EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

Increased bactericidal activity but dose-limiting intolerability at 50 mg·kg^{-1} rifampicin

Lindsey H.M. te Brake, Veronique de Jager, Kim Narunsky, Naadira Vanker, Elin M. Svensson, Patrick P.J. Phillips, Stephen H. Gillespie, Norbert Heinrich, Michael Hoelscher, Rodney Dawson, Andreas H. Diacon, Rob E. Aarnoutse, Martin J. Boeree

Please cite this article as: te Brake LHM, de Jager V, Narunsky K, *et al*. Increased bactericidal activity but dose-limiting intolerability at 50 mg·kg^{-1} rifampicin. *Eur Respir J* 2021; in press (https://doi.org/10.1183/13993003.00955-2020).

Increased bactericidal activity but dose-limiting intolerability at 50 mg/kg rifampicin

Lindsey H.M. te Brake, PhD[1*], Veronique de Jager, MD[2], Kim Narunsky, MD[3], Naadira Vanker, MD[2], Elin M. Svensson, PhD[1,4], Patrick P.J Phillips, PhD[5], Stephen H. Gillespie, DSc[6], Norbert Heinrich, MD[7,8], Michael Hoelscher, FRCP[7,8], Rodney Dawson, PhD[3], Andreas H. Diacon, PhD[2], Rob E. Aarnoutse, PhD[1], Martin J. Boeree, PhD[9], on behalf of the PanACEA Consortium

[1] Department of Pharmacy, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands. [2] TASK Applied Science, Cape Town, South Africa. [3] University of Cape Town Lung institute, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa [4] Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden. [5] UCSF Center for Tuberculosis, University of California San Francisco, San Francisco, The United States. [6] School of Medicine, University of St Andrews, North Haugh, St Andrews, United Kingdom. [7] Division of Infectious Diseases and Tropical Medicine, Medical Centre of the University of Munich, Munich, Germany. [8] German Center for Infection Research (DZIF), Munich, Germany. [9] Department of Lung Diseases, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, The Netherlands.

*** Corresponding author:** Lindsey te Brake, Radboud university medical center, Department of Pharmacy, Geert Grooteplein Zuid 10, 6525 GA, Nijmegen, The Netherlands, Phone: +31-24-3616405, E-mail: Lindsey.teBrake@radboudumc.nl

Take home message: while bactericidal activity continues to increase with dose, for the first time, we identified dose-limiting intolerability for rifampicin dosed at 50mg/kg; rifampicin at 40mg/kg seems the optimal tolerable dose for evaluation in treatment shortening trials

Abstract

Accumulating data have indicated that higher rifampicin doses are more effective and shorten tuberculosis treatment duration. This study evaluated the safety, tolerability, pharmacokinetics, and 7 and 14-day early bactericidal activity (EBA) of increasing doses of rifampicin. Here we report the results of the final cohorts of PanACEA HIGHRIF1, a dose-escalation study in treatment-naive adult smear-positive patients with tuberculosis. Patients received, in consecutive cohorts, 40 or 50mg/kg rifampicin once daily in monotherapy (day 1-7), supplemented with standard dose isoniazid, pyrazinamide and ethambutol between day 8-14. In the 40mg/kg cohort (n=15), 13 patients experienced a total of 36 adverse events (AEs) during monotherapy, resulting in one treatment discontinuation. In the 50mg/kg group (n=17), all patients experienced AEs during monotherapy, 93 in total; 11 patients withdrew or stopped study medication. AEs were mostly mild/moderate and tolerability- rather than safety-related, i.e. gastrointestinal disorders, pruritis, hyperbilirubinemia and jaundice. There was a more than proportional increase in the rifampicin geometric mean AUC_{0-24h} for 50mg/kg compared to 40mg/kg; 571 mg/L*h (range 320-995) versus 387 mg/L*h (201-847), while peak exposures saw proportional increases. Protein-unbound exposure after 50mg/kg (11%, 8-17%) was comparable with lower rifampicin doses. Rifampicin exposures and bilirubin concentrations were correlated (day-3 Spearman's rho 0.670, p<0.001). EBA increased considerably with dose, with the highest seen after 50mg/kg; 14-day EBA -0.427 logCFU/mL/day (95%CI -0.500, -0.355). In conclusion, although associated with an increased bactericidal effect, the 50mg/kg dose was not well tolerated. Rifampicin at 40mg/kg was well tolerated and therefore selected for evaluation in a phase IIC treatment shortening trial.

Word count abstract: 250 words

INTRODUCTION

In 1971 the U.S. Food and Drug Administration approved the pivotal tuberculosis (TB) drug rifampicin at a dose of 10 mg/kg. The recommended dose was chosen on the basis that it was effective at the lowest cost and limited by fear of adverse effects [1]. A dose finding study with an assessment of a maximum tolerated dose had not been performed.

In vitro and mouse models have since revealed that higher doses of rifampicin are associated with improved bactericidal and sterilizing activity, indicating a possibility of shorter treatment for pulmonary TB [2-4]. The End TB Strategy has set targets for treatment coverage as high as ≥90% by 2025, which will increase the number of patients diagnosed with TB, and the number that will receive rifampicin as part of their TB regimen, underlining the urgency for dose optimization of this pivotal drug [5]. Overall, rifampicin is expected to continue to play a fundamental role in TB treatment.

In the PanACEA HIGHRIF1 study in African patients with pulmonary TB, it was shown that doses up to 35 mg/kg given for two weeks resulted in a nine-fold increase in average exposure compared to 10 mg/kg [6], and were safe and well tolerated. Pharmacokinetic-pharmacodynamic (PK-PD) modeling demonstrates that increased rifampicin exposure is likely to be associated with increased early bactericidal activity [7, 8]. In a larger study of 365 patients, high-dose rifampicin (35 mg/kg) combined with isoniazid, pyrazinamide and ethambutol, when administered for a longer period of 3 months, was able to reduce time to sputum culture conversion in pulmonary TB [9].

Given these findings there is an urgent need to assess the safety, tolerability, pharmacokinetics and early bactericidal activity (EBA) of increasing doses of rifampicin to establish the optimum dose. To complete this task we extended the HIGHRIF1 study (clinicaltrials.gov identifier: NCT01392911) by including participants treated with 40 and 50 mg/kg of rifampicin.

METHODS

Study design and participants

We performed an open-label phase II multiple dose-ranging study to evaluate safety, tolerability, pharmacokinetics, and 7 and 14-day early EBA of 40 and 50 mg/kg of rifampicin. Adults (18-65 years) with newly diagnosed, previously untreated, drug-susceptible, sputum smear-positive pulmonary TB, and without medical contraindications were included in the study. Patients were hospitalized in one of two study sites in Cape Town, South Africa. We recruited consecutive cohorts of 15 participants who received monotherapy of rifampicin for 7 days, supplemented with standard doses of isoniazid (5 mg/kg), pyrazinamide (25–30 mg/kg), and ethambutol (15–20 mg/kg) on days 8-14. Patients then continued TB treatment with standard doses of all drugs. Study medication was weight-banded (Figure E1) and taken in the morning with a light breakfast and a glass of water. After completion of each of the cohorts, a safety review was performed by the Trial Steering Committee (TSC) to assess whether a dose increase was possible or whether the maximum tolerable dose (MTD) was assessed. The Maximum Tolerable Dose (MTD) was predefined as the dose level below that producing unacceptable but reversible toxicity and is considered the upper limit of patient tolerance. The study protocol was approved by the applicable ethical review boards and by the South African Health Products Regulatory Authority and was conducted according to international and South African Good Clinical Practice guidelines. Details on eligibility criteria and results of prior HIGHRIF1 cohorts has been published elsewhere [10].

