORBAX C18 RRHD (2.1 X 50mm, 1.8 µm) (Agilent Technologies UK
319 Ltd, Cheshire, UK).

320 Chromatographic separation was achieved using a gradient consisting of 60% A:40%
321 B (0.1% aqueous Trifluoroacetic Acid (TFA) as mobile phase A and 0.1% TFA in acetonitrile as
322 mobile phase B). The mass spectrometer was operated in positive ion mode and a multiple
323 reaction monitoring (MRM) method used for optimum sensitivity and selectivity. The limit
324 of quantitation of both itraconazole and OH-itraconazole was 0.005 µg/mL. The intra-day
325 coefficient of variation (CV) for itraconazole was < 13.5% and the inter-day CV < 10.5%, over
326 the concentration range 0.005 – 8.0 µg/mL. For OH-itraconazole, the intra-day CV was < 9.0
327 % and the inter-day CV was < 8.7% over the same concentration range.

328

329 **Minimum inhibitory concentration testing**

330 The MICs of itraconazole against *Talaromyces marneffei* were determined in
331 duplicate using standardised CLSI methodology for yeasts (24).

332

333 **Population PK modelling**

334 The PK-PD model was fitted to the data in two steps. First, the PK was solved. The
335 mean Bayesian estimates for each individual's PK were fixed and taken forwards for the PD
336 modelling. The PD model was then solved by supplying each patient's PK posterior
337 estimates as covariates alongside the dosing history and individual PD data. Concentration-
338 time data for itraconazole in plasma were modelled using the non-parametric adaptive grid
339 parameter estimation function in Pmetrics (version 1.5.0) (39).

340 The base PK model was itself constructed in a 2-step process, since itraconazole has
341 an active metabolite, OH-itraconazole. Firstly, a PK model was developed to describe the PK
342 of the parent drug. Three clearance models were tested: linear clearance only, Michaelis-
343 Menten clearance (concentration-dependent, saturable clearance) and a combination of
344 both of these mechanisms. The final base model for the PK of the parent drug took the
345 form:

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

347 2. $\frac{dX(2)}{dt} = Ka * X(1) - \left(K23 + \frac{Vmax}{Km*Vcp+X(2)}\right) * X(2) + K32 * X(3)$

348 3. $\frac{dX(3)}{dt} = K23 * X(2) - K32 * X(3)$

349 Where equations 1, 2 and 3 describe the rate of change in amount of itraconazole in
350 milligrams in the gut, central and peripheral compartments, respectively. *Ka* is the
351 absorption rate constant from the gut to the central compartment. X(1), X(2) and X(3) are
352 the amounts of itraconazole in the gut, central and peripheral compartments respectively,
353 in milligrams. K23 and K32 represent first-order transfer constants connecting the central
354 and peripheral compartments. Vmax is the maximal rate of enzymatic metabolism of
355 itraconazole (mg/hr) and Km (mg/L) is the concentration of itraconazole in the central
356 compartment at which enzyme activity is half maximal. Vcp is the volume of the central
357 compartment in litres.

358 The same variations of clearance mechanism were investigated to incorporate the
359 OH-itraconazole (metabolite) data in the PK model. In this case, solely linear clearance,
360 without a saturable clearance component, provided the best fit to the data. The following
361 differential equations were added to the PK model:

362 4. $\frac{dX(4)}{dt} = \left(\frac{Vmax}{Km*Vcp+X(2)}\right) * X(2) - \mathrm{K45} * \mathrm{X(4)} + \mathrm{K54} * \mathrm{X(5)} - \left(\frac{\mathrm{SCLm}}{\mathrm{Vcm}}\right) * \mathrm{X(4)}$

363 5. $\frac{dX(5)}{dt} = K45 * X(4) - K54 * X(5)$

364

365 Where equations 4 and 5 describe the rate of change in amount of OH-itraconazole in
366 milligrams in the central and peripheral compartments, respectively. Accordingly, X(4) and
367 X(5) are the amounts of OH-itraconazole in those compartments in milligrams, with K45 and
368 K54 the first-order intercompartmental rate constants. SCLm is the first-order clearance of
369 OH-itraconazole from the central compartment (litres/hour), and Vcm the volume of the
370 central compartment of OH-itraconazole in litres. Vcm was fixed as a ratio of Vcp, taken
371 from the median ratio of parent to metabolite concentrations at each time point in the data.
372 Multivariate bidirectional linear regression of each subject's covariates against the
373 posterior parameter values was performed to determine whether any clinical variables
374 impacted PK parameters. The fit of the model to the data was assessed using a visual
375 inspection and linear regression of the observed-predicted scatter plots both before and
376 after the Bayesian step. Measures of precision and bias were assessed and weighted
377 residuals were plotted against predicted concentrations and time. Models were compared
378 by assessing 2 x difference in log-likelihood values evaluated against a chi-square
379 distribution with the appropriate number of degrees of freedom (difference in number of
380 parameters between candidate models). Information loss was estimated using the Akaike
381 information criterion. Predictive performance was evaluated in terms of bias and precision
382 through calculation of the mean weighted error and the mean weighted squared error,
383 respectively.
384 There is some uncertainty surrounding the most appropriate PD target for
385 itraconazole and Cmin is generally adopted as a pragmatic target, the PK profile of
386 itraconazole being relatively flat (40). We quantified drug exposure in terms of both Cmin

