

1 **Title page**

2 Title: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and  
3 clinical trial simulations

4 Running title: Bactericidal activity of rifampicin

5 Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H  
6 Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J  
7 Boeree (6) and Ulrika SH Simonsson (1)

8 (1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, 751 24,  
9 Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud  
10 University Medical Center, Nijmegen, 6525 GA, the Netherlands, (3) TASK Foundation,  
11 Cape Town, 7500, South Africa, (4) Division of Pulmonology, Department of Medicine,  
12 University of Cape Town, 7925, and The University of Cape Town Lung Institute, Cape  
13 Town, 7700, South Africa, (5) The School of Medicine, University of St. Andrews, St.  
14 Andrews, KY16 9TF, UK, (6) Department of Lung Diseases, Radboud University Medical  
15 Center, Nijmegen, 6525 GA, the Netherlands and University Centre for Chronic Diseases  
16 Dekkerswald, Groesbeek, the Netherlands

17 Type of article: Major article

18 Word count of abstract: 184

19 Word count of text: 3499

20

21 **Foot note page**

22 **Conflict of interest statement**

23 All authors: No conflict

24 **Funding statement**

25 This work was supported by the Swedish Research Council [521-2011-3442 to R.J.S and  
26 U.S.H.S], the Innovative Medicines Initiative Joint Undertaking [n°115337]  
27 (www.imi.europe.eu, resources of which are composed of financial contribution from the  
28 European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies'  
29 in kind contribution), the European and Developing Countries Clinical Trials Partnership  
30 (EDCTP) [IP.2007.32011.011, IP.2007.32011.012, IP.2007.32011.013], the Netherlands-  
31 African Partnership for Capacity Development and Clinical Interventions Against Poverty-  
32 related Diseases (NACCAP) and the Bill and Melinda Gates Foundation.

33 **Meetings where part of the information has previously been presented:**

34 Part of the results has been presented in the form of an abstract at the 27<sup>th</sup> European Congress  
35 of Clinical Microbiology and Infectious Diseases, 22-25 April 2017 in Vienna, Austria  
36 (abstract P1673), at ASM Microbe, 1-5 June 2017 in New Orleans, Louisiana, USA (abstract  
37 1978) and at the 10<sup>th</sup> International Workshop on Pharmacology of Tuberculosis Drugs in  
38 Atlanta, Georgia, USA (abstract 10).

39 **Corresponding author:** Ulrika SH Simonsson (ulrika.simonsson@farmbio.uu.se)

40 Box 591, 751 24 Uppsala, Sweden

41 Telephone: +46-18-471 46 85

42 Fax: +46-18-471 42 53

43 **Abstract (max 200 words)**

44 **Background:** The current rifampicin dose (10 mg/kg) is sub-optimal for treating tuberculosis.  
45 The PanACEA HIGHRIF1 trial evaluated pharmacokinetics and early bactericidal activity  
46 with rifampicin doses up to 40 mg/kg. Conventional statistics revealed no significant  
47 exposure-response relationship. Our objective was to explore exposure-response for high dose  
48 rifampicin using pharmacokinetic-pharmacodynamic modeling and to predict early  
49 bactericidal activity of 50 mg/kg rifampicin.

50 **Methods:** Data included time-to-positivity of sputum in liquid culture from 83 tuberculosis  
51 patients treated with 10 (n=8), 20, 25, 30, 35 or 40 (n=15/group) mg/kg rifampicin for 7 days  
52 (clinicaltrials.gov, NCT01392911). We used a semi-mechanistic time-to-event approach to  
53 model the time-to-positivity data. Rifampicin exposure and baseline time-to-positivity were  
54 explored as covariates.

55 **Results:** Baseline time-to-positivity was a significant covariate on the predicted initial  
56 bacterial load and rifampicin exposure was significant on bacterial kill in sputum giving  
57 increased early bactericidal activity. The 90% prediction interval for the predicted median day  
58 7 increase in time-to-positivity for 50 mg/kg rifampicin was 7.25-10.3 days.

59 **Conclusions:** A significant exposure-response relationship was found between rifampicin  
60 exposure and early bactericidal activity. Clinical trial simulations showed greater early  
61 bactericidal activity for 50 mg/kg rifampicin. ~~Our semi-mechanistic time-to-event approach is~~  
62 ~~effective for studying exposure-response in tuberculosis and de-risking future drug~~  
63 ~~development.~~

64 **Key words (3-10):** Pharmacodynamics, Tuberculosis, Pharmacokinetics, Patients, Time-to-  
65 positivity, Early bactericidal activity, Models, Bactericidal effect, Mycobacterium  
66 tuberculosis

## 67 Introduction

68 Since the concept of increasing the ~~dose of rifampicin~~ dose for treat~~ingment of~~ tuberculosis  
69 was re-introduced, a battery of trials has been conducted to optimize treatment of pulmonary  
70 tuberculosis and tuberculous meningitis [1–9]. Despite this, a question remains: what is the  
71 optimal dose of rifampicin [10,11]? The answer remains unknown but ~~some~~ recent clinical  
72 trials ~~data has~~ have provided important insight. ~~The~~ PanACEA HIGHRIF1 ~~trial~~ [4] studied  
73 short-term safety, pharmacokinetics and anti-mycobacterial activity of up to 40 mg/kg  
74 rifampicin. No statistically significant relationship was determined between ~~the~~ rifampicin  
75 exposure and ~~the~~ early bactericidal activity [4] in humans despite several lines of evidence  
76 derived from in vitro and animal experiments suggesting a clear relationship between  
77 exposure and mycobacterial killing [12–15].

78 Early bactericidal activity can be quantified using time-to-positivity in liquid culture which is  
79 defined as the time from start of incubation of a sputum specimen in a liquid culture system  
80 until a positive signal is detected. A high bacterial load is expected to lead to short time-to-  
81 positivity and vice versa.

82 Time-to-positivity reflects time-to-event data. For conventional statistics, early bactericidal  
83 activity determined using time-to-positivity is usually analyzed in the context of a series of  
84 data points from daily sputum cultures as the change in time-to-positivity per day of treatment  
85 by regression-based methods [16,17]. Two semi-mechanistic pharmacokinetic-  
86 pharmacodynamic models exist treating time-to-positivity as time-to-event data [18,19].

87 Model-based pharmacokinetic-pharmacodynamic analysis has been shown to be more  
88 powerful ~~in terms of~~ for defining exposure-response than conventional statistical methods  
89 [20]. Semi-mechanistic pharmacokinetic-pharmacodynamic models also allow for meaningful

90 extrapolation ~~of data to~~by simulatinge new scenarios such as predicting early bactericidal  
91 activity of higher than observed doses which can be used to design future clinical trials.

92 ~~The~~Our objective ~~of this study~~ was to apply a semi-mechanistic time-to-event approach to  
93 explore the exposure-response for early bactericidal activity determined using time-to-  
94 positivity in pulmonary tuberculosis patients treated with high dose rifampicin (up to 40  
95 mg/kg) and then, to simulate early bactericidal activity of 45 and 50 mg/kg rifampicin in order  
96 to inform the clinical development process of optimizing a higher rifampicin dose.

97

## 98 **Methods**

### 99 *Ethics*

100 The study ~~protocol~~ was approved by local ethical review boards and by the Medicines Control  
101 Council of South Africa and was conducted according to Good Clinical Practice. All patients  
102 provided written informed consent before enrollment in the study.

### 103 *Patient data*

104 Modeling was performed on one week repeated time-to-positivity data measured from sputum  
105 in patients recruited in ~~the~~ HIGHRIF1 ~~trial~~, a prospective open-label multiple dose-rising trial  
106 registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT01392911 [4]. Smear-positive pulmonary  
107 tuberculosis patients were treated with either 10 (n=8, reference arm), 20, 25, 30, 35 or 40  
108 (n=15/arm) mg/kg daily rifampicin as monotherapy for 7 days. The actual study duration was  
109 14 days with isoniazid, pyrazinamide and ethambutol in standard doses added to the high dose  
110 rifampicin for days 8-14. In this analysis, only data until day 7 was used to define the  
111 exposure-response relationship for rifampicin alone. Overnight sputum sampling was  
112 performed on two consecutive days at baseline and daily for 7 days. Time-to-positivity was  
113 determined in duplicate from each sample using a standardized liquid culture BD BACTEC™  
114 MGIT™ mycobacterial growth indicator tube system (MGIT 960, Becton-Dickinson, Sparks,  
115 MD) in a single laboratory. ~~The~~ HIGHRIF1 ~~trial~~ is described in detail in the relevant  
116 reference ~~references~~ [4].

### 117 *Data analysis*

118 Time-to-positivity data were analyzed with a time-to-event approach using the non-linear  
119 mixed effects modeling software NONMEM 7.3 [21] with the Laplacian estimation method.  
120 Data handling and visualization were done in R version 3.4.3 [22]. Model diagnostics were

121 performed in Xpose 4.6.0 [23,24], in particular visual predictive checks using PsN 4.6.12  
122 [23,25]. Models were compared based on the objective function value (OFV) using the  
123 likelihood ratio test at the 5% significance level.

124 Time-to-positivity replicates at each time point were analyzed without averaging. Time-to-  
125 positivity at baseline was included in the model as a covariate (see below).

### 126 *Structural model*

127 The starting point for model development was a previously developed semi-mechanistic time-  
128 to-event model for time-to-positivity [19]. Briefly, the model structure was derived from  
129 underlying knowledge about (i) how the amount of viable tuberculosis bacteria changes in  
130 human sputum over time, referred to as *sputum model*, (ii) how tuberculosis bacteria are  
131 known to grow in a liquid culture, referred to as *mycobacterial growth model*, and (iii) how  
132 the mycobacterial growth relates to the probability of achieving a positive signal event in the  
133 MGIT, referred to as *hazard model*. The starting model included drug effect without an  
134 exposure-response relationship.

135 Sputum models with one and two mycobacterial subpopulations were tested. The bacterial  
136 load in the single mycobacterial subpopulation model was described using the following  
137 equation by:

$$138 \quad B(t)_{sputum} = B_{0,sputum} \times e^{-k_{kill} \times t_t}$$

139 where  $B_{0,sputum}$  is the predicted bacterial load at start of treatment,  $k_{kill}$  is the first-order  
140 rifampicin bacterial kill rate and  $t_t$  is the time after start of treatment. For the two-  
141 subpopulation model the bacterial load of the first (B1) and second (B2) mycobacterial  
142 subpopulations were described by the following equations:

$$143 \quad B1(t)_{sputum} = B1_{0,sputum} \times e^{-k_{kill,1} \times t_t}$$

144  $B2(t_t)_{sputum} = B2_{0,sputum} \times e^{-k_{kill,2} \times t_t}$

145 where

146  $B(t_t)_{sputum} = B1(t_t)_{sputum} + B2(t_t)_{sputum}$

147 where  $B1_{0,sputum}$  and  $B2_{0,sputum}$  are the predicted bacterial load at start of treatment for the  
148 subpopulations, respectively. Parameters  $k_{kill,1}$  and  $k_{kill,2}$  describe the first-order rifampicin  
149 bacterial kill rates of the subpopulations, respectively.

150 For the mycobacterial growth model in the liquid culture container, a logistic growth model  
151 was used where the change of bacteria in the liquid culture ( $B_{culture}$ ) over time was described  
152 ~~using the following differential equation by:~~

153 
$$\frac{dB_{culture}}{dt_c} = k_G \times (B_{max} - B(t_c)_{culture}) \times B_{culture}$$

154 where the initial bacterial load in the liquid culture ~~were~~ was assumed equal to the number of  
155 bacteria in sputum at the time-point of sputum sampling according to ~~the following:~~

156  $B(t_c = 0)_{culture} = B(t_t = \text{sampling time point})_{sputum}$

157 where  $k_G$  is a predicted maximal mycobacterial growth rate in the liquid,  $B_{max}$  is the maximal  
158 bacterial load in the liquid culture and  $t_c$  is time after inoculation of the liquid culture.

159 Models with two ~~different~~ subpopulations in the liquid culture with different growth rates for  
160 each subpopulation both with and without a possible transfer between subpopulations were  
161 explored. A lag-time for ~~the~~ start of growth in the liquid culture was ~~also~~ explored  
162 ~~implemented~~ as a single lag time for both the one and two subpopulation models. ~~In addition,~~  
163 ~~Time~~Time-dependencies in  $k_G$  were explored including linearly decreasing  $k_G$  with time on



164 treatment and an exponential decline from a baseline value of  $k_G$  ( $k_{G,base}$ ) to a steady state  
165 value of  $k_G$  ( $k_{G,ss}$ ) according to ~~the following~~:

$$166 \quad k(t_t)_G = k_{G,base} + (k_{G,ss} - k_{G,base}) \times (1 - e^{-k_{G,k} \times t_t})$$

167 where  $k_{G,k}$  is the first-order time-dependent decrease of  $k_{G,base}$ .

168 For the hazard model the bacterial load in the liquid culture at any given time-point was equal  
169 to the hazard,  $h(t_c)$ , for the liquid culture to turn into a positive signal described by:

$$170 \quad h(t_c) = B(t_c)_{culture}$$

171 which was used in a next step to calculate the cumulative hazard according to ~~the following~~:

$$172 \quad H(t_c) = \int_0^{t_c} h(t_c) dt$$

173 This finally allowed calculation of the survival, which is the probability of a sample without a  
174 positive signal at time  $t_c$  using the following equation:

$$175 \quad S(t_c) = e^{-H(t_c)}$$

#### 176 *Covariate model*

177 The individual mean time-to-positivity at baseline was not included in the estimation but was  
178 evaluated as a covariate on the predicted bacterial load at start of treatment ( $B_{0,sputum}$  or  
179  $B_{10,sputum}$  and  $B_{20,sputum}$ ) as a power-relationship. The area under the plasma concentration-  
180 time curve between 0 and 24 hours ( $AUC_{0-24h}$ ) at day 7 was evaluated as a covariate on the  
181 rifampicin kill rate parameters ( $k_{kill}$ , or  $k_{kill,1}$ , and  $k_{kill,2}$ ).  $AUC_{0-24h}$  was chosen over  $C_{max}$   
182 because AUC is normally used in PKPD analyses for rifampicin.  $C_{max}$  and  $AUC_{0-24h}$  are  
183 probably highly correlated and would therefore perform similarly when explored in a PKPD  
184 model. Since only once daily dosing was included in the current study design it would

185 probably be difficult to definitely distinguish between  $C_{max}$  and  $AUC_{0-24h}$ . The  $AUC_{0-24h}$  was  
186 calculated for each subject from a full 24 hour pharmacokinetics curve concentration  
187 measurements at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours -using the linear-log trapezoidal  
188 rule in Winnonlin version 5.3 (Pharsight Corp., Mountain View, CA) as described in [4].  
189 Concentrations were measured using validated ultra performance liquid chromatography  
190 (accuracy<4%, limit of quantification= 0.13 mg/L).