Safety and tolerability

Symptom assessments and physical examinations, including vital signs, were performed daily. Haematologic, renal, and liver function tests, glucose, uric acid and urinalysis, as well as electrocardiography were performed at baseline and on Days 1, 3, 6, 10, 14, and 21. Adverse events were graded according to the U.S. National Institute of Health Common Terminology Criteria for

Adverse Events 4.0 (CTCAE v4.0) and were assessed as unrelated, possibly, or definitely related to study therapy by site investigators. A serious adverse event was defined as any untoward medical occurrence that in the opinion of the investigator results in death, is life-threatening, requires (prolongation of) hospitalization, results in persistent or significant disability/incapacity, or is a medically important event. A meeting of the Trial Steering Committee to discuss the continuation or termination of the study would take place if two subjects experiencing a grade 3 adverse event assessed as probably or definitely related to administration of high-dose rifampin, or one subject experiencing a grade 4 or 5 adverse event assessed as definitely related to rifampin, would occur in one dose group.

Pharmacokinetics

Blood samples were taken pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post rifampicin intake with a standardized meal on days 7 and 14 to obtain full pharmacokinetic profiles. Rifampicin total (protein-bound plus unbound) and protein-unbound plasma concentrations were measured after each cohort. Plasma samples of the 40 mg/kg dose group were analysed using the same validated ultra-performance liquid chromatography method with ultraviolet detection as in the preceding cohorts (6). For the 50 mg/kg group, total concentrations of anti-TB drug were analysed using an extensively validated liquid chromatography-mass spectrometry (LC-MS/MS) multi-drug assay. The assay accuracy for rifampicin quantification was 94.24-102.06% dependent on concentration level, the within run imprecision ranged from 0.9-4.89%. Protein-unbound determination of rifampicin occurred via ultrafiltration as previously described (28). Noncompartmental pharmacokinetic analysis was performed with Phoenix WinNonlin 6.4 (Certara USA, INC., Princeton, NJ), as described previously [11]. Unbound fraction in the 50 mg/kg cohort was calculated by dividing the unbound AUC_{0-24h} by the total AUC_{0-24h} for all subjects in this group. A full description of the bio-analytical and pharmacokinetic analyses is provided in the appendix.

Antimycobacterial activity

Pooled overnight (16 h) sputum samples were collected at baseline, daily to day 7, and on days 9 and 14. Samples were processed for culture on selective Middlebrook 7H10S agar plates and in liquid broth using the mycobacterial growth indicator tube (Bactec MGIT960) system. Spot sputum samples were collected before enrolment, at day 19, and 12 weeks after starting study therapy and were prepared for auramine O-stained direct microscopy and rapid resistance testing via the Xpert MTB/RIF assay (Cepheid, USA). All microbiologic testing was performed at the Department of Medical Biochemistry, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa, as described previously [12].

Statistical analyses

This was a descriptive study with no inferential statistics or hypothesis testing [6].

The planned sample size of 15 patients in each group is in keeping with other trials of this type and accounts for the estimate of three dropouts per group. The distribution of TTP on MGIT was positively skewed with log-transformed TTP more closely following a symmetric normal distribution. Mixed effects models with visit day as a discrete random effect were used to estimate the mean logCFU and logTTP in each treatment arm at each visit to describe the data. As in our previous study [6], we found an unexpectedly high number of negative cultures for the short treatment duration. To include these censored observations, Tobit regression [13] was used to estimate the 14-day early bactericidal activity, accounting for negatives cultures using a lower limit of detection censoring for logCFU of 1 and an upper limit for TTP of 42 days. Separate models were fitted for each patient with parameter estimates summarized by treatment group using a random effects model accounting for within- and between-patient variability. We were concerned that data from patients without cultures after day 5 inflated the estimates of the fall in CFU or increase in TTP over time. Given these withdrawals, data from these patients were excluded to account for the loss to follow-up in the 50 mg/kg cohort in an additional analysis. Sensitivity analyses included analysis of the full data as

planned, and a one-stage mixed effects model, allowing for censoring of negative cultures, to assess robustness of the results.

The safety population consisted of all participants who took at least one dose of trial medication. Associations between exposure and liver laboratory assessments were made using Spearman rank correlation. In addition, dose-exposure-tolerability relationships during the monotherapy phase were evaluated post-hoc with an ordered categorical model estimating the probability of having 0, 1, 2, or 3 or more adverse effects (AEs) given the rifampicin dose and exposure. In these analyses, total exposure in plasma ($AUC_{0\text{-}24h}$) at day 7 was used as measure for rifampicin exposure in the study. All system organ classes (SOCs) with tolerability-related AEs were included in the analysis (see Table E2 for details).

Analyses were undertaken using Stata 15.1 (StataCorp LLC, College Station, TX) and NONMEM 7.4 (Icon Development Solutions, Ellicott City, MD).

RESULTS

Patients

A total of 15 culture-positive patients with pulmonary tuberculosis were enrolled in the 40 mg/kg cohort, of whom 14 patients completed the study. One patient was withdrawn because of raised liver enzymes. In the 50 mg/kg cohort, 17 patients were enrolled. Recruitment was temporarily suspended in this cohort for TSC review of interim data. Nine patients (53%) withdrew early from the 50 mg/kg group; 7 during monotherapy and 2 during combination therapy. One additional patient stopped treatment from day 11 onwards but completed study visits (excluding PK assessment) awaiting outcome of the interim TSC review, and one patient had a 3-day dose interruption from day 10-12. Patient characteristics are shown in Table 1.

Safety and tolerability

Of the 15 patients starting on 40 mg/kg rifampicin, 13 (87%) reported AEs during monotherapy (36 events in total). All 17 patients in the 50 mg/kg cohort reported AEs during monotherapy (93 events in total). See Table 2 for an overview of the AEs and their severity during the monotherapy and combination therapy periods in the 40 and 50 mg/kg groups, and previous groups. AEs in both the 40 and 50 mg/kg cohorts were mostly mild/moderate, i.e. >97% of all AEs during monotherapy were grade 1/2 in both cohorts. In addition, AEs were tolerability- rather than safety-related, i.e. gastrointestinal disorders, pruritis, hyperbilirubinemia and jaundice. No grade 4 or 5 adverse events occurred in either cohort.

The most common adverse events in the 40 and 50 mg/kg cohorts were gastrointestinal disorders (grade 1-2), hyperbilirubinemia (grade 1-3), pruritis (grade 1-2), and jaundice (50 mg/kg rifampicin only, grade 1-2), all expected from rifampicin. Overall, the 50 mg/kg cohort contained more cases with gastrointestinal disorders (12 patients (71%) versus 7 patients (47%) during monotherapy), hyperbilirubinemia (10 patients (59%) versus 4 patients (27%), and jaundice (9 patients (53%) versus 0 patients). Elevations in bilirubin (grade 1-3) peaked around day 3-4 after rifampicin start (Figure 2 and Figure E6). See Table E2 and E3 for an overview of the incidence of AEs during monotherapy and combination therapy, respectively, per SOC in both groups.

During 40 mg/kg combination therapy, 4 patients developed a grade 3 AE. In 1/4 patients this was defined as unrelated to high-dose rifampicin (hyperuricaemia), in 3/4 patients this was defined as possibly related (n=1 hyperuricaemia, n=2 hepatic enzyme increased). TSC evaluation of these AEs considered that these grade 3 AEs were either not typical for high-dose rifampicin (n=2 hyperuricemia), and/or considered unrelated or only possibly related to high-dose rifampicin (increased transaminases only developed after introduction of combination therapy). Nonetheless,

the increased transaminases had been classified as 'serious adverse events' and one patient was withdrawn, even though there was no immediate life-threatening risk. All grade 3 AEs resolved.

While no SAEs occurred in the 50 mg/kg cohort, there were nine early withdrawals. Four were withdrawn from the study by the investigator because of adverse events (e.g. grade 2 elevated bilirubin), and five withdrew consent because of social/personal reasons, which were hypothesized by investigators and TSC to be related to experienced intolerability. In addition, one patient was withheld from study treatment between day 10-12 because of intolerability.