387 and AUC. Since the data were collected in a real-world clinical environment, precise drug
388 administration and blood sampling times varied between individuals. Estimates of drug
389 exposure in uniform time intervals across individuals were therefore not possible. The Cmin
390 for each patient was calculated as the mean of the lowest model-estimated PK output per
391 day, over the time frame for which there were data (and therefore model estimates) for
392 that patient. The AUC was calculated as the total average AUC for the treatment course
393 divided by the number of 24-hour intervals for which data were available per patient. This
394 was done in Pmetrics from each patient's posterior mean parameter estimates using the
395 trapezoidal rule in the function 'MakeAUC' (39).

396

397 **Pharmacodynamic modelling**

398 The population PK model described above was used to obtain the mean Bayesian
399 estimates for each patient's PK parameters. These were fixed for each patient and input to
400 the maximum likelihood estimator in ADAPT 5 (41) in order to define the PD parameters and
401 estimate the PD weighting functions. The weighting functions were estimated using the
402 variance model: variance = [intercept + slope**fb*]^2, where *fb* is the fungal burden
403 measured from the quantitative cultures. Each patient's PD data were fitted to the PD
404 model one individual at a time, employing the following structural model:

405

$$\frac{dN}{dt} = -\left[(Kkill_{max}p * \left(\frac{\frac{X(2)^{Hp}}{Vp}}{\frac{X(2)^{Hp}}{Vp} + EC50p^{Hp}} \right)) + (Kkill_{max}m * \left(\frac{\frac{X(4)^{Hm}}{Vm}}{\frac{X(4)^{Hm}}{Vm} + EC50m^{Hm}} \right)) \right] * N$$

406

407 In this model, *N* is the number of CFUs in the bloodstream, *t* is time and *dN/dt* is the rate of
408 change of fungal burden in the bloodstream. $Kkill_{max}$, EC50 and H are the maximal rate of

409 fungal kill, the concentration of drug that induces half maximal rate of killing, and the Hill
410 (slope) function, respectively. The model enabled itraconazole and OH-itraconazole to
411 affect the PD simultaneously and independently: parameters suffixed with 'p' refer to the
412 parent drug, itraconazole; those suffixed with 'm' to the metabolite, OH-itraconazole. As
413 previously, X(2) and X(4) are the amounts of itraconazole and OH-itraconazole in the central
414 compartment respectively, in milligrams. The initial condition, *IC*, represents an estimate of
415 the pre-treatment fungal density in the bloodstream. These PD parameters were estimated
416 for each patient alongside the weighting functions (intercept and slope) from the variance
417 model. These weighting functions were then transcribed into the PD datafile for Pmetrics
418 and the model was run in Pmetrics to arrive at a solution for the population PD. Bayesian
419 posterior estimates of the population PD parameters were then obtained from the final PD
420 model. Population PD model fit was determined according to the same criteria as were
421 used for the population PK model. Internal PK-PD model validation by means of Monte
422 Carlo simulation and visual predictive check demonstrated that 77.8% of observed CFU
423 values fell within the 5th and 95th simulated percentiles (p-value < 0.05).

424 In building the PD model, several methods for handling data below the LLQ were
425 investigated (42). This was necessary because the quantitative cultures were performed by
426 serially diluting 100 μL of blood and the lowest fungal count recorded was 0.699 $\log_{10}$
427 CFU/mL; that is, 5 CFU/mL. It is possible that there were samples with CFU counts below 5
428 CFU/mL but that these colonies were not picked up in the 100μl of blood plated and were
429 therefore recorded as zero. Thus, there is a degree of uncertainty inherent in
430 measurements towards the lower values of the measurement, as is true for many
431 laboratory assays. The PD model was run with these 'zero' CFU counts supplied to Pmetrics
432 as 1 (i.e. unchanged; 0 $\log_{10}$ CFU/mL), as LLQ/2 (0.350 $\log_{10}$ CFU/mL), and by discarding