#### 191 *Stochastic model*

192 Inter-individual variability was investigated in all parameters for the sputum model as well as  
193 for the estimated lag-time for the growth in the mycobacterial growth model. Inter-occasion  
194 variability was investigated in the sputum sampling implemented as a random variability  
195 between occasions for the bacterial load inoculated in the mycobacterial growth model [19].

#### 196 *Model evaluation*

197 The final semi-mechanistic time-to-event model (i.e. the chosen model after structural,  
198 covariate and stochastic model evaluations) was evaluated by performing a 1000 sample  
199 bootstrap stratified on dose group to attain parameter uncertainty in all parameters. A  
200 posterior predictive check was performed by comparing the median time-to-positivity  
201 calculated from 1000 simulated trials with the observed median time-to-positivity.

#### 202 *Clinical trial simulation of pharmacokinetics and time-to-positivity after 45 and 50 mg/kg* 203 *rifampicin*

204 The final semi-mechanistic time-to-event model was used for clinical trial simulation of time-  
205 to-positivity following 45 and 50 mg/kg rifampicin, respectively once daily for 7 days.  
206 Pharmacokinetics as the driver for increasing time-to-positivity were simulated for 45 and 50  
207 mg/kg and the day 7  $AUC_{0-24h}$  was calculated using linear-log trapezoidal rule using ncappc

208 0.2.1.1 within R [26] from 1000 simulated datasets using a pharmacokinetic model developed  
209 on the same patients used in this analysis [27]. For simulating pharmacokinetics, patient  
210 covariates were sampled from the observed population in a bootstrap procedure. The AUC<sub>0-</sub>  
211 <sub>24h</sub> from the 1000 simulated ~~trials~~ions were used to ~~simulate~~predict time-to-positivity after  
212 45 and 50 mg/kg. Baseline time-to-positivity for the simulations were sampled from a log-  
213 normal distribution centered around 4.34 days with a standard deviation of 0.32 days  
214 (estimated from the observed dataset). The same study design as for the HIGHRIF1 trial was  
215 used (i.e. 15 individuals/dose level) [4].

## 216 Results

### 217 *Patients and data*

218 In total, 83 patients and 1102 time-to-positivity measurements were ~~included in this~~  
219 ~~analysis~~analyzed. A few samples (5.2%) were excluded from the analysis; 52 samples were  
220 contaminated (various treatment days) and 8 samples were negative (all occurred before day 7  
221 and were followed by positive samples on later treatment days). Patient characteristics are  
222 summarized in Table 1.

### 223 *Semi-mechanistic time-to-event model*

224 The final semi-mechanistic time-to-event model included one mycobacterial subpopulation,  
225 both ~~in for~~ the sputum and ~~in the~~ mycobacterial growth models. The latter included a time-  
226 varying, exponentially declining  $k_G$ . Figure 1 shows the dynamics within each sub-model for  
227 a typical patient receiving 30 mg/kg. A statistically significant exposure-response relationship  
228 was identified, i.e. the parameter  $k_{kill}$  was found to increase linearly with increasing  $AUC_{0-24h}$   
229 (giving increased early bactericidal activity at higher rifampicin exposures). The baseline  
230 time-to-positivity was a significant covariate on the initial bacterial load in sputum, where a  
231 high time-to-positivity gave a low initial bacterial load. The final model did not include any  
232 random inter-individual or inter-occasion variability as it was not supported by the data.

233 Implementation of two mycobacterial subpopulations in sputum was statistically significant  
234 compared to having one subpopulation ( $dOFV=-37.5$ ,  $p<0.00001$ ). However, adding a two-  
235 subpopulation model to a model with time-varying  $k_G$  was not significant whereas a model  
236 with a one subpopulation model and an exponential decline of  $k_G$  ~~was were~~ significantly  
237 better than a model with only one subpopulation ( $dOFV=-106.1$ ,  $p<0.00001$ ).

238 The  $AUC_{0-24h}$  was found to significantly increase  $k_{kill}$  with a linear relationship ( $dOFV=-88.7$ ,  
239  $p<0.00001$ ). An  $E_{max}$  or a sigmoidal  $E_{max}$  model did not decrease OFV compared to the linear  
240 model. ~~The  $b_{Baseline}$  time-to-positivity was a significant covariate on the initial bacterial~~  
241 ~~load (i.e.  $B_{0,sputum}$ ) in the sputum model ( $dOFV=-357.0$ ,  $p<0.00001$ ) which was described~~  
242 using a power relationship.

### 243 *Model evaluation*

244 The final semi-mechanistic time-to-event model ~~was able to describe~~ the observed data well  
245 ~~which is shown in~~ according to a posterior predictive check (~~in~~ Figure 2). The predicted  
246 median time-to-positivity based on the final model (grey shaded area) agrees well with the  
247 observed median time-to-positivity (black lines) in all dose groups. This was also seen when  
248 performing a visual predictive check (Supplementary Figure 1). The parameter estimates and  
249 corresponding precision in estimated parameters is shown in Table 2. The parameter precision  
250 was overall low.

### 251 *Clinical trial simulation of pharmacokinetics and time-to-positivity after 45 and 50 mg/kg* 252 *rifampicin*

253 The predictions of early bactericidal activity after 45 and 50 mg/kg rifampicin are  
254 summarized as a 90% prediction interval (PI) for the median change from baseline time-to-  
255 positivity at day 7 in Figure 3 and Table 3. The observed median values are included to  
256 provide a point of reference. The median simulated day 7  $AUC_{0-24hs}$  (Table 3) displayed  
257 larger relative increases in  $AUC_{0-24h}$  than the relative increases in dose due to dose-dependent  
258 bioavailability and saturable elimination [4,27].

259 The final semi-mechanistic time-to-event model predicted a bacterial kill rate in sputum of  
260  $0.0608 \text{ days}^{-1}$  (90% confidence interval [CI]:  $0.0345-0.0882 \text{ days}^{-1}$ ) at  $42.8 \text{ h} \cdot \text{mg/L}$  (median  
261 predicted  $AUC_{0-24h}$  for 10 mg/kg) which is considerably lower than  $0.481 \text{ days}^{-1}$  (90% CI:

262 0.273-0.698 days<sup>-1</sup>) at 338.7 h·mg/L (median predicted AUC<sub>0-24h</sub> for 40 mg/kg). This  
263 corresponds to half-lives of bacterial elimination of 11.4 vs 1.44 days, respectively. In other  
264 words, 40 mg/kg gave 7.9 times faster kill than 10 mg/kg where the AUC<sub>0-24h</sub> was also 7.9  
265 times higher for 40 mg/kg. For 50 mg/kg, the model predicted a median AUC<sub>0-24h</sub> of 481  
266 h·mg/L which corresponds to a bacterial kill rate of 0.684 days<sup>-1</sup> (90% CI: 0.388-0.991 days<sup>-1</sup>;  
267 half-life of bacterial elimination=1.01 days).

268 The exposure predictions are summarized in more detail in Supplementary Figure 2.  
269 Supplementary Table 1 summarizes the simulated AUC<sub>0-24h</sub>s. Supplementary Figure 3  
270 summarizes the complete time course of the predicted median time-to-positivity.

## 271 Discussion

272 Our semi-mechanistic time-to-event model was developed to describe early bactericidal  
273 activity determined using time-to-positivity measurements from pulmonary tuberculosis  
274 patients treated with 10 to 40 mg/kg rifampicin. A statistically significant exposure-response  
275 relationship was detected between rifampicin and bacterial kill in sputum giving greater early  
276 bactericidal activity at higher exposures. Exposure-response was not detected using  
277 conventional statistics and a likely explanation for this is that in contrast to conventional  
278 statistics, we used pharmacokinetic-pharmacodynamic modeling which has been shown to be  
279 more powerful than conventional statistical methods and that we used a time-to-event  
280 approach which is reflective of time-to-positivity data. The final model described the  
281 observed data well and was used for clinical trial simulations in order to predict early  
282 bactericidal activity following 50 mg/kg rifampicin. The clinical trial simulations of 45 and 50  
283 mg/kg predict a further increase in early bactericidal activity compared with 40 mg/kg  
284 (highest observed dose). The predicted pharmacokinetic exposure ( $AUC_{0-24h}$ ) at day 7 which  
285 was the driver for increase in early bactericidal activity increased more than proportional  
286 compared to the increase in dose (Table 3) [27].

287 The predicted early bactericidal activity expressed as a day 7 median increase in time-to-  
288 positivity was 4.37-6.30 days (90% ~~prediction interval~~PI) for 40 mg/kg compared with 2.11-  
289 3.97 days for 10 mg/kg (Table 3). The early bactericidal activity for 40 mg/kg is clearly  
290 greater than for 10 mg/kg but this increase in early bactericidal activity (about two-fold) is  
291 smaller than the relative increase in predicted exposure between 10 and 40 mg/kg (almost 8-  
292 fold, Table 3). This may appear unexpectedly low since the final model includes a linear  
293 exposure-response relationship between exposure and bacterial kill in sputum which means  
294 that for example, an 8 times higher  $AUC_{0-24h}$  will give 8 times greater bacterial kill. However,

295 the bacterial kill in sputum does not have a linear relationship with day 7 increase in time-to-  
296 positivity.

297 In this work the bacterial load in sputum and liquid culture are reported as a probability per  
298 time unit (risk of sample turning positive per day) which are difficult to interpret. This is  
299 because the model was built only using time-to-positivity measurements. The MGIT manual  
300 states that the liquid culture container contains approximately  $10^5$ - $10^6$  colony forming  
301 units/ml when the system signals positive which may reflect the  $B_{\max}$  value.  $B_{\max}$  describes  
302 the maximal bacterial load in the liquid culture container. The numbers presented as risk per  
303 time bear no meaning as such but they can be viewed on a relative scale, i.e. looking at  
304 percentage change of bacterial load from the initial load.

305 The data supported a linear exposure-response relationship between rifampicin  $AUC_{0-24h}$  and  
306 bacterial kill in sputum. This implies that the model ~~will predict~~ an increased ~~in~~ time-to-  
307 positivity for any increase in  $AUC_{0-24h}$  and thus a dose where no further increase in early  
308 bactericidal activity is expected cannot be predicted from this work, and neither can any  
309 limitation arising from intolerability or adverse events with higher doses. The  $E_{\max}$  model (see  
310 e.g. page 1057 in [28]) can be used to predict maximal effect doses but was not supported ~~by~~  
311 ~~the data~~. When the  $E_{\max}$  model cannot be supported in favor of a linear model, ~~such as here~~, it  
312 may indicate that the data (or doses/exposures ~~contained in the dataset~~) do not cover the upper  
313 end of a sigmoidal exposure-response curve. Thus our results indicate that 40 mg/kg is located  
314 in the ascending part of the exposure-response curve which ~~is in agreement~~ agrees with with in  
315 vitro, in vivo and clinical studies [13,29]. Once 50 mg/kg data becomes available, the model  
316 can be updated to see if an  $E_{\max}$  model can be identified ~~with the expanded exposure range~~.  
317 The Our results ~~presented here~~ suggest ~~a large~~ overlapping ~~in the~~ distributions of individual  
318 predicted time-to-positivity between 45 and 50 mg/kg (Figure 3) which was also reflected in



319 the simulated pharmacokinetic exposures (Supplementary Figure 2). Given this large overlap  
320 in response (and exposure) it may be rational to include only 50 mg/kg in a future clinical  
321 trial. This exemplifies a strength of modeling and simulation and how it can be used to better  
322 design clinical trials.

323 The pharmacokinetic parameters of the non-linear relation of elimination with respect to dose  
324 were estimated with high precision [27]. As such, the predicted exposures at high doses are  
325 regarded as reliable.

326 Inter-individual variability or inter-occasion variability was not supported in the final model  
327 which may be because the variability was low or that the data was unable to support it.

328 However, variability is included through baseline time-to-positivity and exposure which were  
329 included as covariates in the final model.

330 ~~A similar model structure has been used in~~ other ~~time-to-event~~ models for time-to-  
331 positivity exist [18,19]. Chigutsa et al. [18] included two mycobacterial subpopulations  
332 whereas ~~in our analysis we found only model includes~~ one. In contrast to our study (83  
333 patients during one week), Chigutsa et al. performed a longer and larger trial (140 patients  
334 during 8 weeks) using standard drug combination. A biphasic pattern in the time-to-positivity  
335 data over time on treatment is probably necessary to support two mycobacterial  
336 subpopulations. Time-to-positivity data following rifampicin in monotherapy may have less  
337 biphasic pattern than the standard drug combination modelled in [18] which gave insufficient  
338 support for two mycobacterial subpopulations in our model. In order to detect biphasic killing  
339 the treatment must have pronounced killing of multiple mycobacterial sub-populations which  
340 may be case for the standard combination but not for rifampicin monotherapy. -The  
341 mycobacterial growth rate in the MGIT ~~was~~ decreaseding with time in our ~~final~~ model ~~which~~  
342 was also the case for the model presented by similar to Chigutsa et al. [18]. In the model by

343 Svensson and Karlsson [19] the growth rate ~~in the MGIT remained was~~ constant ~~over time~~.  
344 Despite ~~the use using of~~ time-to-positivity to develop the latter model, the bacterial load was  
345 predicted using the unit of bacteria per milliliter of sputum. This ~~contrastseonstrats\_ with~~ our  
346 model where the bacterial load is presented as a probability per time unit ~~as described above~~.  
347 It was possible by Svensson and Karlsson to predict bacterial load as bacteria/ml since many  
348 samples were negative in addition to a series of assumptions by the authors (explained in  
349 [19]). In ~~the current our~~ dataset, ~~only~~ 8 samples were negative, ~~which was~~ too few to use the  
350 ~~same~~ approach that Svensson and Karlsson used which would ~~have~~ allowed predicting  
351 bacterial load ~~in the more intuitive unit of as~~ bacteria/ml. ~~Thus, there are other models with~~  
352 ~~some differences. However, our model is the first time to event model for time to positivity~~  
353 ~~describing the short term response for rifampicin monotherapy.~~  
354 ~~More simplistic~~ Simpler models for time-to-positivity data use linear or bi-linear regression  
355 with time as the independent variable and ~~trat~~ treating time-to-positivity as a continuous  
356 variable including repeated measurements [16,30–32]. Different treatments, doses or  
357 exposures can ~~potentially~~ be explored as predictors for ~~the estimated~~ coefficients for ~~the~~  
358 increase in time-to-positivity. However, these simpler regression-based models ~~have been~~  
359 ~~shown to have lower power~~ less powerful than model-based pharmacokinetic-  
360 pharmacodynamic methods for finding exposure-response for tuberculosis ~~in general~~ [20].  
361 This is further supported by our semi-mechanistic time-to-event analysis which could  
362 demonstrate exposure-response but the conventional regression-based statistical analysis did  
363 not [4].