Based on the high incidence of adverse effects and the many withdrawals in the 50 mg/kg cohort, the TSC assessed that the 50 mg/kg dose was not tolerable and that the 40 mg/kg dose was to be regarded as the maximum tolerable dose. The TSC considered that the safety profile of 40 mg/kg rifampicin was acceptable, mostly mild/moderate and reversible, and therefore 40 mg/kg was considered the MTD. In addition, the profile was considered to be comparable to that of 35 mg/kg, a dose that also has been found to be safe and effective when given for 12 weeks in a randomized controlled trial [9].

Pharmacokinetics

The geometric mean AUC_{0-24h} and C_{max} values of rifampicin at day 7 and 14 are presented in Table 3. On adding 25% of the dose of rifampicin from 40 to 50 mg/kg, the geometric mean AUC_{0-24} increased approximately 50%, which reflects a more than dose-proportional increase of exposure of a similar magnitude as previously observed (Figure E2) [10]. In contrast, rifampicin peak exposure increased proportional with the dose. Of note, large inter-individual variability in AUC_{0-24h} and C_{max} was observed, with exposures between groups overlapping considerably (Table 3 and Figure E2). Protein-unbound rifampicin exposure, or free fraction, was comparable to other (lower) doses of rifampicin

[14]. For rifampicin PK profiles and PK parameters of other study drugs we refer to Figure E3 and Table E1, respectively.

Antimycobacterial activity

One patient from the 50 mg/kg group had consistent negative cultures at baseline and throughout treatment and was therefore removed from all analyses. Figure 1 summarizes the change in viable bacterial load in sputum over 7 and 14 days expressed as fall of CFU and increase of TTP for patients from all cohorts. The 7 days EBA shows that the fall in bacterial load in the first week is due to rifampicin alone and is extended in the second week. In a post-hoc analysis, 6 patients in the 50 mg/kg group without cultures after day 5 were excluded because they only contributed data up to day 4 thereby making it challenging to estimate change over a 14 day period (Figure E4). This was a post hoc analysis not anticipated in the statistical analysis plan, but was conservative, resulting in smaller estimates of slope than our planned primary analysis. Overall, bactericidal activity as measured on both solid and liquid media increased over the 10 to 50 mg/kg cohorts with the highest 14-day activity seen in the highest dose cohort in all analyses. Sensitivity analyses using a one-stage mixed effect model showed consistent results when all negative cultures were imputed with the lower limit of detection of the respective culture method, when the first negative culture was imputed with the lower limit of detection, and when negative cultures were ignored, supporting the robustness of our findings. For the differences in bacterial load in CFU and TTP compared to baseline over time we refer to Figure E5.

Exposure-safety analyses

Figure 2 shows the relationship between rifampicin $AUC_{0\text{-}24h}$ at day 7 and total serum bilirubin for all scheduled safety visits across HIGHRIF1 cohorts (n= 93). Rifampicin exposures and bilirubin concentrations were correlated (Spearman's rho on day 3 of 0.670, $p<0.001$). ALT and AST concentrations were not correlated with rifampicin exposure (Figure E7 and E8).

With respect to the dose-exposure-tolerability evaluation, a linear relation on logit scale described the data appropriately (goodness-of-fit pots available in supplementary material (Figure E9). Dose and exposure ($AUC_{0\text{-}24h}$ at day 7) were both separately strong predictors of the probability of developing tolerability AEs (likelihood ratio test, $p<0.0001$ for both). A 50 mg/kg dose was associated with 76% (90% confidence interval (CI) 56-88%) risk of three or more tolerability related (rather than safety-related) adverse events, for 40 mg/kg and 10 mg/kg the corresponding risks were 47% (29-64%) and 2.0% (0.1-5.4%), respectively. The relationships with associated uncertainty are illustrated in Figure 3.

DISCUSSION

More than 40 years after the introduction of rifampicin, during which time it has become the most important drug for the treatment of TB, we have now identified a maximum tolerated dose. In our first reports of the HIGHRIF1 study, we showed that high-dose rifampicin up to 35 mg/kg was safe and well tolerated, exposure increased more than proportional with dose, and there was greater early bactericidal activity at higher exposures [6-8]. We have now shown continued increases in drug exposures and extended EBA in the 40 mg/kg and 50 mg/kg cohorts. The 40 mg/kg cohort was in line with previous cohorts with AEs of only mild to moderate severity. Rifampicin dosed at 50 mg/kg once daily, however, was poorly tolerated, with a sharp increase in frequency and severity of AEs as well as subject withdrawals compared to 40mg/kg. Thus, we consider that rifampicin dosed at 40 mg/kg is the maximum tolerated dose.

Overall, experienced adverse events were mild or moderate and mostly tolerability related, i.e. gastrointestinal disorders (grade 1-2), pruritis (grade 1-2), and jaundice (grade 1-2, in the 50 mg/kg group only, related to hyperbilirubinemia). It is the large number rather than the severity of individual AEs that caused poor tolerability and withdrawals in the 50 mg/kg group.
While minor bilirubin elevations were common in all groups, a remarkably high incidence of hyperbilirubinemia (grade 1-3) was observed in the highest dose group. The elevations in bilirubin peaked around day 3-4 after start of rifampicin, were exposure dependent, and were not associated with other liver enzyme elevations (Figure 2, Figure E6-E8). Strikingly, in the 50 mg/kg arm normalization of bilirubin levels was slower compared to other arms (Figure E6). McColl et al. (1987) found that in healthy subjects unconjugated bilirubin rises after starting rifampicin, which we now believe is because of inhibition of bilirubin hepatocellular uptake via OATP and/or glucuronidation by UGTA1A1 [15]. Bilirubin levels then decline to less than pretreatment values upon rifampicin continuation, suggestive for induction of net bilirubin clearance [16]. In contrast, increased bilirubin levels were all conjugated (direct) in the subset of patients tested in the 50 mg/kg arm (Figure E10),

suggesting reduced biliary clearance by MRP2 after intracellular conjugation in the liver [17]. In line with this, we may anticipate other liver transporters to also be inhibited by rifampicin at these high intracellular exposures [17].

Rifampicin exposures following 40 and 50 mg/kg were high, but within the expected range based on previous results and modeling predictions [7], and again without a ceiling effect as reported in the pharmacokinetics of rifapentine [18]. More importantly, apart from average exposures, the lowest observed AUC_{0-24h} and C_{max} values also increased with almost every dose step (Figure E2). These low exposures may cause treatment failures and relapses, and create conditions for the emergence of resistance [19, 20]. Strikingly, at high exposure levels no saturation of plasma proteins occurred, as the fraction protein-unbound (free, active drug) was comparable with other reports [14, 21].

Early phase clinical tuberculosis studies usually only include small numbers of patients selected on the basis of very strict criteria who are treated for only a short period of time. Our EBA findings, therefore, need to be confirmed in phase II studies with less narrow inclusion criteria and adequate patient numbers. However, even though this study was not powered or designed to test statistical differences between groups, the presented EBA results are striking. There is a clear increase in EBA with dose and exposure, with the highest EBA so far seen in the 50 mg/kg cohort. The broad trend in increasing EBA with dose was seen on both liquid and solid media; there was a suggestion that the increase up to 40mg/kg was less conspicuous on liquid than solid media, although our study was too small to draw definitive conclusions. Inclusion in the analysis of the six patients who withdrew early from the 50mg/kg arm may have artificially inflated the estimate of the 14-day bactericidal activity; our sensitivity analysis excluding these patients did show slightly lower 14-day activity although it was still clearly higher than any other dose group. We chose to retain the analysis including all patients since this was an observational, hypothesis generating study and we were keen to include all the data in the primary analysis. In addition, we cannot exclude that participants withdrawing early

may also have had increased EBA because of elevated rifampicin exposures, possibly explaining tolerability-related early withdrawal from the study. In general, our EBA results are in line with findings from other high-dose rifampicin studies in pulmonary TB [8, 9, 22, 23] and in TB mouse models [4, 24]. In our previous study in patients with pulmonary TB, high-dose rifampicin at 35 mg/kg for 12 weeks was found to be safe and reduce the time to culture conversion, an intermediate clinical endpoint [9]. Our current work further supports that higher rifampicin doses perform better and thus have the potential to improve clinical outcome, decrease relapse rates, reduce the emergence of rifampicin resistance and reduce treatment duration.