433 these datapoints altogether, to determine which of these 3 methods provided the best
434 model fit. EFA was calculated by performing a linear regression on $\log_{10}$ CFU/mL versus day
435 of CSF culture, taking the slope of the regression line as the EFA for each patient.
436
437 **Statistical modelling**
438 For patients who had both PK and PD data available, Cox proportional hazard models
439 were fitted to examine the effect of AUC/MIC and Cmin/MIC for itraconazole on the time to
440 sterilization of fungal cultures and the time to death. The Cox models took the form: $h(t) =$
441 $h_0(t) exp (\beta_1 * PDI + \beta_2 * BFB)$ where t is time to event, $h(t)$ is the hazard function and $h_0(t)$ is
442 the baseline hazard. β_1 and β_2 are the coefficients for regression. The hazard ratio is
443 estimated by $exp(\beta_i)$. *PDI* and *BFB* are the pharmacodynamic index (either AUC/MIC or
444 Cmin/MIC) and the baseline fungal burden, respectively. Baseline fungal burden was
445 stratified according to its mean due to violation of the proportional hazards assumption,
446 although this did not alter the non-stratified effect sizes of either pharmacodynamic index.
447 The relationship between each pharmacodynamic index and EFA was assessed using a linear
448 regression model, which took the form: $EFA = \beta_0 + \beta_1 * PDI + \beta_2 * BFB + \varepsilon$, where β_0 is the
449 intercept, β_1 and β_2 are the regression coefficients for *PDI* and *BFB* respectively, and ε is the
450 model error term.
451
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Parameter (Units)	Mean	Median (95% credibility interval§)	Standard deviation
Ka (h^{-1})	1.781	0.238 (0.103-0.397)	4.275
Vcp (liters)	783.762	668.104 (500.251-1060.468)	287.724
K23 (h^{-1})	16.200	20.831 (1.782-28.103)	12.804
K32 (h^{-1})	6.449	1.152 (0.411-3.131)	9.554
Vmax (mg/hour)	55.836	37.767 (25.013-65.382)	36.512
Km (mg/liter)	0.426	0.223 (0.162-0.435)	0.473
K45 (h^{-1})	1.562	0.005 (0.005-0.005)	5.501
K54 (h^{-1})	25.713	29.986 (29.977-29.990)	9.191
SCLm (liters/hour)	133.351	135.899 (100.738-199.961)	70.765
Vcm (liters)	866.057*	738.255* (552.777-1171.817)	287.724

611

612 Table 1

613 Parameter estimates for the final pharmacokinetic model. Ka, absorption rate constant
614 from the gut to the central compartment; Vcp, volume of the central compartment for
615 itraconazole; K23, first-order transfer constant of itraconazole from the central to the
616 peripheral compartment; K32, first-order transfer constant of itraconazole from the
617 peripheral to the central compartment; Vmax, maximal rate of enzymatic metabolism of
618 itraconazole; Km, concentration of itraconazole in the central compartment at which
619 clearance is half maximal; K45, first-order transfer constant of hydroxyitraconazole from the
620 central to the peripheral compartment; K54, first-order transfer constant of
621 hydroxyitraconazole from the peripheral to the central compartment; SCLm; first-order
622 clearance of hydroxyitraconazole from the central compartment; Vcm, volume of the
623 central compartment for hydroxyitraconazole.

624 [§]95% credibility interval: used in Bayesian statistics to represent the interval within which an

625 unobserved value falls with a 95% probability

626 *fixed as 1.105*Vcp

Parameter	Mean	Median	Standard deviation
$Kkill_{max}p$ (log_{10}CFU/mL/h)	0.206	0.010	0.436
Hp	2.194	2.999	1.176
EC50p (mg/liter)	13.449	14.976	3.222
$Kkill_{max}m$ (log_{10}CFU/mL/h)	0.208	0.055	0.422
Hm	1.325	0.671	0.957
EC50m (mg/liter)	8.640	6.697	3.515
IC (CFU/mL)	1442.141	5.909	4412.916

627

628 Table 2

629 Parameter estimates for the final pharmacodynamic model. $Kkill_{max}$, maximum rate of drug-
630 induced killing of *T. marneffei*; H, Hill/ slope function; EC50, plasma concentration of drug
631 that induces half-maximal kill rate; IC, estimated fungal density just prior to initiation of
632 itraconazole. Parameters suffixed with 'p' describe the parent drug, itraconazole.
633 Parameters suffixed with 'm' refer to the metabolite, hydroxyitraconazole.