364 ~~The d~~ Day 7 AUC<sub>0-24h</sub> was ~~used as~~ a covariate for the rate of decline of bacterial load in  
365 sputum. Rifampicin ~~is known to have~~ has time-dependent pharmacokinetics (due to ~~an~~ auto-  
366 induction ~~phenomenon~~) and the AUC<sub>0-24h</sub> will gradually decrease from day 1 and onwards  
367 [27]. This was a potential source of bias ~~given that~~ since the extent and time-course of auto-

368 induction may differ between dose ~~es~~age groups. We have however ~~previously~~ shown (in the  
369 same patients) that auto-induction is similar between dose groups (extent and time-course)  
370 [27]. In this work we chose not to use the predicted  $AUC_{0-24h}$  on day 1 as this only shifts the  
371 value for the coefficient for the bacterial decline ( $k_{kill}$ ) without altering the predictions.

372 The non-compartmental analysis  $AUC_{0-24h}$  (i.e. non-model-based) was used here as a  
373 secondary summary variable for the pharmacokinetic exposure and input to the semi-  
374 mechanistic time-to-event model. ~~An alternative method would be to derive the~~ Alternatively,  
375 model-based  $AUC_{0-24h}$  ~~and could be used~~ as input, using the previously developed population  
376 pharmacokinetic model [27]. ~~In this case t~~ The non-compartmental analysis  $AUC_{0-24h}$  was  
377 however calculated from a full pharmacokinetic curve and with such rich sampling, non-  
378 compartmental analysis  $AUC_{0-24h}$  is expected to perform similar to model-based  $AUC_{0-24h}$   
379 ~~predicted from a population pharmacokinetic model.~~

380 The model was built on time-to-positivity data from patients treated for 7 days with daily  
381 rifampicin. We have used the model for extrapolation in terms of predicting early bactericidal  
382 activity for 7 days for an increased dose ~~of~~ (50 mg/kg), but we are unable to predict activity  
383 ~~for periods in excess of~~ longer than 7 days. While our findings are certainly not discouraging  
384 for the exploration of even higher doses, no association with sterilizing activity can be made.

385 This study defines a statistically significant short-term exposure-response relationship for up  
386 to 40 mg/kg rifampicin and shows that a further increase in early bactericidal activity can be  
387 expected beyond 40 mg/kg.

388 In conclusion, this study has established the exposure-response between rifampicin exposure  
389 from 10 to 40 mg/kg and increase in early bactericidal activity determined using time-to-  
390 positivity. These results give further weight to studying higher doses of rifampicin in longer  
391 and larger clinical trials. Model-based clinical trial simulations of pharmacokinetics and time-

392 to-positivity following 50 mg/kg rifampicin predict further increase in early bactericidal  
393 activity compared with 40 mg/kg.

394

395 **Conflict of interests statement**

396 All authors: No conflict

397 **Funding statement**

398 This work was supported by the Swedish Research Council [521-2011-3442 to R.J.S and  
399 U.S.H.S], the Innovative Medicines Initiative Joint Undertaking [n°115337]  
400 ([www.imi.europe.eu](http://www.imi.europe.eu), resources of which are composed of financial contribution from the  
401 European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies'  
402 in kind contribution), the European and Developing Countries Clinical Trials Partnership  
403 (EDCTP) [IP.2007.32011.011, IP.2007.32011.012, IP.2007.32011.013], the Netherlands-  
404 African Partnership for Capacity Development and Clinical Interventions Against Poverty-  
405 related Diseases (NACCAP) and the Bill and Melinda Gates Foundation.

406

407 **Acknowledgement**

408 The authors thank the patients and site staff for their participation in the underlying study.

409 **References**

- 410 1. Diacon AH, Patientia RF, Venter A, et al. Early bactericidal activity of high-dose  
411 rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears.  
412 *Antimicrob Agents Chemother.* **2007**; 51(8):2994–2996.
- 413 2. Ruslami R, Nijland HMJ, Alisjahbana B, Parwati I, Crevel R van, Aarnoutse RE.  
414 Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in  
415 pulmonary tuberculosis patients. *Antimicrob Agents Chemother.* **2007**; 51(7):2546–  
416 2551.
- 417 3. Ruslami R, Ganiem AR, Dian S, et al. Intensified regimen containing rifampicin and  
418 moxifloxacin for tuberculous meningitis: an open-label, randomised controlled phase 2  
419 trial. *Lancet Infect Dis.* **2013**; 13(1):27–35.
- 420 4. Boeree MJ, Diacon AH, Dawson R, et al. A dose-ranging trial to optimize the dose of  
421 rifampin in the treatment of tuberculosis. *Am J Respir Crit Care Med.* **2015**;  
422 191(9):1058–1065.
- 423 5. Heemskerck AD, Bang ND, Mai NTH, et al. Intensified Antituberculosis Therapy in  
424 Adults with Tuberculous Meningitis. *N Engl J Med.* **2016**; 374(2):124–134.
- 425 6. Milstein M, Lecca L, Peloquin C, et al. Evaluation of high-dose rifampin in patients with  
426 new, smear-positive tuberculosis (HIRIF): study protocol for a randomized controlled  
427 trial. *BMC Infect Dis.* **2016**; 16(1):453.
- 428 7. Yunivita V, Dian S, Ganiem AR, et al. Pharmacokinetics and safety/tolerability of  
429 higher oral and intravenous doses of rifampicin in adult tuberculous meningitis patients.  
430 *Int J Antimicrob Agents.* **2016**; 48(4):415–421.
- 431 8. Boeree MJ, Heinrich N, Aarnoutse R, et al. High-dose rifampicin, moxifloxacin, and  
432 SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial.  
433 *Lancet Infect Dis.* **2017**; 17(1):39–49.
- 434 9. Aarnoutse RE, Kibiki GS, Reither K, et al. Pharmacokinetics, tolerability and  
435 bacteriological response of 600, 900 and 1200 mg rifampicin daily in patients with  
436 pulmonary TB. *Antimicrob Agents Chemother.* **2017**; .
- 437 10. Peloquin C. What is the “right” dose of rifampin? *Int J Tuberc Lung Dis Off J Int Union*  
438 *Tuberc Lung Dis.* **2003**; 7(1):3–5.
- 439 11. Ingen J van, Aarnoutse RE, Donald PR, et al. Why Do We Use 600 mg of Rifampicin in  
440 Tuberculosis Treatment? *Clin Infect Dis Off Publ Infect Dis Soc Am.* **2011**; 52(9):e194-  
441 199.
- 442 12. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent *Mycobacterium*  
443 tuberculosis killing and prevention of resistance by rifampin. *Antimicrob Agents*  
444 *Chemother.* **2007**; 51(11):3781–3788.



- 445 13. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin  
446 in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother.* **2003**;  
447 47(7):2118–2124.
- 448 14. Rosenthal IM, Tasneen R, Peloquin CA, et al. Dose-ranging comparison of rifampin and  
449 rifapentine in two pathologically distinct murine models of tuberculosis. *Antimicrob*  
450 *Agents Chemother.* **2012**; 56(8):4331–4340.
- 451 15. Steenwinkel JEM de, Aarnoutse RE, Knecht GJ de, et al. Optimization of the rifampin  
452 dosage to improve the therapeutic efficacy in tuberculosis treatment using a murine  
453 model. *Am J Respir Crit Care Med.* **2013**; 187(10):1127–1134.
- 454 16. Diacon AH, Dawson R, Hanekom M, et al. Early bactericidal activity and  
455 pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrob Agents*  
456 *Chemother.* **2010**; 54(8):3402–3407.
- 457 17. Phillips PPJ, Mendel CM, Burger DA, et al. Limited role of culture conversion for  
458 decision-making in individual patient care and for advancing novel regimens to  
459 confirmatory clinical trials. *BMC Med.* **2016**; 14(1):19.
- 460 18. Chigutsa E, Patel K, Denti P, et al. A time-to-event pharmacodynamic model describing  
461 treatment response in patients with pulmonary tuberculosis using days to positivity in  
462 automated liquid mycobacterial culture. *Antimicrob Agents Chemother.* **2013**;  
463 57(2):789–795.
- 464 19. Svensson EM, Karlsson MO. Modelling of mycobacterial load reveals bedaquiline’s  
465 exposure–response relationship in patients with drug-resistant TB. *J Antimicrob*  
466 *Chemother* [Internet]. [cited 2017 Sep 21]; . Available from:  
467 [https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-](https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-mycobacterial-load-reveals)  
468 [mycobacterial-load-reveals](https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-mycobacterial-load-reveals)
- 469 20. Svensson RJ, Gillespie SH, Simonsson USH. Improved power for TB Phase IIa trials  
470 using a model-based pharmacokinetic–pharmacodynamic approach compared with  
471 commonly used analysis methods. *J Antimicrob Chemother.* **2017**; 72(8):2311–2319.
- 472 21. Beal SL, Sheiner LB, Boeckmann AJ, and Bauer RJ (eds) NONMEM 7.3.0 Users  
473 Guides. (1989-2013). ICON Development Solutions, Hanover, MD.
- 474 22. R Core Team (2015). R: A language and environment for statistical computing. R  
475 Foundation for Statistical Computing, Vienna, Austria.
- 476 23. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for  
477 NONMEM: Tutorial on Pirana, PsN, and Xpose. *CPT Pharmacomet Syst Pharmacol.*  
478 **2013**; 2:e50.
- 479 24. [<http://xpose.sourceforge.net/>] (accessed on 17/10/25).
- 480 25. [<https://uupharmacometrics.github.io/PsN/>] (accessed on 17/10/25).
- 481 26. Acharya C, Hooker AC, Türkyılmaz GY, Jönsson S, Karlsson MO. A diagnostic tool for  
482 population models using non-compartmental analysis: The ncappc package for R.  
483 *Comput Methods Programs Biomed.* **2016**; 127:83–93.

- 484 27. Svensson RJ, Aarnoutse RE, Diacon AH, et al. A population pharmacokinetic model  
485 incorporating saturable pharmacokinetics and auto-induction for high rifampicin doses.  
486 Clin Pharmacol Ther. **2018** ; 103(4):674-683.
- 487 28. Nielsen EI, Friberg LE. Pharmacokinetic-pharmacodynamic modeling of antibacterial  
488 drugs. Pharmacol Rev. **2013**; 65(3):1053–1090.
- 489 29. Svensson E, Svensson R, Brake L te, et al. The potential for treatment shortening with  
490 higher rifampicin doses: relating drug exposure to treatment response in patients with  
491 pulmonary tuberculosis. Clin Infect Dis. **2018** [cited 2018 Mar 29]; Available from:  
492 <https://doi.org/10.1093/cid/ciy026>
- 493 30. Sloan DJ, Mwandumba HC, Garton NJ, et al. Pharmacodynamic Modeling of Bacillary  
494 Elimination Rates and Detection of Bacterial Lipid Bodies in Sputum to Predict and  
495 Understand Outcomes in Treatment of Pulmonary Tuberculosis. Clin Infect Dis. **2015**;  
496 61(1):1–8.
- 497 31. Kayigire XA, Friedrich SO, Merwe L van der, Donald PR, Diacon AH. Simultaneous  
498 staining of sputum smears for acid-fast and lipid-containing Myobacterium tuberculosis  
499 can enhance the clinical evaluation of antituberculosis treatments. Tuberc Edinb Scotl.  
500 **2015**; 95(6):770–779.
- 501 32. Burger DA. Bayesian non-linear models for the bactericidal activity of tuberculosis  
502 drugs [Internet] [Thesis]. University of the Free State; 2015 [cited 2017 Oct 25].  
503 Available from: <http://scholar.ufs.ac.za:8080/xmlui/handle/11660/1169>

504

505

506 **Figure legends**

507 Figure 1 Dynamics of sputum model (a), mycobacterial growth model (b) and hazard model  
508 (c) for a typical patient receiving a dose of 30 mg/kg with  $AUC_{0-24h}$  of 298 h·mg/L and a  
509 baseline time-to-positivity of 4 days. Open squares shows the dynamics for an early (day 1)  
510 sample and open triangles show the dynamics for a late (day 7) sample.

511  $AUC_{0-24h}$ , rifampicin area under the plasma concentration-time curve during 24 hours;  $B_{sputum}$ ,  
512 predicted bacterial load in sputum;  $B_{culture}$ , predicted bacterial load in liquid culture

513 Figure 2 Posterior predictive check for median time-to-positivity in each observed dose group.  
514 Black dots connected with solid black lines are observed median time-to-positivity. Shaded  
515 areas are 90% prediction interval from 1000 simulated trials.

516 Figure 3 Model predictions of day 7 median change from baseline time-to-positivity for  
517 different doses of rifampicin monotherapy. Shaded area is 90% prediction interval based on  
518 1000 simulated trials. Observed day 7 median change from baseline time-to-positivity is  
519 shown as black dots connected by solid black lines (no model fit performed for this plot, the  
520 observed data is just overlaid the predictions).

521

522 **Table legends**

523 Table 1 Summary of baseline characteristics shown as median and range for continuous

524 variables and number and % for categorical variables

525 Table 2 Parameter estimates from the final semi-mechanistic time-to-event model

526 Table 3 Predicted median day 7 change from baseline time-to-positivity and  $AUC_{0-24h}$

527

528 **Supplementary appendix**

529 Supplementary Figure 1 Kaplan-Meier visual predictive check of final model. Solid black  
530 lines are the percent negative samples. Blue shaded areas are the 95% confidence interval of  
531 1000 simulations.

532 Supplementary Figure 2 Visual representation of the predicted and observed rifampicin non-  
533 compartmental analysis area under plasma concentration time curve for 24 hours ( $AUC_{0-24h}$ )  
534 for day 7 (left plot) and day 14 (right plot). Black dots with error bars are observed median  
535 and range of  $AUC_{0-24h}$  and red dots with error bars are predicted median and range of  $AUC_{0-}$   
536  $_{24h}$ . No model fitting was performed for the predictions, the observed data is just overlaid the  
537 predictions for reference. Red and blue shaded areas are the variability in the predicted  
538 median (red) and range (blue) shown as inter-quartile range from 1000 simulations.

539 Supplementary Figure 3 Predictions of median time-to-positivity for 10-50 mg/kg rifampicin.  
540 The shaded areas are the 90% prediction interval of the median time-to-positivity at each  
541 time-point from 1000 simulations. The black dots connected with solid black lines are the  
542 observed median time-to-positivity (no model fit performed for this plot, the observed data is  
543 just overlaid the predictions).