To further optimize the rifampicin dose and its dosing strategy, multiple approaches could be considered. The most promising strategy from a programmatic point of view would be to start therapy with lower rifampicin doses, allowing the body to get used to rifampicin in terms of gastrointestinal tolerance while also facilitating induction of rifampicin and bilirubin clearance. After this initial period, a higher dose of rifampicin could be introduced in all patients. Personalized medicine with titration for individual maximum exposures is promising because of the increase in variation of exposures found with higher doses and higher rifampicin exposures in turn are associated with improved bactericidal activity and culture conversion [8, 22]. Unfortunately, a maximal effect has not yet been identified and as such there are no clear exposure targets. A low-cost point of care device to estimate rifampicin concentrations in real-time could support the implementation of high-dose rifampicin treatment in programmatic settings [25]. Finally, just recently our group published that weight-band dosing yields a small and non-clinically relevant decrease in variability of AUC0–24h compared to flat-dosing [26]. This supports the implementation use of flat-dosing, and we are planning to implement this in our follow-up trial.

For now we have sufficient data to move high-dose rifampicin forward to a study with less narrow inclusion criteria, increased patient numbers, longer treatment duration, and clinical endpoints, i.e. a

so-called Phase IIC Selection Trial with Extended Post-treatment follow-up (STEP) design [27]. In this trial, the experimental regimen is given for the duration for which it will be studied in phase III (presently 3 or 4 months) and patients are followed for clinical outcomes of treatment failure and relapse for a total of 12 months from randomisation. Generated data will provide valuable information about the likelihood of success of high-dose rifampicin containing regimen in a future phase III trial.

Currently, one phase III study with the objective to reduce treatment duration by increasing the dose of rifampicin is enrolling (RIFASHORT, ClinicalTrials.gov Identifier: NCT02581527). The primary endpoints are treatment failure and relapse after 12 months. Based on the data presented here rifampicin dose increase evaluated in RIFASHORT, i.e. 1200 or 1800 mg of rifampicin corresponding to around 20 to 30 mg/kg, may seem modest[28, 29], but will still provide important input to support the ability of higher doses to prevent failure and relapse.

In conclusion, rifampicin dosed at 50 mg/kg once daily, introduced at once at the start of TB therapy, was poorly tolerated. It was associated with a remarkably improved fall in bacterial load compared to other dosages. The 40 mg/kg dose was safe, tolerable and associated with improved bactericidal effect and is, therefore, the appropriate dose to be evaluated in a follow-up phase IIC trial investigating treatment shortening potential of high-dose rifampicin. Such a study also provides the opportunity to study tolerability of 40 mg/kg in a larger population when given for a longer duration.

Our research concludes a journey that started in the 1960s. We need to move forward with confirmative clinical trials to further inform implementation of high dose rifampicin in programs and guidelines.

Funding

This publication was produced by PanACEA, which is part of the European and Developing Countries Clinical Trials Partnership (EDCTP) 1 programme [project code IP.2007.32011.012 (HIGHRIF)] and the EDCTP2 programme supported by the European Union (grant number TRIA2015-1102-PanACEA). The funding source had no influence on study design; in the collection, analysis, and interpretation of data; in the writing of the report. Publication of the article is defined as a deliverable under the Grant Agreement.

Declaration of interests

We declare no competing interests.

REFERENCES

1. van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, et al. Why Do We Use 600 mg of Rifampicin in Tuberculosis Treatment? *Clin Infect Dis*. 2011;**52**(9):e194-9.
2. Jayaram R, Gaonkar S, Kaur P, Suresh BL, Mahesh BN, Jayashree R, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother*. 2003;**47**(7):2118-24.
3. Rosenthal IM, Tasneen R, Peloquin CA, Zhang M, Almeida D, Mdluli KE, et al. Dose-ranging comparison of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis. *Antimicrob Agents Chemother*. 2012;**56**(8):4331-40.
4. de Steenwinkel JE, Aarnoutse RE, de Knegt GJ, ten Kate MT, Teulen M, Verbrugh HA, et al. Optimization of the rifampin dosage to improve the therapeutic efficacy in tuberculosis treatment using a murine model. *Am J Respir Crit Care Med*. 2013;**187**(10):1127-34.
5. WHO. Global Tuberculosis Report 2018. 2018.
6. Boeree MJ, Diacon AH, Dawson R, Narunsky K, du Bois J, Venter A, et al. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. *Am J Respir Crit Care Med*. 2015;**191**(9):1058-65.
7. Svensson RJ, Aarnoutse RE, Diacon AH, Dawson R, Gillespie SH, Boeree MJ, et al. A Population Pharmacokinetic Model Incorporating Saturable Pharmacokinetics and Autoinduction for High Rifampicin Doses. *Clin Pharmacol Ther*. 2018;**103**(4):674-83.
8. Svensson RJ, Svensson EM, Aarnoutse RE, Diacon AH, Dawson R, Gillespie SH, et al. Greater Early Bactericidal Activity at Higher Rifampicin Doses Revealed by Modeling and Clinical Trial Simulations. *J Infect Dis*. 2018;**218**(6):991-9.
9. Boeree MJ, Heinrich N, Aarnoutse R, Diacon AH, Dawson R, Rehal S, et al. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. *The Lancet Infectious diseases*. 2017;**17**(1):39-49.
10. Boeree MJ, Diacon AH, Dawson R, Narunsky K, du Bois J, Venter A, et al. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. *Am J Respir Crit Care Med*. 2015;**191**(9):1058-65.
11. Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, Aarnoutse RE. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. *Antimicrob Agents Chemother*. 2007;**51**(7):2546-51.
12. Donald PR, Sirgel FA, Venter A, Parkin DP, Seifart HI, van de Wal BW, et al. Early bactericidal activity of antituberculosis agents. *Expert review of anti-infective therapy*. 2003;**1**(1):141-55.
13. Long JS. Regression models for categorical and limited dependent variables. Thousand Oaks, CA: Sage Publications; 1997.
14. Litjens CHC, Aarnoutse RE, van Ewijk-Beneken Kolmer EWJ, Svensson EM, Colbers A, Burger DM, et al. Protein binding of rifampicin is not saturated when using high-dose rifampicin. *J Antimicrob Chemother*. 2019;**74**(4):986-90.
15. Chiou WJ, de Morais SM, Kikuchi R, Voorman RL, Li X, Bow DA. In vitro OATP1B1 and OATP1B3 inhibition is associated with observations of benign clinical unconjugated hyperbilirubinemia. *Xenobiotica*. 2014;**44**(3):276-82.
16. McColl KE, Thompson GG, el Omar E, Moore MR, Park BK, Brodie MJ. Effect of rifampicin on haem and bilirubin metabolism in man. *Br J Clin Pharmacol*. 1987;**23**(5):553-9.
17. Te Brake LH, Russel FG, van den Heuvel JJ, de Knegt GJ, de Steenwinkel JE, Burger DM, et al. Inhibitory potential of tuberculosis drugs on ATP-binding cassette drug transporters. *Tuberculosis (Edinburgh, Scotland)*. 2016;**96**:150-7.
18. Savic RM, Lu Y, Bliven-Sizemore E, Weiner M, Nuermberger E, Burman W, et al. Population pharmacokinetics of rifapentine and desacetyl rifapentine in healthy volunteers: nonlinearities in clearance and bioavailability. *Antimicrob Agents Chemother*. 2014;**58**(6):3035-42.

19. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J Infect Dis*. 2011;**204**(12):1951-9.
20. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clin Infect Dis*. 2012;**55**(2):169-77.
21. te Brake LH, Ruslami R, Later-Nijland H, Mooren F, Teulen M, Apriani L, et al. Exposure to total and protein-unbound rifampin is not affected by malnutrition in Indonesian tuberculosis patients. *Antimicrob Agents Chemother*. 2015;**59**(6):3233-9.
22. Svensson EM, Svensson RJ, Te Brake LHM, Boeree MJ, Heinrich N, Konsten S, et al. The Potential for Treatment Shortening With Higher Rifampicin Doses: Relating Drug Exposure to Treatment Response in Patients With Pulmonary Tuberculosis. *Clin Infect Dis*. 2018;**67**(1):34-41.
23. Velasquez GE, Brooks MB, Coit JM, Pertinez H, Vargas Vasquez D, Sanchez Garavito E, et al. Efficacy and Safety of High-Dose Rifampin in Pulmonary Tuberculosis. A Randomized Controlled Trial. *Am J Respir Crit Care Med*. 2018;**198**(5):657-66.
24. Liu Y, Pertinez H, Ortega-Muro F, Alameda-Martin L, Harrison T, Davies G, et al. Optimal doses of rifampicin in the standard drug regimen to shorten tuberculosis treatment duration and reduce relapse by eradicating persistent bacteria. *J Antimicrob Chemother*. 2018;**73**(3):724-31.
25. De Jager V, Le Roux S, Mnunu M, Niesler T, Diacon A. Transcutaneous rifampicin concentration monitoring. Abstract 10th international workshop on Clinical Pharmacology of Tuberculosis drugs, Atlanta, GA, USA, 2017.
26. Susanto BO, Svensson RJ, Svensson EM, Aarnoutse R, Boeree MJ, Simonsson USH. Rifampicin can be given as flat-dosing instead of weight-band dosing. *Clin Infect Dis*. 2019.
27. Phillips PP, Dooley KE, Gillespie SH, Heinrich N, Stout JE, Nahid P, et al. A new trial design to accelerate tuberculosis drug development: the Phase IIC Selection Trial with Extended Post-treatment follow-up (STEP). *BMC medicine*. 2016;**14**:51.
28. Aarnoutse RE, Kibiki GS, Reither K, Semvua HH, Haraka F, Mtabho CM, et al. Pharmacokinetics, tolerability and bacteriological response of 600, 900 and 1200 mg rifampicin daily in patients with pulmonary TB. *Antimicrob Agents Chemother*. 2017.
29. Te Brake LHM, Boeree MJ, Aarnoutse RE. Conflicting Findings on an Intermediate Dose of Rifampicin for Pulmonary Tuberculosis. *Am J Respir Crit Care Med*. 2019;**199**(9):1166-7.

Table 1| Demographic and baseline characteristics of study participants in HIGHRIF1.

	10 mg/kg	**20 mg/kg**	**25 mg/kg**	**30 mg/kg**	**35 mg/kg**	**40 mg/kg**	**50 mg/kg**	**All**
	n=8	*n=15*	*n=15*	*n=15*	*n=15*	*n=15*	*n=17*	*n=100*
Age, year	28 (20-49)	28 (18-47)	26 (20-47)	40 (20-60)	38 (21-60)	35 (23-58)	25 (20-55)	31 (18-60)
Weight, kg	57 (47-65)	52 (42-63)	53 (40-68)	54 (46-84)	57 (41-74)	59 (47-65)	53 (43-64)	53 (40-84)
BMI, kg/m^3	21 (16-26)	18 (17-26)	19 (15-25)	21 (16-31)	19 (15-25)	19 (17-25)	18 (16-23)	19 (15-31)
Male, n (%)	6 (75%)	11 (73%)	10 (67%)	11 (73%)	10 (67%)	11 (73%)	15 (88%)	74 (74%)
Race, n (%)								
Black	3 (38%)	7 (47%)	4 (27%)	9 (60%)	5 (33%)	10 (67%)	8 (47%)	46 (46%)
Colored	5 (63%)	8 (53%)	11 (73%)	6 (40%)	10 (67%)	5 (33%)	8 (47%)	53 (53%)
Caucasian							1 (6%)	1 (1%)
HIV^+, n (%)	0	0	0	3 (20%)	1 (7%)	1 (7%)	0	5 (5%)
Baseline log_{10} CFU	5.4 (4.0-6.4)	5.0 (2.6-7.3)	6.5 (5.4-7.6)	6.4 (5.3-7.4)	5.8 (1.0-7.2)	6.5 (5.1-7.3)	6.4 (4.8-8.5)	6.1 (1.0-8.5)
Baseline TTP	4.0 (3.3-5.3)	4.7 (3.0-9.1)	4.0 (3.4-6.6)	3.9 (2.9-6.0)	3.9 (2.6-19.3)	4.0 (2.2-7.6)	4.4 (2.7-7.3)	4.0 (2.2-19.3)

BMI = Body Mass Index; CFU = colony-forming units; RIF = rifampicin; TTP = time to positivity in days

Results are expressed in median (range), unless stated otherwise

Table 2| Total number of adverse events per severity, dose group and treatment period.

	10-30 mg/kg RIF	**35 mg/kg RIF**	**40 mg/kg RIF**	**50 mg/kg RIF**
AE monotherapy	*n=53*	*n=15*	*n=15*	*n=17*
Total, n	46	25	36	93[*]
Not specified	0	0	0	10
Unrelated	13	4	9	10
Possibly related	28	19	19	24
Related	5	2	8	49
Grade 1, n	38	18	26	60
Grade 2, n	6	7	9	21
Grade 3, n	2	0	1	1
SAE, n	0	0	0	0
AE combination therapy	*n=53*	*n=15*	*n=15*	*n=10*
Total, n	62	32	24	34
Not specified	0	0	0	0
Unrelated	23	13	10	11
Possibly related	38	19	13	14
Related	1	0	1	9
Grade 1, n	52	22	19	28
Grade 2, n	8	9	1	6
Grade 3, n	2	1	4[**]	0
SAE, n	1	0	2	0

AE = Adverse Event; RIF = rifampicin; SAE = Serious Adverse Event

[*] 11 adverse events were not graded, and of 1 event severity was not indicated

[**] In 1/4 patients with a grade 3 AE developing in the combination phase, this was defined as unrelated to high-dose rifampicin (hyperuricaemia). In 3/4 patients with a grade 3 AE in the combination phase, this was defined as possibly related (n=1 hyperuricaemia, n=2 hepatic enzyme increased).

Table 3| Rifampicin pharmacokinetics during monotherapy (day 7) and combination therapy (day 14).

PK parameter	40 mg/kg RIF	50 mg/kg RIF
Monotherapy	*n=15*	*n=10*
AUC_{0-24h} (mg/L*h)	387 (201-847)	571 (320-995)
C_{max} (mg/L)	53.9 (40.0-80.8)	63.4 (42.3-85.0)
CL/F	5.9 (3.2-9.7)	4.6 (2.6-6.6)
Vd/F	30.9 (18.5-50.7)	32.4 (23.9-45.2)
Half-life (h)	3.7 (1.7-4.1)	4.9 (2.5-8.5)
AUC free fraction (%) - average	NA	10.8 (8.4-16.9)
Combination therapy (steady state)[*]	*n=14*	*n=7*
AUC_{0-24h} (mg/L*h)	257 (173-349)	370 (231-559)
C_{max} (mg/L)	41.4 (26.4-56.6)	53.2 (39.7-73.6)
CL/F	8.7 (7.0-11.7)	6.9 (5.4-9.1)
Vd/F	31.6 (18.5-45.3)	26.3 (20.0-39.5)
Half-life (h)	5.6 (2.1-9.5)	2.6 (2.0-5.1)
AUC free fraction (%) - average	NA	10.6 (8.8-13.2)

AUC = area under the concentration-time curve; Cmax = peak plasma concentration; RIF = rifampicin
Results are given in geometric mean and range, unless stated otherwise

[*] rifampicin clearance increases during multiple dose therapy due to its known induction of hepatic enzymes, which leads to autoinduction of its own metabolism.