634

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637
638 Figure 1
639
640 Drug concentrations in 76 patients. Black diamonds represent itraconazole concentrations.
641 White triangles represent hydroxyitraconazole concentrations. Grey arrows represent
642 approximate times of itraconazole administration. The median concentration of
643 hydroxyitraconazole to itraconazole per time point is 0.905.
644
645
646 Figure 2
647 Structure of the pharmacokinetic-pharmacodynamic model for itraconazole in
648 talaromycosis. After ingestion, itraconazole is absorbed from the gastrointestinal tract into
649 the bloodstream according to the absorption rate constant, *Ka*. Saturable hepatic
650 metabolism of itraconazole results in the presence of hydroxyitraconazole in the
651 bloodstream. Both itraconazole and hydroxyitraconazole undergo bidirectional transfer
652 between the central and peripheral compartments. Hydroxyitraconazole is partially
653 removed from the central compartment through first-order clearance. The
654 pharmacodynamic effect on the burden of talaromycosis in the bloodstream is produced by
655 the additive effect of itraconazole and hydroxyitraconazole in the bloodstream.
656 Black dashed arrows indicate clearance mechanisms. Grey dashed arrows indicate the
657 pharmacokinetic compartments that produce pharmacodynamic effects.
658 * Saturable clearance of parent drug by hepatic metabolism ($\frac{Vmax}{Km*Vcp+X(2)}$). ** First order
659 clearance of metabolite (CLm/Vcm). GI: gastrointestinal. PD: pharmacodynamic. X(1):
660 amount of itraconazole in the gut. X(2): amount of itraconazole in the bloodstream. X(3):
661 amount of itraconazole in the peripheral compartment. X(4): amount of

662 hydroxyitraconazole in the bloodstream. X(5): amount of hydroxyitraconazole in the
663 peripheral compartment. K23, K32, K45, K54: first-order transfer constants between central
664 and peripheral compartments. *N*: number of colony-forming units in the bloodstream.

665

666 Figure 3 A

667 Scatter plots of observed versus predicted values for the chosen population
668 pharmacokinetic model after the Bayesian step. Left panel: itraconazole concentrations. r^2
669 0.69; intercept -0.03 (95% confidence interval -0.09 to 0.03); regression slope 1.01 (95% CI -
670 0.95 to 1.06). Right panel: OH-itraconazole concentrations. r^2 0.73; intercept -0.05 (95% CI -
671 0.12 to 0.00); regression slope 0.99 (95% CI 0.94 to 1.04).

672

673 Figure 3 B

674 Each panel displays the weighted residual error values against predicted concentrations in
675 the scatterplot on the left and against time in the center. On the right is a histogram of
676 residuals with normal curve superimposed. Top panel: itraconazole concentrations. Mean
677 weighted residual error: 0.01 (p-value 0.78, standard deviation 0.77). Shapiro-Wilk test for
678 normality: p = 0. Bottom panel: OH-itraconazole concentrations. Mean weighted residual
679 error: 0.11 (p-value 0.04, standard deviation 1.35). Shapiro-Wilk test for normality: p = 0.

680

681 Figure 4

682 Scatter plots of observed versus predicted values for the chosen population
683 pharmacodynamic model after the Bayesian step. For the linear regression, r^2 0.68;
684 intercept -0.07 (95% confidence interval -0.09 to 0.22); slope 0.99 (95% CI 0.92 to 1.07).

685

686
687 Figure 5 A and B

688 (a) Kaplan-Meier plot of the time to sterilisation (limited to the 14-day induction phase
689 of treatment).

690 (b) Time course of reduction in fungal burden for the 65 patients who provided PD data.
691 Open triangles are observed data points from individual patients; solid lines are
692 model estimates of each patient's PD profile.

693

694
695 Figure 6
696 Cox model predictions of hazard ratios depending on PD index. All models are adjusted for
697 the median baseline fungal burden of 2.2 log10 CFU/ml. A: The hazard ratio for time to
698 sterility with increasing Cmin:MIC is 1.01 (95% confidence interval 0.99 to 1.03), p=0.38. B:
699 The hazard ratio for time to sterility with increasing AUC:MIC is 1.00 (95% confidence
700 interval 0.99 to 1.00), p=0.46. C: The hazard ratio for time to death with increasing
701 Cmin:MIC is 0.99 (95% confidence interval 0.96 to 1.02), p=0.71. D: The hazard ratio for
702 time to death with increasing AUC:MIC, adjusted for the fungal burden, is 1.00 (95%
703 confidence interval 0.99 to 1.00), p=0.99.
704
705
706 Figure 7
707 Relationship between pharmacodynamic indices and early fungicidal activity, adjusted for a
708 median baseline fungal burden of 2.2 log_{10} CFU/ml. (A) Predicted log_{10} EFA = -0.64 - 0.004 *
709 (Cmin:MIC), p = 0.18. A one-unit increase in Cmin:MIC decreases the log_{10} EFA by -0.004
710 CFU/mL/day (95% confidence interval -0.010 to 0.002). (B) Predicted log_{10} EFA = -0.64 -
711 0.0001 * (AUC:MIC), p = 0.19. A one-unit increase in AUC:MIC decreases the log_{10} EFA by -
712 0.0001 CFU/mL/day (95% confidence interval -0.0003 to 0.0001).

713