544 Supplementary Table 1 Simulated day 7  $AUC_{0-24h}$  values used for prediction of high dose  
545 rifampicin

546

1 **Title page**

2 Title: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and  
3 clinical trial simulations

4 Running title: Bactericidal activity of rifampicin

5 Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H  
6 Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J  
7 Boeree (6) and Ulrika SH Simonsson (1)

8 (1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, 751 24,  
9 Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud  
10 University Medical Center, Nijmegen, 6525 GA, the Netherlands, (3) TASK Foundation,  
11 Cape Town, 7500, South Africa, (4) Division of Pulmonology, Department of Medicine,  
12 University of Cape Town, 7925, and The University of Cape Town Lung Institute, Cape  
13 Town, 7700, South Africa, (5) The School of Medicine, University of St. Andrews, St.  
14 Andrews, KY16 9TF, UK, (6) Department of Lung Diseases, Radboud University Medical  
15 Center, Nijmegen, 6525 GA, the Netherlands and University Centre for Chronic Diseases  
16 Dekkerswald, Groesbeek, the Netherlands

17 Type of article: Major article

18 Word count of abstract: 184

19 Word count of text: 3499

20

21 **Foot note page**

22 **Conflict of interest statement**

23 All authors: No conflict

24 **Funding statement**

25 This work was supported by the Swedish Research Council [521-2011-3442 to R.J.S and  
26 U.S.H.S], the Innovative Medicines Initiative Joint Undertaking [n°115337]  
27 (www.imi.europe.eu, resources of which are composed of financial contribution from the  
28 European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies'  
29 in kind contribution), the European and Developing Countries Clinical Trials Partnership  
30 (EDCTP) [IP.2007.32011.011, IP.2007.32011.012, IP.2007.32011.013], the Netherlands-  
31 African Partnership for Capacity Development and Clinical Interventions Against Poverty-  
32 related Diseases (NACCAP) and the Bill and Melinda Gates Foundation.

33 **Meetings where part of the information has previously been presented:**

34 Part of the results has been presented in the form of an abstract at the 27<sup>th</sup> European Congress  
35 of Clinical Microbiology and Infectious Diseases, 22-25 April 2017 in Vienna, Austria  
36 (abstract P1673), at ASM Microbe, 1-5 June 2017 in New Orleans, Louisiana, USA (abstract  
37 1978) and at the 10<sup>th</sup> International Workshop on Pharmacology of Tuberculosis Drugs in  
38 Atlanta, Georgia, USA (abstract 10).

39 **Corresponding author:** Ulrika SH Simonsson (ulrika.simonsson@farmbio.uu.se)

40 Box 591, 751 24 Uppsala, Sweden

41 Telephone: +46-18-471 46 85

42 Fax: +46-18-471 42 53

43 **Abstract (max 200 words)**

44 **Background:** The current rifampicin dose (10 mg/kg) is sub-optimal for treating tuberculosis.  
45 The PanACEA HIGHRIF1 trial evaluated pharmacokinetics and early bactericidal activity  
46 with rifampicin doses up to 40 mg/kg. Conventional statistics revealed no significant  
47 exposure-response relationship. Our objective was to explore exposure-response for high dose  
48 rifampicin using pharmacokinetic-pharmacodynamic modeling and to predict early  
49 bactericidal activity of 50 mg/kg rifampicin.

50 **Methods:** Data included time-to-positivity of sputum in liquid culture from 83 tuberculosis  
51 patients treated with 10 (n=8), 20, 25, 30, 35 or 40 (n=15/group) mg/kg rifampicin for 7 days  
52 (clinicaltrials.gov, NCT01392911). We used a semi-mechanistic time-to-event approach to  
53 model the time-to-positivity data. Rifampicin exposure and baseline time-to-positivity were  
54 explored as covariates.

55 **Results:** Baseline time-to-positivity was a significant covariate on the predicted initial  
56 bacterial load and rifampicin exposure was significant on bacterial kill in sputum giving  
57 increased early bactericidal activity. The 90% prediction interval for the predicted median day  
58 7 increase in time-to-positivity for 50 mg/kg rifampicin was 7.25-10.3 days.

59 **Conclusions:** A significant exposure-response relationship was found between rifampicin  
60 exposure and early bactericidal activity. Clinical trial simulations showed greater early  
61 bactericidal activity for 50 mg/kg rifampicin.

62 **Key words (3-10):** Pharmacodynamics, Tuberculosis, Pharmacokinetics, Patients, Time-to-  
63 positivity, Early bactericidal activity, Models, Bactericidal effect, Mycobacterium  
64 tuberculosis



65 **Introduction**

66 Since the concept of increasing the rifampicin dose for treating tuberculosis was re-  
67 introduced, a battery of trials has been conducted to optimize treatment of pulmonary  
68 tuberculosis and tuberculous meningitis [1–9]. Despite this, a question remains: what is the  
69 optimal dose of rifampicin [10,11]? The answer remains unknown but recent clinical trials  
70 have provided important insight. PanACEA HIGHRIF1 [4] studied short-term safety,  
71 pharmacokinetics and anti-mycobacterial activity of up to 40 mg/kg rifampicin. No  
72 statistically significant relationship was determined between rifampicin exposure and early  
73 bactericidal activity [4] in humans despite several lines of evidence derived from in vitro and  
74 animal experiments suggesting a clear relationship between exposure and mycobacterial  
75 killing [12–15].

76 Early bactericidal activity can be quantified using time-to-positivity in liquid culture which is  
77 defined as the time from start of incubation of a sputum specimen in a liquid culture system  
78 until a positive signal is detected. A high bacterial load is expected to lead to short time-to-  
79 positivity and vice versa.

80 Time-to-positivity reflects time-to-event data. For conventional statistics, early bactericidal  
81 activity determined using time-to-positivity is usually analyzed in the context of a series of  
82 data points from daily sputum cultures as the change in time-to-positivity per day of treatment  
83 by regression-based methods [16,17]. Two semi-mechanistic pharmacokinetic-  
84 pharmacodynamic models exist treating time-to-positivity as time-to-event data [18,19].

85 Model-based pharmacokinetic-pharmacodynamic analysis has been shown to be more  
86 powerful for defining exposure-response than conventional statistical methods [20]. Semi-  
87 mechanistic pharmacokinetic-pharmacodynamic models also allow for extrapolation by

88 simulating new scenarios such as predicting early bactericidal activity of higher than observed  
89 doses which can be used to design future clinical trials.

90 Our objective was to apply a semi-mechanistic time-to-event approach to explore the  
91 exposure-response for early bactericidal activity determined using time-to-positivity in  
92 pulmonary tuberculosis patients treated with high dose rifampicin (up to 40 mg/kg) and then,  
93 to simulate early bactericidal activity of 45 and 50 mg/kg rifampicin in order to inform the  
94 clinical development process of optimizing a higher rifampicin dose.

95

96 **Methods**

97 *Ethics*

98 The study was approved by local ethical review boards and by the Medicines Control Council  
99 of South Africa and was conducted according to Good Clinical Practice. All patients provided  
100 written informed consent before enrollment in the study.

101 *Patient data*

102 Modeling was performed on one week repeated time-to-positivity data measured from sputum  
103 in patients recruited in HIGHRIF1, a prospective open-label multiple dose-rising trial  
104 registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT01392911 [4]. Smear-positive pulmonary  
105 tuberculosis patients were treated with either 10 (n=8, reference arm), 20, 25, 30, 35 or 40  
106 (n=15/arm) mg/kg daily rifampicin as monotherapy for 7 days. The actual study duration was  
107 14 days with isoniazid, pyrazinamide and ethambutol in standard doses added to the high dose  
108 rifampicin for days 8-14. In this analysis, only data until day 7 was used to define the  
109 exposure-response relationship for rifampicin alone. Overnight sputum sampling was  
110 performed on two consecutive days at baseline and daily for 7 days. Time-to-positivity was  
111 determined in duplicate from each sample using a standardized liquid culture BD BACTEC™  
112 MGIT™ mycobacterial growth indicator tube system (MGIT 960, Becton-Dickinson, Sparks,  
113 MD) in a single laboratory. HIGHRIF1 is described in detail in the relevant reference [4].

114 *Data analysis*

115 Time-to-positivity data were analyzed with a time-to-event approach using the non-linear  
116 mixed effects modeling software NONMEM 7.3 [21] with the Laplacian estimation method.  
117 Data handling and visualization were done in R version 3.4.3 [22]. Model diagnostics were  
118 performed in Xpose 4.6.0 [23,24], in particular visual predictive checks using PsN 4.6.12

119 [23,25]. Models were compared based on the objective function value (OFV) using the  
120 likelihood ratio test at the 5% significance level.

121 Time-to-positivity replicates at each time point were analyzed without averaging. Time-to-  
122 positivity at baseline was included in the model as a covariate (see below).

### 123 *Structural model*

124 The starting point for model development was a previously developed semi-mechanistic time-  
125 to-event model for time-to-positivity [19]. Briefly, the model structure was derived from  
126 underlying knowledge about (i) how the amount of viable tuberculosis bacteria changes in  
127 human sputum over time, referred to as *sputum model*, (ii) how tuberculosis bacteria are  
128 known to grow in a liquid culture, referred to as *mycobacterial growth model*, and (iii) how  
129 the mycobacterial growth relates to the probability of achieving a positive signal event in the  
130 MGIT, referred to as *hazard model*. The starting model included drug effect without an  
131 exposure-response relationship.

132 Sputum models with one and two mycobacterial subpopulations were tested. The bacterial  
133 load in the single mycobacterial subpopulation model was described by:

$$134 \quad B(t_t)_{sputum} = B_{0,sputum} \times e^{-k_{kill} \times t_t}$$

135 where  $B_{0,sputum}$  is the predicted bacterial load at start of treatment,  $k_{kill}$  is the first-order  
136 rifampicin bacterial kill rate and  $t_t$  is the time after start of treatment. For the two-  
137 subpopulation model the bacterial load of the first (B1) and second (B2) mycobacterial  
138 subpopulations were described by:

$$139 \quad B1(t_t)_{sputum} = B1_{0,sputum} \times e^{-k_{kill,1} \times t_t}$$

$$140 \quad B2(t_t)_{sputum} = B2_{0,sputum} \times e^{-k_{kill,2} \times t_t}$$

141 where

$$142 \quad B(t_t)_{sputum} = B1(t_t)_{sputum} + B2(t_t)_{sputum}$$

143 where  $B1_{0,sputum}$  and  $B2_{0,sputum}$  are the predicted bacterial load at start of treatment for the  
144 subpopulations, respectively. Parameters  $k_{kill,1}$  and  $k_{kill,2}$  describe the first-order rifampicin  
145 bacterial kill rates of the subpopulations, respectively.

146 For the mycobacterial growth model in the liquid culture container, a logistic growth model  
147 was used where the change of bacteria in the liquid culture ( $B_{culture}$ ) over time was described  
148 by:

$$149 \quad \frac{dB_{culture}}{dt_c} = k_G \times (B_{max} - B(t_c)_{culture}) \times B_{culture}$$

150 where the initial bacterial load in the liquid culture was assumed equal to the number of  
151 bacteria in sputum at the time-point of sputum sampling according to:

$$152 \quad B(t_c = 0)_{culture} = B(t_t = \text{sampling time point})_{sputum}$$

153 where  $k_G$  is a predicted maximal mycobacterial growth rate in the liquid,  $B_{max}$  is the maximal  
154 bacterial load in the liquid culture and  $t_c$  is time after inoculation of the liquid culture.

155 Models with two subpopulations in the liquid culture with different growth rates for each  
156 subpopulation both with and without a possible transfer between subpopulations were  
157 explored. A lag-time for start of growth in the liquid culture was explored as a single lag time  
158 for both the one and two subpopulation models. Time-dependencies in  $k_G$  were explored  
159 including linearly decreasing  $k_G$  with time on treatment and an exponential decline from a  
160 baseline value of  $k_G$  ( $k_{G,base}$ ) to a steady state value of  $k_G$  ( $k_{G,ss}$ ) according to:

$$161 \quad k(t_t)_G = k_{G,base} + (k_{G,ss} - k_{G,base}) \times (1 - e^{-k_{G,k} \times t_t})$$

162 where  $k_{G,k}$  is the first-order time-dependent decrease of  $k_{G,base}$ .

163 For the hazard model the bacterial load in the liquid culture at any given time-point was equal  
164 to the hazard,  $h(t_c)$ , for the liquid culture to turn into a positive signal described by:

$$165 \quad h(t_c) = B(t_c)_{culture}$$

166 which was used in a next step to calculate the cumulative hazard according to:

$$167 \quad H(t_c) = \int_0^{t_c} h(t_c) dt$$

168 This finally allowed calculation of the survival, which is the probability of a sample without a  
169 positive signal at time  $t_c$  using the following equation:

$$170 \quad S(t_c) = e^{-H(t_c)}$$

#### 171 *Covariate model*

172 The individual mean time-to-positivity at baseline was not included in the estimation but was  
173 evaluated as a covariate on the predicted bacterial load at start of treatment ( $B_{0,sputum}$  or  
174  $B_{1,0,sputum}$  and  $B_{2,0,sputum}$ ) as a power-relationship. The area under the plasma concentration-  
175 time curve between 0 and 24 hours ( $AUC_{0-24h}$ ) at day 7 was evaluated as a covariate on the  
176 rifampicin kill rate parameters ( $k_{kill}$  or  $k_{kill,1}$  and  $k_{kill,2}$ ).  $AUC_{0-24h}$  was chosen over  $C_{max}$   
177 because AUC is normally used in PKPD analyses for rifampicin.  $C_{max}$  and  $AUC_{0-24h}$  are  
178 probably highly correlated and would therefore perform similarly when explored in a PKPD  
179 model. Since only once daily dosing was included in the current study design it would  
180 probably be difficult to definitely distinguish between  $C_{max}$  and  $AUC_{0-24h}$ . The  $AUC_{0-24h}$  was  
181 calculated for each subject from concentration measurements at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12  
182 and 24 hours using the linear-log trapezoidal rule in Winnonlin version 5.3 (Pharsight Corp.,

183 Mountain View, CA) as described in [4]. Concentrations were measured using validated ultra  
184 performance liquid chromatography (accuracy<4%, limit of quantification= 0.13 mg/L).

#### 185 *Stochastic model*

186 Inter-individual variability was investigated in all parameters for the sputum model as well as  
187 for the estimated lag-time for the growth in the mycobacterial growth model. Inter-occasion  
188 variability was investigated in the sputum sampling implemented as a random variability  
189 between occasions for the bacterial load inoculated in the mycobacterial growth model [19].

#### 190 *Model evaluation*

191 The final semi-mechanistic time-to-event model (i.e. the chosen model after structural,  
192 covariate and stochastic model evaluations) was evaluated by performing a 1000 sample  
193 bootstrap stratified on dose group to attain parameter uncertainty. A posterior predictive check  
194 was performed by comparing the median time-to-positivity calculated from 1000 simulated  
195 trials with the observed median time-to-positivity.

#### 196 *Clinical trial simulation of pharmacokinetics and time-to-positivity after 45 and 50 mg/kg* 197 *rifampicin*

198 The final semi-mechanistic time-to-event model was used for clinical trial simulation of time-  
199 to-positivity following 45 and 50 mg/kg rifampicin, respectively once daily for 7 days.  
200 Pharmacokinetics as the driver for increasing time-to-positivity were simulated for 45 and 50  
201 mg/kg and the day 7  $AUC_{0-24h}$  was calculated using linear-log trapezoidal rule using ncappc  
202 0.2.1.1 within R [26] from 1000 simulated datasets using a pharmacokinetic model developed  
203 on the same patients used in this analysis [27]. For simulating pharmacokinetics, patient  
204 covariates were sampled from the observed population in a bootstrap procedure. The  $AUC_{0-}$   
205  $_{24h}$  from the 1000 simulations were used to predict time-to-positivity after 45 and 50 mg/kg.