A) 7 and 14-day EBA with 95% confidence intervals, log_{10}CFU/ml/day.

B) 7 and 14-day EBA with 95% confidence intervals, log_{10}TTP/day.

Figure 1| Early bactericidal activity (0-7 and 0-14 EBA) of rifampicin based on CFU (A) and TTP (B).

Data from all patients included with the exception of data from one patient from each of 20mg/kg and 50mg/kg group that had consistent negative cultures at baseline and throughout.

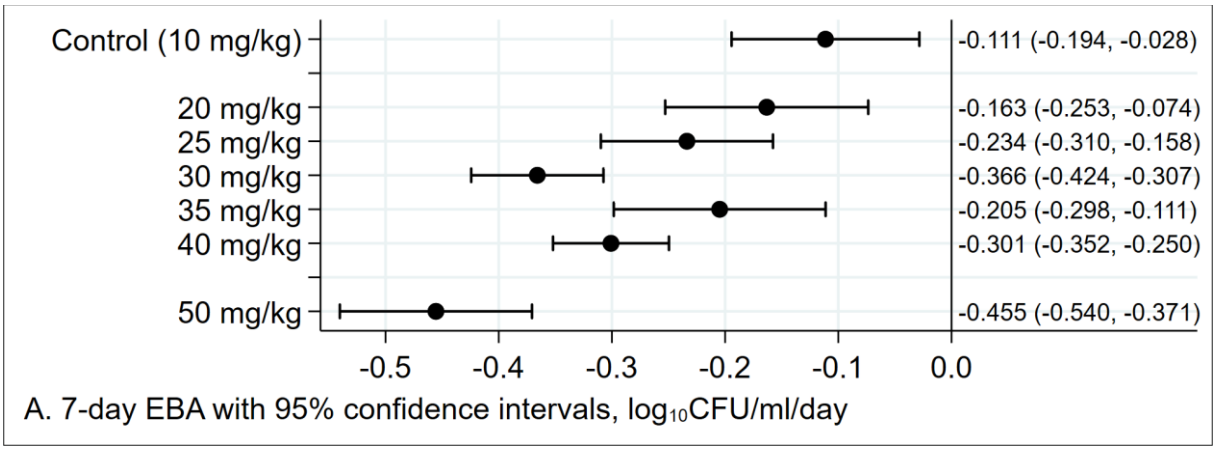
The slight change in the estimates for 10 mg/kg and 20 mg/kg compared to previously [6] are because of data corrections (4 CFU cultures were recorded as negative while they were actually missing).

CFU = colony-forming units; TTP = time to positivity

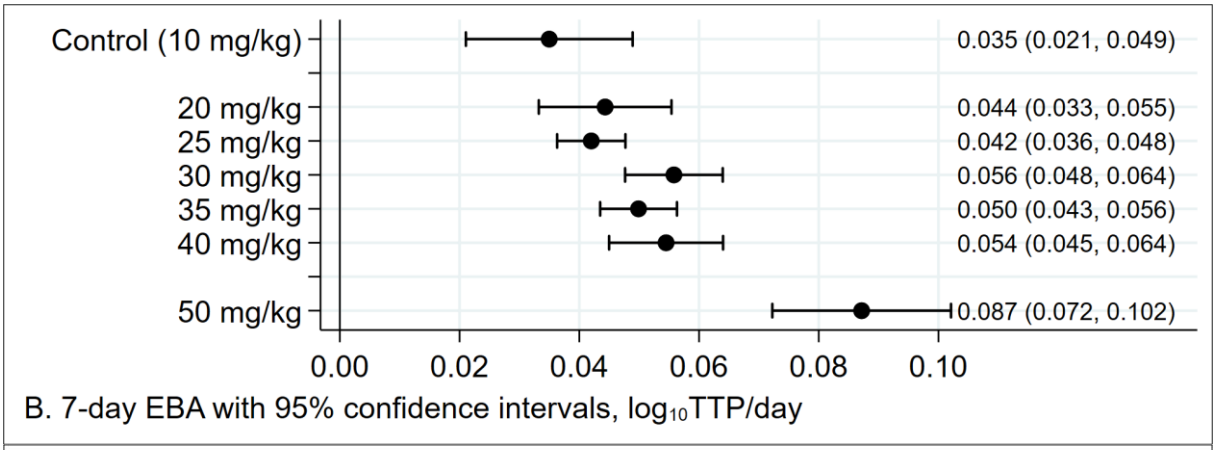
Figure 2| Total serum bilirubin per day on rifampicin plotted against rifampicin total exposures ($AUC_{0\text{-}24h}$ at day 7) in all patients with PK results (n=93) in the HIGHRIF1 study.

The lines represent linear regressions (for illustration, statistical testing described in the text). The y-axis is capped at 70 µmol/L for readability, excluding three outlying points (day 3 in one patient in the 40 mg/kg group with $AUC_{0\text{-}24h}$ 338 mg/L*h and bilirubin 111 µmol/L; day 7 and 10 in one patient in the 50 mg/kg group with $AUC_{0\text{-}24h}$ 980 mg/L*h and bilirubin 100 and 175 µmol/L, respectively).

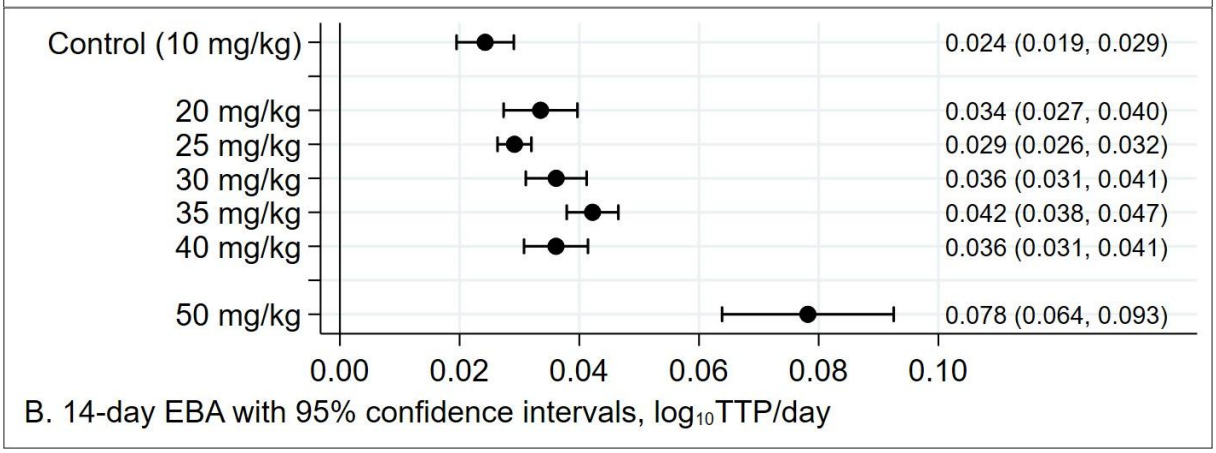
Figure 3 | Probability of tolerability-related adverse events (AEs) during the first week of rifampicin monotherapy related to the rifampicin dose or exposure ($AUC_{0\text{-}24h}$ at day 7). The shaded areas represents 90% confidence intervals based on the estimated parameter uncertainty.



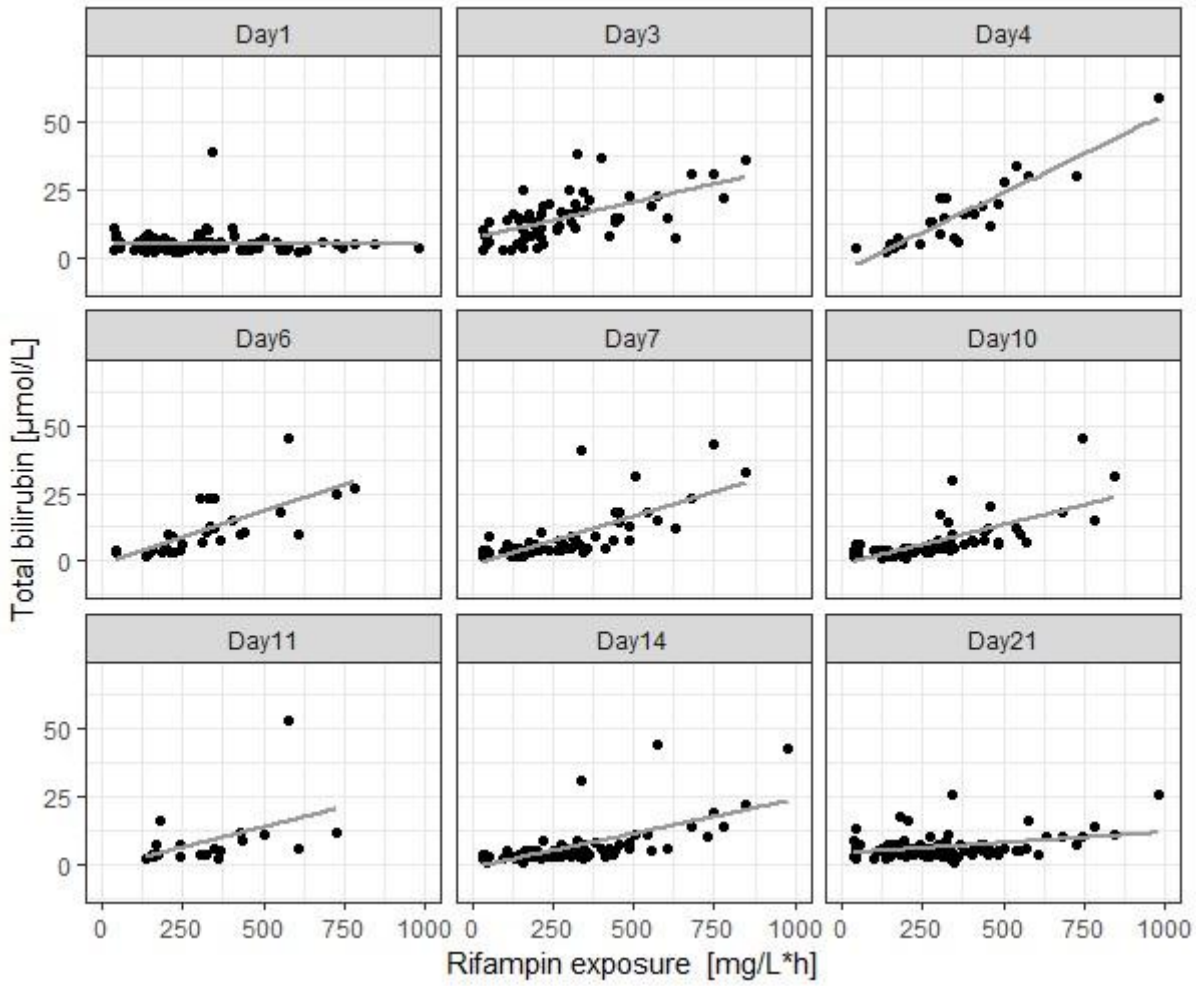
A. 7-day EBA with 95% confidence intervals, log_{10}CFU/ml/day

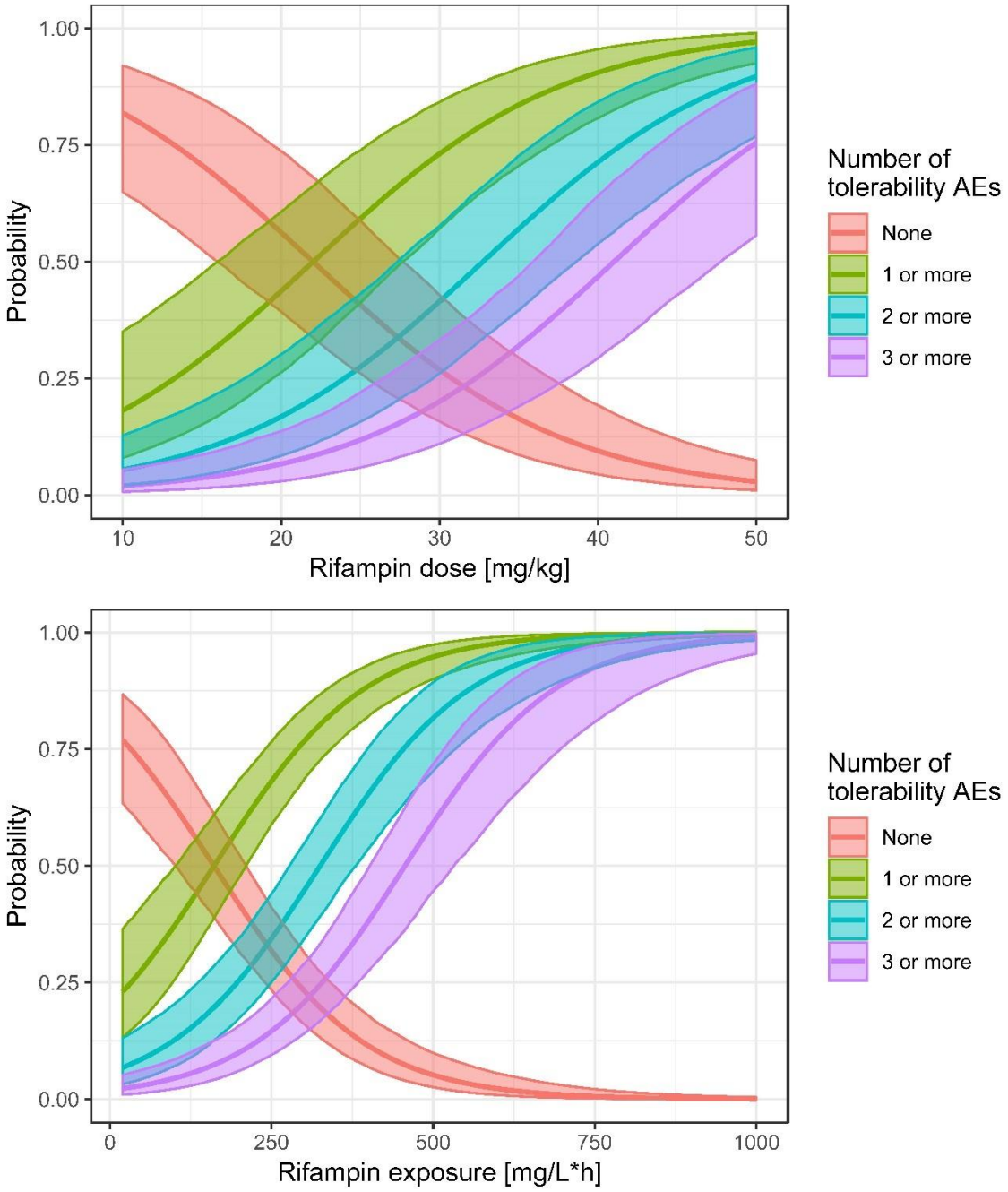


B. 7-day EBA with 95% confidence intervals, log_{10}TTP/day



B. 14-day EBA with 95% confidence intervals, log_{10}TTP/day


Day1
Day3
Day4
Day6
Day7
Day10
Day11
Day14
Day21
Total bilirubin [µmol/L]
Rifampin exposure [mg/L*h]
0
25
50
0
250
500
750
1000