206 Baseline time-to-positivity for the simulations were sampled from a log-normal distribution  
207 centered around 4.34 days with a standard deviation of 0.32 days (estimated from the  
208 observed dataset). The same study design as for the HIGHRIF1 trial was used (i.e. 15  
209 individuals/dose level) [4].



## 210 **Results**

### 211 *Patients and data*

212 In total, 83 patients and 1102 time-to-positivity measurements were analyzed. A few samples  
213 (5.2%) were excluded from the analysis; 52 samples were contaminated (various treatment  
214 days) and 8 samples were negative (all occurred before day 7 and were followed by positive  
215 samples on later treatment days). Patient characteristics are summarized in Table 1.

### 216 *Semi-mechanistic time-to-event model*

217 The final semi-mechanistic time-to-event model included one mycobacterial subpopulation,  
218 both for the sputum and mycobacterial growth models. The latter included a time-varying,  
219 exponentially declining  $k_G$ . Figure 1 shows the dynamics within each sub-model for a typical  
220 patient receiving 30 mg/kg. A statistically significant exposure-response relationship was  
221 identified, i.e. the parameter  $k_{kill}$  was found to increase linearly with increasing  $AUC_{0-24h}$   
222 (giving increased early bactericidal activity at higher rifampicin exposures). The baseline  
223 time-to-positivity was a significant covariate on the initial bacterial load in sputum, where a  
224 high time-to-positivity gave a low initial bacterial load. The final model did not include any  
225 random inter-individual or inter-occasion variability as it was not supported by the data.

226 Implementation of two mycobacterial subpopulations in sputum was statistically significant  
227 compared to having one subpopulation (dOFV=-37.5,  $p<0.00001$ ). However, adding a two-  
228 subpopulation model to a model with time-varying  $k_G$  was not significant whereas a model  
229 with a one subpopulation model and an exponential decline of  $k_G$  was significantly better than  
230 a model with only one subpopulation (dOFV=-106.1,  $p<0.00001$ ).

231 The  $AUC_{0-24h}$  was found to significantly increase  $k_{kill}$  with a linear relationship (dOFV=-88.7,  
232  $p<0.00001$ ). An  $E_{max}$  or a sigmoidal  $E_{max}$  model did not decrease OFV compared to the linear

233 model. Baseline time-to-positivity was a significant covariate on the initial bacterial load (  
234  $dOFV=-357.0$ ,  $p<0.00001$ ) which was described using a power relationship.

### 235 *Model evaluation*

236 The final semi-mechanistic time-to-event model described the observed data well according to  
237 a posterior predictive check (Figure 2). The predicted median time-to-positivity based on the  
238 final model (grey shaded area) agrees well with the observed median time-to-positivity (black  
239 lines) in all dose groups. This was also seen when performing a visual predictive check  
240 (Supplementary Figure 1). The parameter estimates and corresponding precision is shown in  
241 Table 2. The parameter precision was overall low.

### 242 *Clinical trial simulation of pharmacokinetics and time-to-positivity after 45 and 50 mg/kg* 243 *rifampicin*

244 The predictions of early bactericidal activity after 45 and 50 mg/kg rifampicin are  
245 summarized as a 90% prediction interval (PI) for the median change from baseline time-to-  
246 positivity at day 7 in Figure 3 and Table 3. The observed median values are included to  
247 provide a point of reference. The median simulated day 7  $AUC_{0-24h}$  (Table 3) displayed  
248 larger relative increases in  $AUC_{0-24h}$  than the relative increases in dose due to dose-dependent  
249 bioavailability and saturable elimination [4,27].

250 The final semi-mechanistic time-to-event model predicted a bacterial kill rate in sputum of  
251  $0.0608 \text{ days}^{-1}$  (90% confidence interval [CI]:  $0.0345-0.0882 \text{ days}^{-1}$ ) at  $42.8 \text{ h}\cdot\text{mg/L}$  (median  
252 predicted  $AUC_{0-24h}$  for 10 mg/kg) which is considerably lower than  $0.481 \text{ days}^{-1}$  (90% CI:  
253  $0.273-0.698 \text{ days}^{-1}$ ) at  $338.7 \text{ h}\cdot\text{mg/L}$  (median predicted  $AUC_{0-24h}$  for 40 mg/kg). This  
254 corresponds to half-lives of bacterial elimination of 11.4 vs 1.44 days, respectively. In other  
255 words, 40 mg/kg gave 7.9 times faster kill than 10 mg/kg where the  $AUC_{0-24h}$  was also 7.9  
256 times higher for 40 mg/kg. For 50 mg/kg, the model predicted a median  $AUC_{0-24h}$  of 481

257 h·mg/L which corresponds to a bacterial kill rate of 0.684 days<sup>-1</sup> (90% CI: 0.388-0.991 days<sup>-1</sup>;  
258 half-life of bacterial elimination=1.01 days).

259 The exposure predictions are summarized in more detail in Supplementary Figure 2.

260 Supplementary Table 1 summarizes the simulated AUC<sub>0-24h</sub>. Supplementary Figure 3

261 summarizes the complete time course of the predicted median time-to-positivity.

## 262 **Discussion**

263 Our semi-mechanistic time-to-event model was developed to describe early bactericidal  
264 activity determined using time-to-positivity measurements from pulmonary tuberculosis  
265 patients treated with 10 to 40 mg/kg rifampicin. A statistically significant exposure-response  
266 relationship was detected between rifampicin and bacterial kill in sputum giving greater early  
267 bactericidal activity at higher exposures. Exposure-response was not detected using  
268 conventional statistics and a likely explanation for this is that in contrast to conventional  
269 statistics, we used pharmacokinetic-pharmacodynamic modeling which has been shown to be  
270 more powerful than conventional statistical methods and that we used a time-to-event  
271 approach which is reflective of time-to-positivity data. The final model described the  
272 observed data well and was used for clinical trial simulations in order to predict early  
273 bactericidal activity following 50 mg/kg rifampicin. The clinical trial simulations of 45 and 50  
274 mg/kg predict a further increase in early bactericidal activity compared with 40 mg/kg  
275 (highest observed dose). The predicted pharmacokinetic exposure ( $AUC_{0-24h}$ ) at day 7 which  
276 was the driver for increase in early bactericidal activity increased more than proportional  
277 compared to the increase in dose (Table 3) [27].

278 The predicted early bactericidal activity expressed as a day 7 median increase in time-to-  
279 positivity was 4.37-6.30 days (90% PI) for 40 mg/kg compared with 2.11-3.97 days for 10  
280 mg/kg (Table 3). The early bactericidal activity for 40 mg/kg is clearly greater than for 10  
281 mg/kg but this increase in early bactericidal activity (about two-fold) is smaller than the  
282 relative increase in predicted exposure between 10 and 40 mg/kg (almost 8-fold, Table 3).  
283 This may appear unexpectedly low since the final model includes a linear exposure-response  
284 relationship between exposure and bacterial kill in sputum which means that for example, an  
285 8 times higher  $AUC_{0-24h}$  will give 8 times greater bacterial kill. However, the bacterial kill in  
286 sputum does not have a linear relationship with day 7 increase in time-to-positivity.

287 In this work the bacterial load in sputum and liquid culture are reported as a probability per  
288 time unit (risk of sample turning positive per day) which are difficult to interpret. This is  
289 because the model was built only using time-to-positivity measurements. The MGIT manual  
290 states that the liquid culture container contains approximately  $10^5$ - $10^6$  colony forming  
291 units/ml when the system signals positive which may reflect the  $B_{\max}$  value.  $B_{\max}$  describes  
292 the maximal bacterial load in the liquid culture container. The numbers presented as risk per  
293 time bear no meaning as such but they can be viewed on a relative scale, i.e. looking at  
294 percentage change of bacterial load from the initial load.

295 The data supported a linear exposure-response relationship between rifampicin  $AUC_{0-24h}$  and  
296 bacterial kill in sputum. This implies that the model predicts increased time-to-positivity for  
297 any increase in  $AUC_{0-24h}$  and thus a dose where no further increase in early bactericidal  
298 activity is expected cannot be predicted from this work, and neither can any limitation arising  
299 from intolerability or adverse events with higher doses. The  $E_{\max}$  model (see e.g. page 1057 in  
300 [28]) can be used to predict maximal effect doses but was not supported. When the  $E_{\max}$   
301 model cannot be supported in favor of a linear model it may indicate that the data (or  
302 doses/exposures) do not cover the upper end of a sigmoidal exposure-response curve. Thus  
303 our results indicate that 40 mg/kg is located in the ascending part of the exposure-response  
304 curve which agrees with with in vitro, in vivo and clinical studies [13,29]. Once 50 mg/kg  
305 data becomes available, the model can be updated to see if an  $E_{\max}$  model can be identified.

306 Our results suggest overlapping distributions of individual predicted time-to-positivity  
307 between 45 and 50 mg/kg (Figure 3) which was also reflected in the simulated  
308 pharmacokinetic exposures (Supplementary Figure 2). Given this large overlap in response  
309 (and exposure) it may be rational to include only 50 mg/kg in a future clinical trial. This  
310 exemplifies a strength of modeling and simulation and how it can be used to better design  
311 clinical trials.

312 The pharmacokinetic parameters of the non-linear relation of elimination with respect to dose  
313 were estimated with high precision [27]. As such, the predicted exposures at high doses are  
314 regarded as reliable.

315 Inter-individual variability or inter-occasion variability was not supported in the final model  
316 which may be because the variability was low or that the data was unable to support it.  
317 However, variability is included through baseline time-to-positivity and exposure which were  
318 included as covariates in the final model. Similar model structures for other models for time-  
319 to-positivity exist [18,19]. Chigutsa et al. [18] included two mycobacterial subpopulations  
320 whereas our model includes one. In contrast to our study (83 patients during one week),  
321 Chigutsa et al. performed a longer and larger trial (140 patients during 8 weeks) using  
322 standard drug combination. A biphasic pattern in the time-to-positivity data over time on  
323 treatment is probably necessary to support two mycobacterial subpopulations. Time-to-  
324 positivity data following rifampicin in monotherapy may have less biphasic pattern than the  
325 standard drug combination modelled in [18] which gave insufficient support for two  
326 mycobacterial subpopulations in our model. In order to detect biphasic killing the treatment  
327 must have pronounced killing of multiple mycobacterial sub-populations which may be case  
328 for the standard combination but not for rifampicin monotherapy. The mycobacterial growth  
329 rate in the MGIT decreased with time in our model similar to Chigutsa et al. [18]. In the  
330 model by Svensson and Karlsson [19] the growth rate was constant. Despite using time-to-  
331 positivity to develop the latter model, the bacterial load was predicted using the unit of  
332 bacteria per milliliter of sputum. This contrasts our model where the bacterial load is  
333 presented as a probability per time unit. It was possible by Svensson and Karlsson to predict  
334 bacterial load as bacteria/ml since many samples were negative in addition to a series of  
335 assumptions by the authors (explained in [19]). In our dataset, 8 samples were negative, too

336 few to use the approach that Svensson and Karlsson used which would allow predicting  
337 bacterial load as bacteria/ml.

338 Simpler models for time-to-positivity data use linear or bi-linear regression with time as the  
339 independent variable and treat time-to-positivity as a continuous variable including repeated  
340 measurements [16,30–32]. Different treatments, doses or exposures can be explored as  
341 predictors for coefficients for increase in time-to-positivity. However, these simpler  
342 regression-based models less powerful than model-based pharmacokinetic-pharmacodynamic  
343 methods for finding exposure-response for tuberculosis [20]. This is further supported by our  
344 semi-mechanistic time-to-event analysis which could demonstrate exposure-response but the  
345 conventional regression-based statistical analysis did not [4].

346 Day 7  $AUC_{0-24h}$  was a covariate for the rate of decline of bacterial load in sputum. Rifampicin  
347 has time-dependent pharmacokinetics (due to auto-induction) and the  $AUC_{0-24h}$  will gradually  
348 decrease from day 1 and onwards [27]. This was a potential source of bias since the extent  
349 and time-course of auto-induction may differ between doses. We have however shown (in the  
350 same patients) that auto-induction is similar between dose groups (extent and time-course)  
351 [27]. In this work we chose not to use the predicted  $AUC_{0-24h}$  on day 1 as this only shifts the  
352 value for the coefficient for the bacterial decline ( $k_{kill}$ ) without altering the predictions.

353 The non-compartmental analysis  $AUC_{0-24h}$  (i.e. non-model-based) was used here as a  
354 secondary summary variable for the pharmacokinetic exposure and input to the semi-  
355 mechanistic time-to-event model. Alternatively, model-based  $AUC_{0-24h}$  could be used as  
356 input, using the previously developed population pharmacokinetic model [27]. The non-  
357 compartmental analysis  $AUC_{0-24h}$  was however calculated from a full pharmacokinetic curve  
358 and with such rich sampling, non-compartmental analysis  $AUC_{0-24h}$  is expected to perform  
359 similar to model-based  $AUC_{0-24h}$ .

360 The model was built on time-to-positivity data from patients treated for 7 days with daily  
361 rifampicin. We have used the model for extrapolation in terms of predicting early bactericidal  
362 activity for 7 days for an increased dose (50 mg/kg), but we are unable to predict activity  
363 longer than 7 days. While our findings are certainly not discouraging for the exploration of  
364 even higher doses, no association with sterilizing activity can be made. This study defines a  
365 statistically significant short-term exposure-response relationship for up to 40 mg/kg  
366 rifampicin and shows that a further increase in early bactericidal activity can be expected  
367 beyond 40 mg/kg.

368 In conclusion, this study has established the exposure-response between rifampicin exposure  
369 from 10 to 40 mg/kg and increase in early bactericidal activity determined using time-to-  
370 positivity. These results give further weight to studying higher doses of rifampicin in longer  
371 and larger clinical trials. Model-based clinical trial simulations of pharmacokinetics and time-  
372 to-positivity following 50 mg/kg rifampicin predict further increase in early bactericidal  
373 activity compared with 40 mg/kg.

374



375 **Conflict of interests statement**

376 All authors: No conflict

377 **Funding statement**

378 This work was supported by the Swedish Research Council [521-2011-3442 to R.J.S and  
379 U.S.H.S], the Innovative Medicines Initiative Joint Undertaking [n°115337]  
380 (www.imi.europe.eu, resources of which are composed of financial contribution from the  
381 European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies'  
382 in kind contribution), the European and Developing Countries Clinical Trials Partnership  
383 (EDCTP) [IP.2007.32011.011, IP.2007.32011.012, IP.2007.32011.013], the Netherlands-  
384 African Partnership for Capacity Development and Clinical Interventions Against Poverty-  
385 related Diseases (NACCAP) and the Bill and Melinda Gates Foundation.

386

387 **Acknowledgement**

388 The authors thank the patients and site staff for their participation in the underlying study.

389 **References**

- 390 1. Diacon AH, Patientia RF, Venter A, et al. Early bactericidal activity of high-dose  
391 rifampin in patients with pulmonary tuberculosis evidenced by positive sputum smears.  
392 *Antimicrob Agents Chemother.* **2007**; 51(8):2994–2996.
- 393 2. Ruslami R, Nijland HMJ, Alisjahbana B, Parwati I, Crevel R van, Aarnoutse RE.  
394 Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in  
395 pulmonary tuberculosis patients. *Antimicrob Agents Chemother.* **2007**; 51(7):2546–  
396 2551.
- 397 3. Ruslami R, Ganiem AR, Dian S, et al. Intensified regimen containing rifampicin and  
398 moxifloxacin for tuberculous meningitis: an open-label, randomised controlled phase 2  
399 trial. *Lancet Infect Dis.* **2013**; 13(1):27–35.
- 400 4. Boeree MJ, Diacon AH, Dawson R, et al. A dose-ranging trial to optimize the dose of  
401 rifampin in the treatment of tuberculosis. *Am J Respir Crit Care Med.* **2015**;  
402 191(9):1058–1065.
- 403 5. Heemskerck AD, Bang ND, Mai NTH, et al. Intensified Antituberculosis Therapy in  
404 Adults with Tuberculous Meningitis. *N Engl J Med.* **2016**; 374(2):124–134.
- 405 6. Milstein M, Lecca L, Peloquin C, et al. Evaluation of high-dose rifampin in patients with  
406 new, smear-positive tuberculosis (HIRIF): study protocol for a randomized controlled  
407 trial. *BMC Infect Dis.* **2016**; 16(1):453.
- 408 7. Yunivita V, Dian S, Ganiem AR, et al. Pharmacokinetics and safety/tolerability of  
409 higher oral and intravenous doses of rifampicin in adult tuberculous meningitis patients.  
410 *Int J Antimicrob Agents.* **2016**; 48(4):415–421.
- 411 8. Boeree MJ, Heinrich N, Aarnoutse R, et al. High-dose rifampicin, moxifloxacin, and  
412 SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial.  
413 *Lancet Infect Dis.* **2017**; 17(1):39–49.
- 414 9. Aarnoutse RE, Kibiki GS, Reither K, et al. Pharmacokinetics, tolerability and  
415 bacteriological response of 600, 900 and 1200 mg rifampicin daily in patients with  
416 pulmonary TB. *Antimicrob Agents Chemother.* **2017**; .
- 417 10. Peloquin C. What is the “right” dose of rifampin? *Int J Tuberc Lung Dis Off J Int Union*  
418 *Tuberc Lung Dis.* **2003**; 7(1):3–5.
- 419 11. Ingen J van, Aarnoutse RE, Donald PR, et al. Why Do We Use 600 mg of Rifampicin in  
420 Tuberculosis Treatment? *Clin Infect Dis Off Publ Infect Dis Soc Am.* **2011**; 52(9):e194-  
421 199.
- 422 12. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent *Mycobacterium*  
423 tuberculosis killing and prevention of resistance by rifampin. *Antimicrob Agents*  
424 *Chemother.* **2007**; 51(11):3781–3788.

- 425 13. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin  
426 in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother.* **2003**;  
427 47(7):2118–2124.
- 428 14. Rosenthal IM, Tasneen R, Peloquin CA, et al. Dose-ranging comparison of rifampin and  
429 rifapentine in two pathologically distinct murine models of tuberculosis. *Antimicrob*  
430 *Agents Chemother.* **2012**; 56(8):4331–4340.
- 431 15. Steenwinkel JEM de, Aarnoutse RE, Knegt GJ de, et al. Optimization of the rifampin  
432 dosage to improve the therapeutic efficacy in tuberculosis treatment using a murine  
433 model. *Am J Respir Crit Care Med.* **2013**; 187(10):1127–1134.
- 434 16. Diacon AH, Dawson R, Hanekom M, et al. Early bactericidal activity and  
435 pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrob Agents*  
436 *Chemother.* **2010**; 54(8):3402–3407.
- 437 17. Phillips PPJ, Mendel CM, Burger DA, et al. Limited role of culture conversion for  
438 decision-making in individual patient care and for advancing novel regimens to  
439 confirmatory clinical trials. *BMC Med.* **2016**; 14(1):19.
- 440 18. Chigutsa E, Patel K, Denti P, et al. A time-to-event pharmacodynamic model describing  
441 treatment response in patients with pulmonary tuberculosis using days to positivity in  
442 automated liquid mycobacterial culture. *Antimicrob Agents Chemother.* **2013**;  
443 57(2):789–795.
- 444 19. Svensson EM, Karlsson MO. Modelling of mycobacterial load reveals bedaquiline’s  
445 exposure–response relationship in patients with drug-resistant TB. *J Antimicrob*  
446 *Chemother* [Internet]. [cited 2017 Sep 21]; . Available from:  
447 [https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-](https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-mycobacterial-load-reveals)  
448 [mycobacterial-load-reveals](https://academic.oup.com/jac/article/doi/10.1093/jac/dkx317/4157900/Modelling-of-mycobacterial-load-reveals)
- 449 20. Svensson RJ, Gillespie SH, Simonsson USH. Improved power for TB Phase IIa trials  
450 using a model-based pharmacokinetic–pharmacodynamic approach compared with  
451 commonly used analysis methods. *J Antimicrob Chemother.* **2017**; 72(8):2311–2319.
- 452 21. Beal SL, Sheiner LB, Boeckmann AJ, and Bauer RJ (eds) NONMEM 7.3.0 Users  
453 Guides. (1989-2013). ICON Development Solutions, Hanover, MD.
- 454 22. R Core Team (2015). R: A language and environment for statistical computing. R  
455 Foundation for Statistical Computing, Vienna, Austria.
- 456 23. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for  
457 NONMEM: Tutorial on Pirana, PsN, and Xpose. *CPT Pharmacomet Syst Pharmacol.*  
458 **2013**; 2:e50.
- 459 24. [<http://xpose.sourceforge.net/>] (accessed on 17/10/25).
- 460 25. [<https://uupharmacometrics.github.io/PsN/>] (accessed on 17/10/25).
- 461 26. Acharya C, Hooker AC, Türkyılmaz GY, Jönsson S, Karlsson MO. A diagnostic tool for  
462 population models using non-compartmental analysis: The ncappc package for R.  
463 *Comput Methods Programs Biomed.* **2016**; 127:83–93.

- 464 27. Svensson RJ, Aarnoutse RE, Diacon AH, et al. A population pharmacokinetic model  
465 incorporating saturable pharmacokinetics and auto-induction for high rifampicin doses.  
466 Clin Pharmacol Ther. **2018** ; 103(4):674-683.
- 467 28. Nielsen EI, Friberg LE. Pharmacokinetic-pharmacodynamic modeling of antibacterial  
468 drugs. Pharmacol Rev. **2013**; 65(3):1053–1090.
- 469 29. Svensson E, Svensson R, Brake L te, et al. The potential for treatment shortening with  
470 higher rifampicin doses: relating drug exposure to treatment response in patients with  
471 pulmonary tuberculosis. Clin Infect Dis. **2018** [cited 2018 Mar 29]; Available from:  
472 <https://doi.org/10.1093/cid/ciy026>
- 473 30. Sloan DJ, Mwandumba HC, Garton NJ, et al. Pharmacodynamic Modeling of Bacillary  
474 Elimination Rates and Detection of Bacterial Lipid Bodies in Sputum to Predict and  
475 Understand Outcomes in Treatment of Pulmonary Tuberculosis. Clin Infect Dis. **2015**;  
476 61(1):1–8.
- 477 31. Kayigire XA, Friedrich SO, Merwe L van der, Donald PR, Diacon AH. Simultaneous  
478 staining of sputum smears for acid-fast and lipid-containing Myobacterium tuberculosis  
479 can enhance the clinical evaluation of antituberculosis treatments. Tuberc Edinb Scotl.  
480 **2015**; 95(6):770–779.
- 481 32. Burger DA. Bayesian non-linear models for the bactericidal activity of tuberculosis  
482 drugs [Internet] [Thesis]. University of the Free State; 2015 [cited 2017 Oct 25].  
483 Available from: <http://scholar.ufs.ac.za:8080/xmlui/handle/11660/1169>

484

485

486 **Figure legends**

487 Figure 1 Dynamics of sputum model (a), mycobacterial growth model (b) and hazard model  
488 (c) for a typical patient receiving a dose of 30 mg/kg with  $AUC_{0-24h}$  of 298 h·mg/L and a  
489 baseline time-to-positivity of 4 days. Open squares shows the dynamics for an early (day 1)  
490 sample and open triangles show the dynamics for a late (day 7) sample.

491  $AUC_{0-24h}$ , rifampicin area under the plasma concentration-time curve during 24 hours;  $B_{sputum}$ ,  
492 predicted bacterial load in sputum;  $B_{culture}$ , predicted bacterial load in liquid culture

493 Figure 2 Posterior predictive check for median time-to-positivity in each observed dose group.  
494 Black dots connected with solid black lines are observed median time-to-positivity. Shaded  
495 areas are 90% prediction interval from 1000 simulated trials.

496 Figure 3 Model predictions of day 7 median change from baseline time-to-positivity for  
497 different doses of rifampicin monotherapy. Shaded area is 90% prediction interval based on  
498 1000 simulated trials. Observed day 7 median change from baseline time-to-positivity is  
499 shown as black dots connected by solid black lines (no model fit performed for this plot, the  
500 observed data is just overlaid the predictions).

501

502 **Table legends**

503 Table 1 Summary of baseline characteristics shown as median and range for continuous  
504 variables and number and % for categorical variables

505 Table 2 Parameter estimates from the final semi-mechanistic time-to-event model

506 Table 3 Predicted median day 7 change from baseline time-to-positivity and  $AUC_{0-24h}$

507



508 **Supplementary appendix**

509 Supplementary Figure 1 Kaplan-Meier visual predictive check of final model. Solid black  
510 lines are the percent negative samples. Blue shaded areas are the 95% confidence interval of  
511 1000 simulations.

512 Supplementary Figure 2 Visual representation of the predicted and observed rifampicin non-  
513 compartmental analysis area under plasma concentration time curve for 24 hours ( $AUC_{0-24h}$ )  
514 for day 7 (left plot) and day 14 (right plot). Black dots with error bars are observed median  
515 and range of  $AUC_{0-24h}$  and red dots with error bars are predicted median and range of  $AUC_{0-}$   
516  $_{24h}$ . No model fitting was performed for the predictions, the observed data is just overlaid the  
517 predictions for reference. Red and blue shaded areas are the variability in the predicted  
518 median (red) and range (blue) shown as inter-quartile range from 1000 simulations.

519 Supplementary Figure 3 Predictions of median time-to-positivity for 10-50 mg/kg rifampicin.  
520 The shaded areas are the 90% prediction interval of the median time-to-positivity at each  
521 time-point from 1000 simulations. The black dots connected with solid black lines are the  
522 observed median time-to-positivity (no model fit performed for this plot, the observed data is  
523 just overlaid the predictions).

524 Supplementary Table 1 Simulated day 7  $AUC_{0-24h}$  values used for prediction of high dose  
525 rifampicin

526

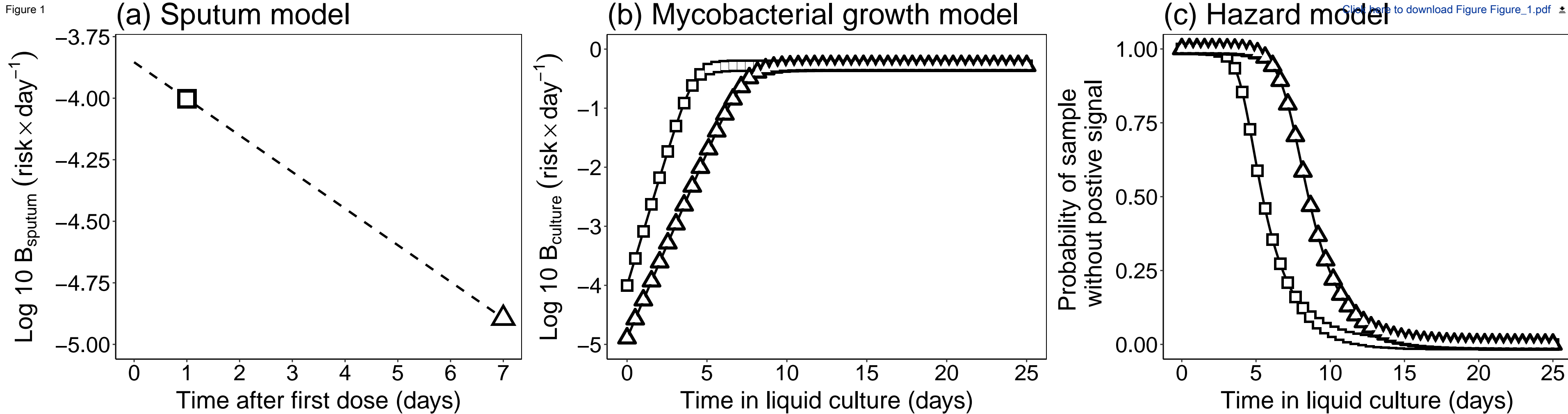


Figure 2

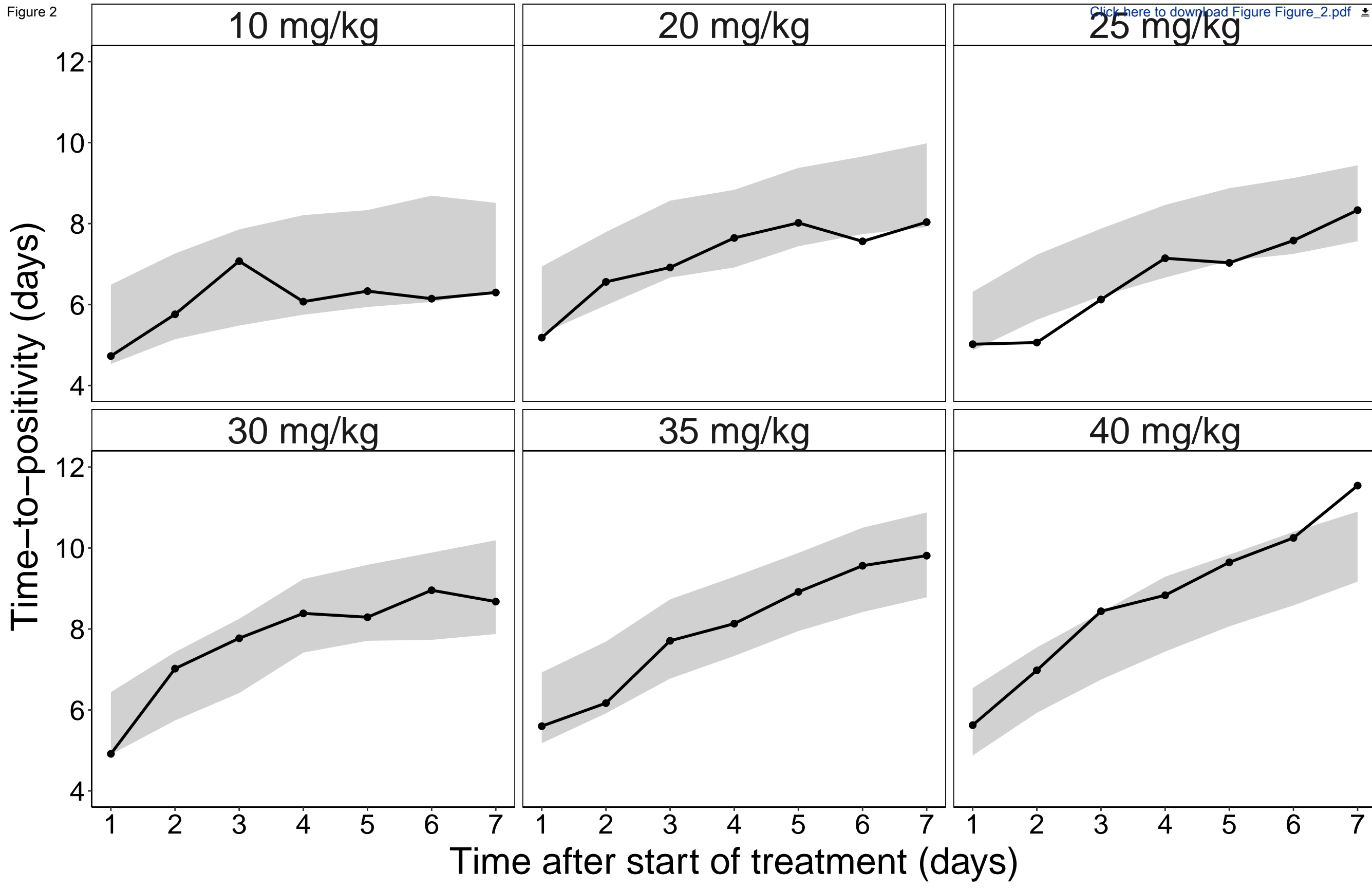


Figure 3

[Click here to download Figure Figure\\_3.pdf](#)

Change from baseline  
Time-to-positivity (days)

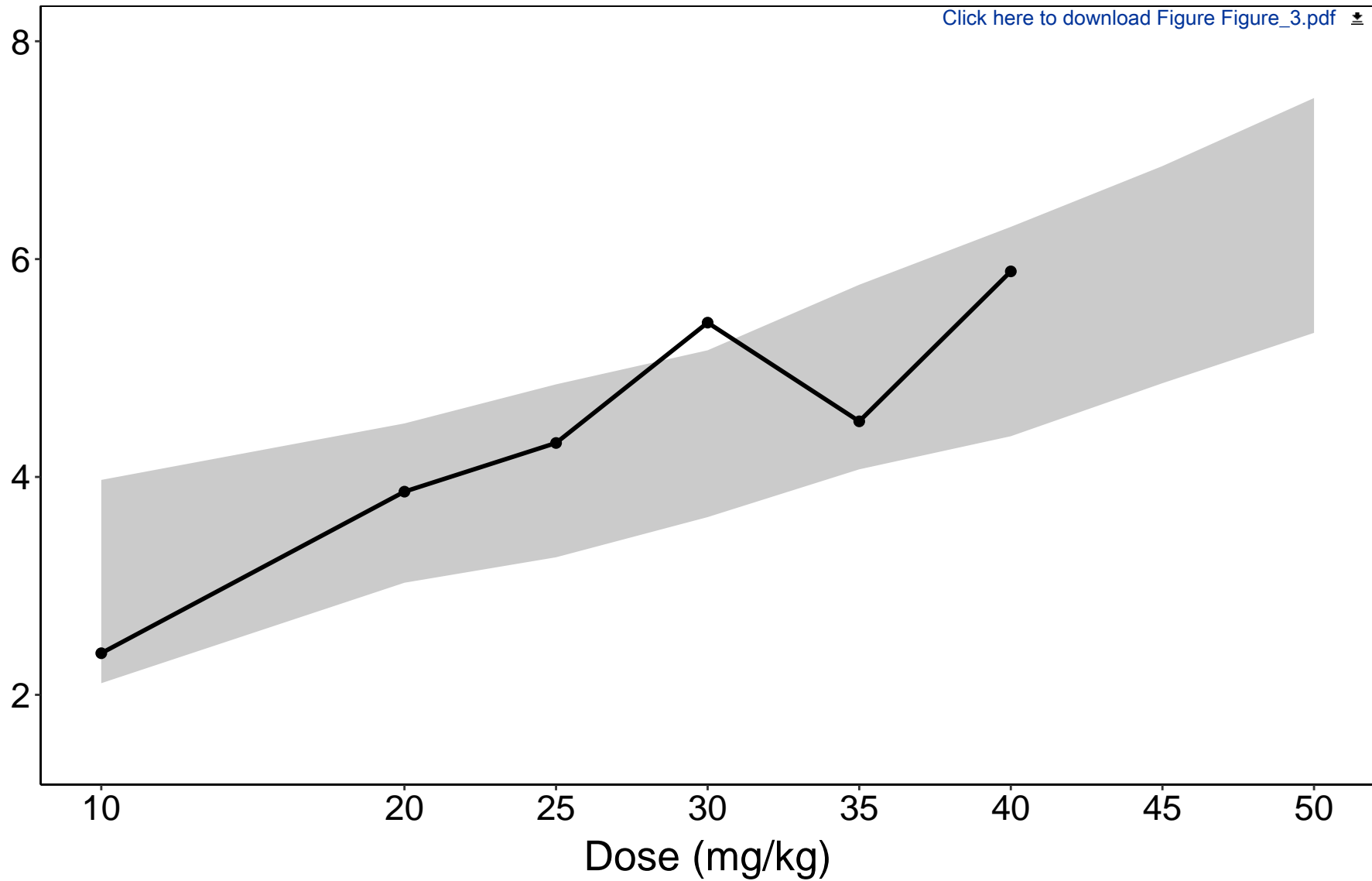


Table 1 Summary of baseline patient characteristics shown as median and range for continuous variables and number and % for categorical variables

<b>Parameter</b>	<b>All</b>	<b>10 mg/kg</b>	<b>20 mg/kg</b>	<b>25 mg/kg</b>	<b>30 mg/kg</b>	<b>35 mg/kg</b>	<b>40 mg/kg</b>
N	83	8	15	15	15	15	15
Weight (kg)	53.9 (40.2-84.2)	56.9 (46.8-64.9)	52.6 (41.8-62.7)	52.8 (40.2-67.9)	54.0 (45.7-84.2)	57.0 (40.5-74.0)	58.9 (46.7-64.8)
Age (years)	31.0 (18.0-59.0)	27.5 (19.0-49.0)	27.0 (18.0-46.0)	25.0 (19.0-46.0)	40.0 (19.0-59.0)	37.0 (21.0-59.0)	34.0 (23.0-58.0)
Body mass index (kg/m <sup>2</sup> )	19.4 (14.7-30.9)	20.5 (15.8-26.3)	18.6 (16.8-26.2)	19.3 (15.1-25.2)	20.9 (16.4-30.9)	19.4 (14.7-25.2)	19.4 (17.2-19.4)
Patients with male sex	59 (71.1)	6 (75.0)	11 (73.3)	10 (66.7)	11 (73.3)	10 (66.7)	11 (73.3)
Patients with black race*	38 (45.8)	3 (37.5)	7 (46.7)	4 (26.4)	9 (60.0)	5 (33.3)	10 (66.7)
Patients with colored race*	45 (54.2)	5 (62.5)	8 (53.3)	11 (73.3)	6 (40.0)	10 (66.7)	5 (33.3)

Patients with HIV- infection (%)	3 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)	1 (6.7)	0 (0.0)
Baseline time-to- positivity (days)	4.0 (2.2-18.2)	4.0 (3.3-5.2)	4.9 (3.0-9.1)	4.0 (3.4-6.5)	4.0 (2.9-6.3)	3.9 (2.6-18.2)	4.0 (2.2-7.6)
Day 7 AUC <sub>0-24h</sub> (h·mg/L)	241 (34-847)	43 (34-53)	155 (96-221)	178 (134-380)	298 (177-781)	321 (145-555)	357 (201-847)

---

\*“Colored race” is a population group genetically descended from South-East Asia, whereas “black race” refers to African natives.

Abbreviations: AUC<sub>0-24h</sub>, area under the plasma concentration-time curve during 24 hours

Table 2 Parameter estimates from the final semi-mechanistic time-to-event model

	Parameter	Description	Estimate	90% confidence interval <sup>2</sup>
<b>Sputum model</b>	$B_{0,culture}$ (risk·day <sup>-1</sup> ) <sup>3</sup>	Baseline bacterial load	$1.40 \cdot 10^{-4}$	$1.67 \cdot 10^{-5} - 8.78 \cdot 10^{-4}$
	$\Theta_{TTP}$	Effect of baseline time-to-positivity on $B_0$	-7.13	-9.30 - -5.18
	$k_{kill}$ (L·h <sup>-1</sup> ·mg <sup>-1</sup> ·day <sup>-1</sup> )	First-order rifampicin bacterial kill rate	$1.42 \cdot 10^{-3}$	$8.06 \cdot 10^{-4} - 2.06 \cdot 10^{-3}$
<b>Mycobacterial growth model</b>	$k_{G,base}$ (day <sup>-1</sup> )	Baseline mycobacterial growth rate	4.90	3.18 - 6.52
	$k_{G,ss}$ (day <sup>-1</sup> )	Steady state mycobacterial growth rate	2.74	1.57 - 3.78
	$k_{G,k}$ (day <sup>-1</sup> )	Rate constant for decrease of mycobacterial growth rate	0.580	0.387 - 0.870
	$B_{max}$ (risk·day <sup>-1</sup> ) <sup>3</sup>	Maximal bacterial load in liquid container	0.523	0.459 - 0.665

<sup>1</sup>Mathematical structure for the final model:

$$B(t)_{sputum} = B_{0,sputum} \times \left( \frac{TTP_{baseline}}{median(TTP_{baseline})} \right)^{\Theta_{TTP}} \times e^{-k_{kill} \times AUC_{0-24h} \times t} \text{ (sputum model)}$$

$$\frac{dB_{culture}}{dt_c} = \left( k_{G,base} + (k_{G,ss} - k_{G,base}) \times (1 - e^{-k_{G,k} \times t_c}) \right) \times (B_{max} - B(t_c)_{culture}) \times$$

$B_{culture}$  (mycobacterial growth model)

$h(t_c) = B(t_c)_{culture}$  (hazard model)

<sup>2</sup>Obtained from a 1000 sample non-parametric bootstrap, <sup>3</sup>Risk refers to risk of a positive signal from the liquid culture system



Table 3 Predicted median day 7 change from baseline time-to-positivity and AUC<sub>0-24h</sub>

Dose (mg/kg)	Median day 7 change from baseline time-to-positivity (days)		Median day 7 AUC <sub>0-24h</sub> (h·mg/L)	
	Observed	Predicted*	Predicted	% increase in AUC <sub>0-24h</sub> from 10 mg/kg
10	2.38	2.11 - 3.97	42.8	-
20	3.87	3.03 - 4.49	139.2	225.2
25	4.31	3.26 - 4.85	172.7	303.5
30	5.42	3.63 - 5.16	226.3	429.4
35	4.51	4.07 - 5.76	286.2	568.7
40	5.89	4.37 - 6.30	338.7	691.4
45	-	4.86 - 6.85	413.2	865.4
50	-	5.32 - 7.48	481.4	1024.8

\*90% prediction interval based on 1000 simulated datasets

Abbreviations: AUC<sub>0-24h</sub>, area under the plasma concentration-time curve during 24 hours

## Supplementary Figure 1

Supplement to: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and clinical trial simulations

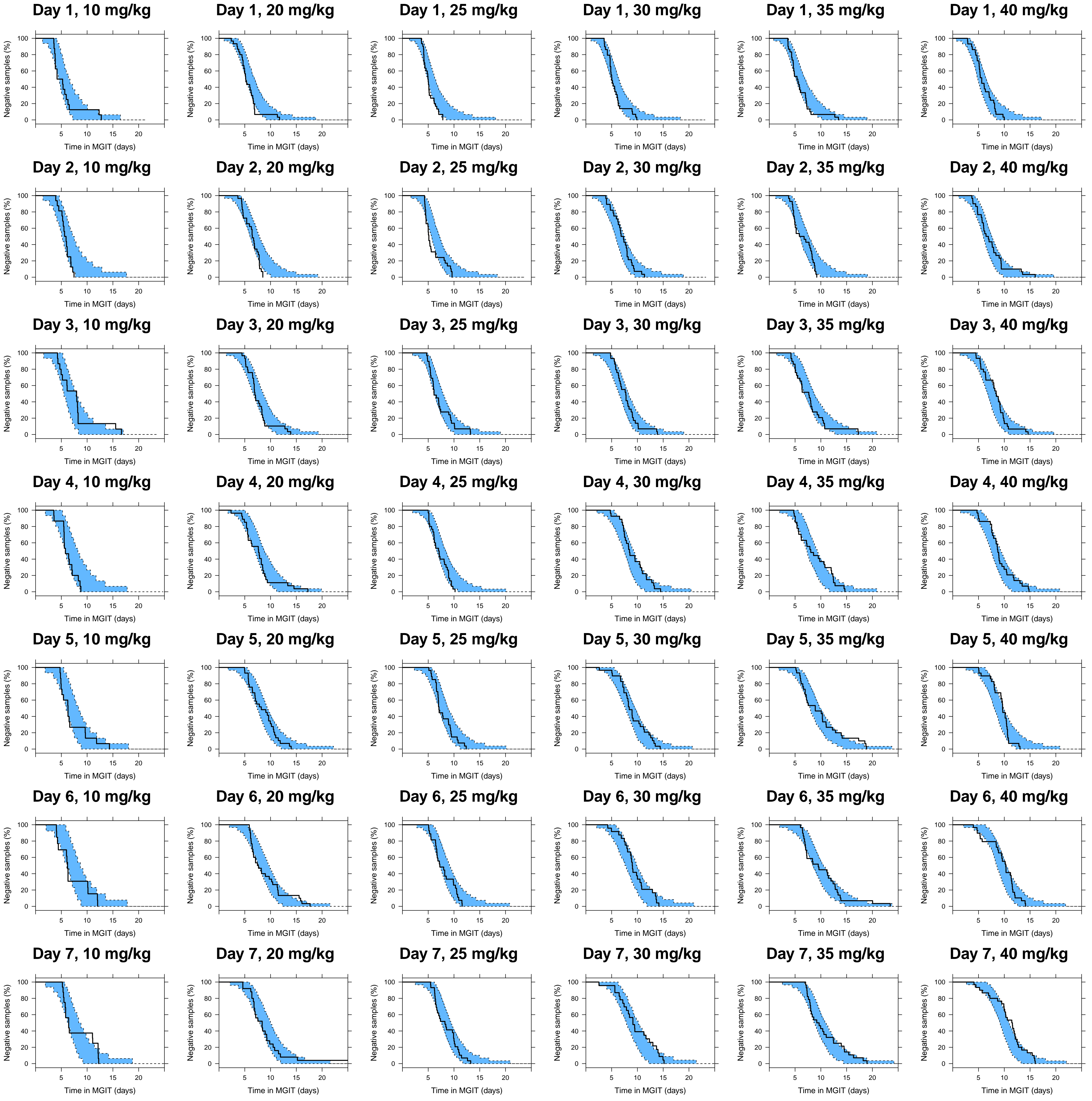
Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J Boeree (6) and Ulrika SH Simonsson (1)

(1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands, (3) TASK Foundation, Cape Town, South Africa, (4) Division of Pulmonology, Department of Medicine, University of Cape Town, and The University of Cape Town Lung Institute, Cape Town, South Africa, (5) The School of Medicine, University of St. Andrews, St. Andrews, UK, (6) Department of Lung Diseases, Radboud University Medical Center, Nijmegen, the Netherlands and University Centre for Chronic Diseases Dekkerswald, Groesbeek, the Netherlands

**Corresponding author:** Ulrika SH Simonsson ([ulrika.simonsson@farmbio.uu.se](mailto:ulrika.simonsson@farmbio.uu.se))

### Figure legend

Supplementary Figure 1 Kaplan-Meier visual predictive check of final model. Solid black lines are the percent negative samples. Blue shaded areas are the 95% confidence interval of 1000 simulations.



### Supplementary Figure 3

Supplement to: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and clinical trial simulations

Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J Boeree (6) and Ulrika SH Simonsson (1)

(1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands, (3) TASK Foundation, Cape Town, South Africa, (4) Division of Pulmonology, Department of Medicine, University of Cape Town, and The University of Cape Town Lung Institute, Cape Town, South Africa, (5) The School of Medicine, University of St. Andrews, St. Andrews, UK, (6) Department of Lung Diseases, Radboud University Medical Center, Nijmegen, the Netherlands and University Centre for Chronic Diseases Dekkerswald, Groesbeek, the Netherlands

**Corresponding author:** Ulrika SH Simonsson ([ulrika.simonsson@farmbio.uu.se](mailto:ulrika.simonsson@farmbio.uu.se))

### Figure legend

Supplementary Figure 3 Predictions of median time-to-positivity for 10-50 mg/kg rifampicin. The shaded areas are the 90% prediction interval of the median time-to-positivity at each time-point from 1000 simulations. The black dots connected with solid black lines are the observed median time-to-positivity (no model fit performed for this plot, the observed data is just overlaid the predictions).



## Supplementary Figure 2

Supplement to: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and clinical trial simulations

Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J Boeree (6) and Ulrika SH Simonsson (1)

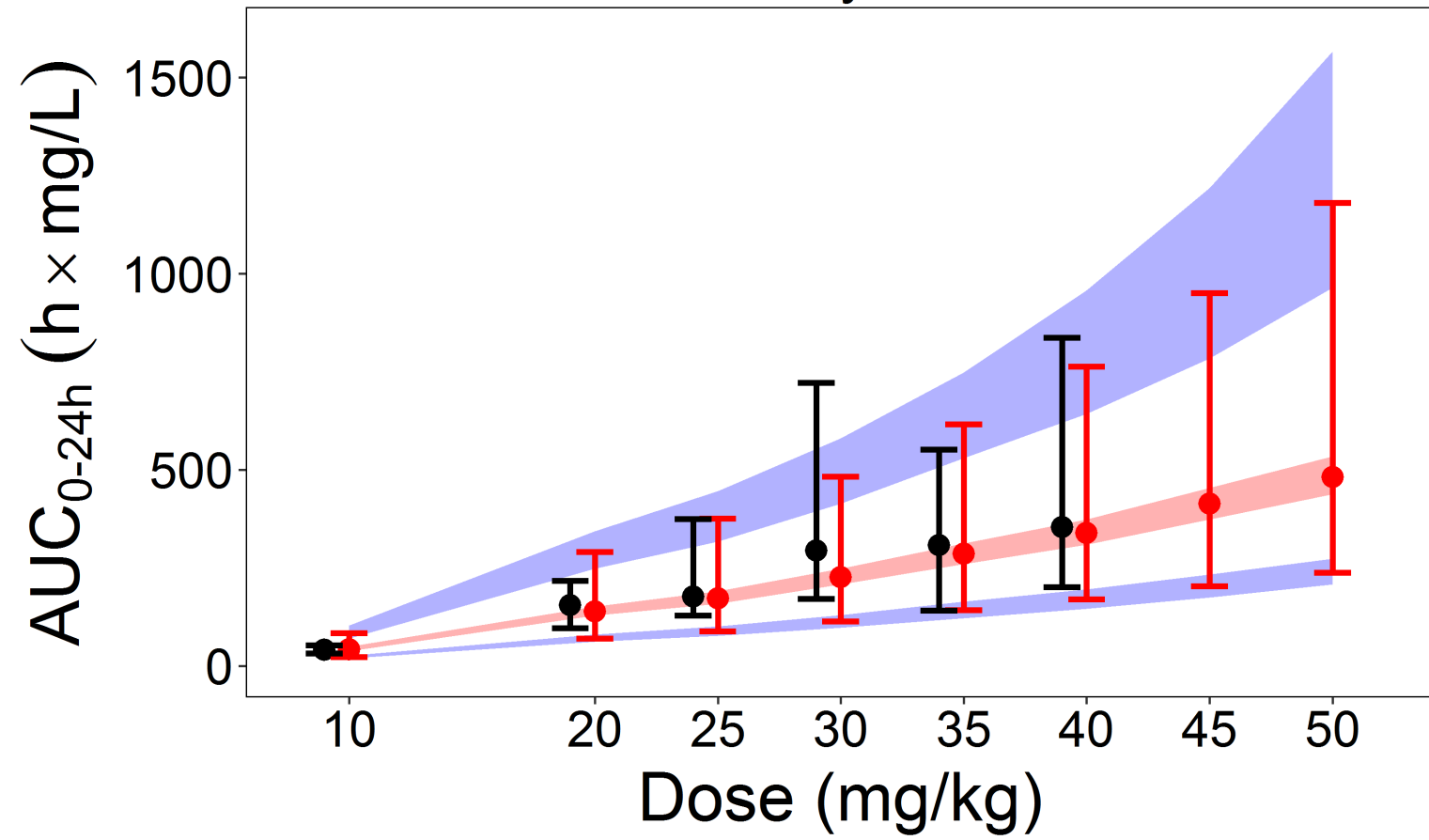
(1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands, (3) TASK Foundation, Cape Town, South Africa, (4) Division of Pulmonology, Department of Medicine, University of Cape Town, and The University of Cape Town Lung Institute, Cape Town, South Africa, (5) The School of Medicine, University of St. Andrews, St. Andrews, UK, (6) Department of Lung Diseases, Radboud University Medical Center, Nijmegen, the Netherlands and University Centre for Chronic Diseases Dekkerswald, Groesbeek, the Netherlands

**Corresponding author:** Ulrika SH Simonsson ([ulrika.simonsson@farmbio.uu.se](mailto:ulrika.simonsson@farmbio.uu.se))

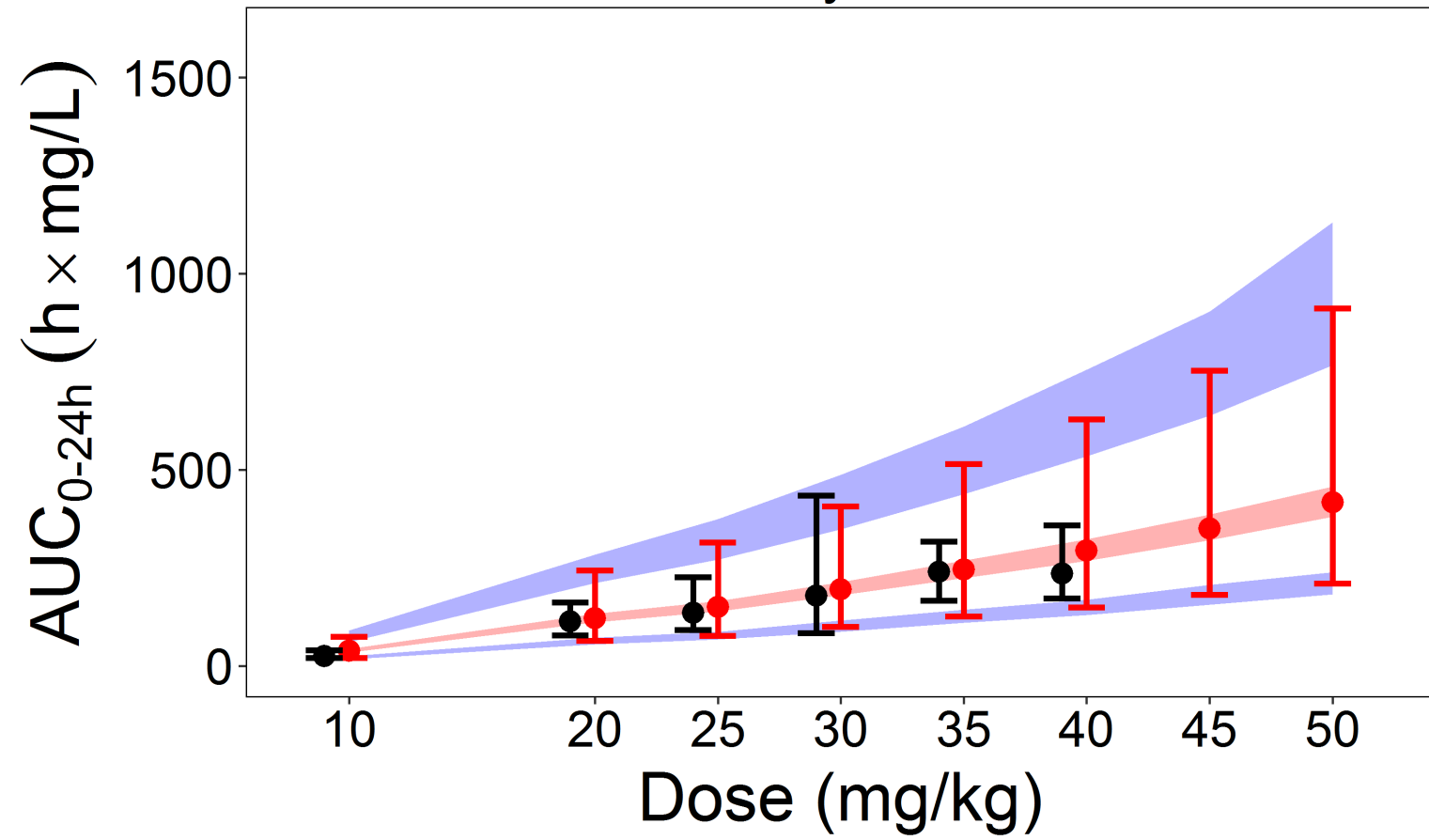
### Figure legend

Supplementary Figure 2 Visual representation of the predicted and observed rifampicin non-compartmental analysis area under plasma concentration time curve for 24 hours ( $AUC_{0-24h}$ ) for day 7 (left plot) and day 14 (right plot). Black dots with error bars are observed median and range of  $AUC_{0-24h}$  and red dots with error bars are predicted median and range of  $AUC_{0-24h}$ . No model fitting was performed for the predictions, the observed data is just overlaid the predictions for reference. Red and blue shaded areas are the variability in the predicted median (red) and range (blue) shown as inter-quartile range from 1000 simulations.

Day 7



Day 14



## Supplementary Table 1

Supplement to: Greater early bactericidal activity at higher rifampicin doses revealed by modeling and clinical trial simulations

Authors: Robin J Svensson (1), Elin M Svensson (1,2), Rob E Aarnoutse (2), Andreas H Diacon (3), Rodney Dawson (4), Stephen H Gillespie (5), Mischka Moodley (3), Martin J Boeree (6) and Ulrika SH Simonsson (1)

(1) Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, (2) Department of Pharmacy, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands, (3) TASK Foundation, Cape Town, South Africa, (4) Division of Pulmonology, Department of Medicine, University of Cape Town, and The University of Cape Town Lung Institute, Cape Town, South Africa, (5) The School of Medicine, University of St. Andrews, St. Andrews, UK, (6) Department of Lung Diseases, Radboud University Medical Center, Nijmegen, the Netherlands and University Centre for Chronic Diseases Dekkerswald, Groesbeek, the Netherlands

**Corresponding author:** Ulrika SH Simonsson ([ulrika.simonsson@farmbio.uu.se](mailto:ulrika.simonsson@farmbio.uu.se))



Supplementary Table 1 Simulated day 7 AUC<sub>0-24h</sub> values used for prediction of high dose rifampicin

Dose group (mg/kg)	Day 7 AUC <sub>0-24h</sub> (h·mg/L)					
	Observed			Simulated*		
	Median	Max	Min	Median (IQR)	Max (IQR)	Min (IQR)
10	43.0	53.0	34.0	42.8 (37.7-48.8)	83.2 (69.8-103.8)	22.7 (18.8-26.3)
20	155.0	221.0	96.0	139.2 (128.3-151.0)	290.8 (248.4-343.8)	70.1 (61.3-79.8)
25	178.0	380.0	134.0	172.7 (158.1-190.3)	375.9 (316.7-446.1)	88.2 (76.3-100.5)
30	298.0	781.0	177.0	226.3 (208.2-247.6)	482.6 (414.2-580.8)	113.0 (97.8-130.1)
35	321.0	555.0	145.0	286.2 (259.9-312.0)	616.1 (529.8-748.1)	142.7 (122.1-163.9)
40	357.0	847.0	201.0	338.7 (308.9-373.0)	763.3 (642.9-957.4)	170.5 (145.4-194.7)
45	-	-	-	413.2 (373.5-453.7)	950.5 (784.0-1218.6)	203.2 (174.5-232.8)
50	-	-	-	481.4 (437.6-534.3)	1181.0 (964.6-1567.2)	237.4 (207.6-273.5)

\*All values are based on 1000 simulated trials

Abbreviations: AUC<sub>0-24h</sub>, area under the plasma concentration-time curve during 24 hours; IQR, inter-quartile range