1.00
0.75
0.50
0.25
0.00
Probability
10
20
30
40
50
Rifampin dose [mg/kg]
Number of tolerability AEs
None
1 or more
2 or more
3 or more
1.00
0.75
0.50
0.25
0.00
Probability
0
250
500
750
1000
Rifampin exposure [mg/L*h]
Number of tolerability AEs
None
1 or more
2 or more
3 or more

Supplementary methods: pharmacokinetic methods

Blood samples pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours post-dose for drug quantification were taken on days 7 and 14. Rifampicin concentrations were measured after each cohort at the Department of Pharmacy of the Radboud university medical center, Nijmegen, the Netherlands. Plasma samples of the 40 mg/kg dose group were analyzed using the same validated ultra-performance liquid chromatography method with ultraviolet detection as in the preceding cohorts (6). For the 50 mg/kg group, total (protein-bound plus unbound) and protein-unbound plasma concentrations of rifampicin at day 7 and 14, as well as total plasma concentrations of isoniazid, pyrazinamide and ethambutol on day 14 were measured. To determine total concentrations of anti-TB drugs in the 50 mg/kg cohort an extensively validated liquid chromatography-mass spectrometry (LC-MS/MS) multi-drug assay was used. The assay accuracy for rifampicin quantification was 94.24-102.06% dependent on concentration level, the within run imprecision ranged from 0.9-4.89%, the rifampicin lower limit of quantification (LLOQ) was 0.09 mg/L, and the higher limit of quantification (HLOQ) was 60 mg/L. The multi-drug assay performed well in an external proficiency testing program. Protein-unbound determination of rifampicin occurred via ultrafiltration as previously described (28). Noncompartmental analysis was performed with Phoenix WinNonlin 6.4 (Certara USA, INC., Princeton, NJ) to calculate relevant pharmacokinetic parameters, including the area under the plasma concentration-time curve (AUC_{0-24h} or total exposure) and highest observed plasma concentration (C_{max}), as described previously (11).

Table E1 | Isoniazid, pyrazinamide and ethambutol pharmacokinetics during combination therapy with 50 mg/kg rifampicin at day 14 after start of rifampicin treatment.

PK parameter	50 mg/kg RIF (n=7)
Isoniazid	
AUC_{0-24h} (mg/L*h)	7.9 (4.2-16.3)
C_{max} (mg/L)	2.1 (1.2-5.8)
Pyrazinamide	
AUC_{0-24h} (mg/L*h)	295 (240-386)
C_{max} (mg/L)	30.3 (27.0-42.6)
Ethambutol	
AUC_{0-24h} (mg/L*h)	13.5 (9.4-19.6)
C_{max} (mg/L)	2.2 (1.7-3.2)

Results are given in geometric mean and range, unless stated otherwise.

Table E2 | Incidence of treatment emergent adverse events by System Organ Class during monotherapy.

	40 mg/kg RIF N=15	50 mg/kg RIF N=17
Gastrointestinal disorders[#], n (%)	**7 (47)**	**12 (71)**
Abdominal discomfort, n	1	
Abdominal distension, n	1	1
Abdominal pain, n	3	3
Constipation, n	1	
Upper abdominal pain, n		1
Diarrhoea, n		2
Dysgeusia, n	1	3
Dyspepsia, n		6
Faeces discoloured, n		1
Flatulence, n		1
Frequent bowel movements, n	1	
Nausea, n	2	5
Vomiting, n		5
Investigations, n (%)	**4 (27)**	**10 (59)**
ALT increased, n		2
AST increased, n		2
Alkaline phosphatase increased, n		1
Bilirubin increased, n	4	10
Hepatic enzymes increased, n		1
Skin and subcutaneous tissue disorders[#], n (%)	**9 (60)**	**10 (59)**
Pruritis, n	9	6
Generalised pruritis, n		3
Rash, n		1
Rash pruritic, n		1
Hepatobiliary disorders, n (%)		**9 (53)**
Hepatitis, n		1
Jaundice, n		9
Nervous System Disorders[#], n (%)	**4 (27)**	**6 (35)**
Dizziness, n		2
Headache, n	3	4
Musculoskeletal chest pain, n	1	
Neuropathy peripheral, n	1	
Somnolence, n		1

Musculoskeletal and connective tissue disorders[#], n (%)	**4 (27)**	**5 (29)**
Arthralgia, n	1	2
Backpain, n	2	1
Muscular weakness, n		1
Musculoskeletal pain, n		1
Myalgia, n	1	
Eye disorders[#], n (%)	**2 (13)**	**4 (24)**
Dry eye, n	1	1
Eye irritation, n		3
Eye swelling, n	1	
General disorders / administration site conditions[#], n (%)	**1 (7)**	**3 (18)**
Fatigue, n		1
Malaise, n		1
Night sweats, n		1
Peripheral swelling, n	1	
Pyrexia, n		1
Respiratory, thoracic and mediastinal disorders[#], n (%)		**2 (12)**
Epistaxis, n		1
Oropharyngeal pain, n		1
Renal and urinary disorders, n (%)		**3 (18)**
Dysuria, n		2
Proteinuria, n		1
Blood and lymph disorders, n (%)		**1 (6)**
Anaemia, n		1

Results are given in number (%) of patients. Patients can experience multiple events within a System Organ Class.

[#] System Organ Class included in composite tolerability endpoint. Classes and all their events were included if they contained at least one tolerability-related event. Laboratory abnormalities and related disorders were specifically excluded.

Table E3 | Incidence of treatment emergent adverse events by System Organ Class during combination therapy.

	40 mg/kg RIF N=15	50 mg/kg RIF N=10
Gastrointestinal disorders, n (%)	**4 (27)**	**4 (40)**
Abdominal discomfort, n	1	
Abdominal pain, n		2
Constipation, n	1	1
Diarrhoea, n	1	2
Dyspepsia, n	1	
Nausea, n		1
Vomiting, n	1	1
Investigations, n (%)	**2 (13)**	**3 (30)**
ALT increased, n		1
AST increased, n	1	
Bilirubin increased, n		1
Creatinine increased, n		1
Hepatic enzymes increased, n	2	
Potassium increased		1
Skin and subcutaneous tissue disorders, n (%)	**4 (27)**	**6 (60)**
Pruritis, n	4	6
Rash, n		2
Urticaria, n		1
Hepatobiliary disorders, n (%)	**2 (13)**	
Hepatitis, n		
Jaundice, n	2	
Nervous System Disorders, n (%)	**3 (20)**	**2 (20)**
Dizziness, n		1
Headache, n	3	2
Musculoskeletal and connective tissue disorders, n (%)	**1 (7)**	**1 (10)**
Musculoskeletal pain, n		1
Musculoskeletal chest pain, n	1	
Pain in extremity, n	1	
Eye disorders, n (%)		**1 (10)**
Conjunctivitis, n		1
General disorders / administration site conditions, n (%)		**2 (20)**
Fatigue, n		1
Puncture site pain, n		1

Respiratory, thoracic and mediastinal disorders, n (%)	**2 (13)**	**2 (20)**
Chest pain, n	1	1
Haemoptysis, n	1	
Oropharyngeal pain, n		1
Metabolism and nutrition disorders, n (%)	**3 (20)**	
Decreased appetite, n	1	
Hyperuricaemia, n	2	
Blood and lymph disorders, n (%)		**1 (10)**
Anaemia, n		1

Results are given in number (%) of patients. Patients can experience multiple events within a System Organ Class.

Figure E1| Rifampicin doses according to body weight for all HIGHRIF1 cohorts.

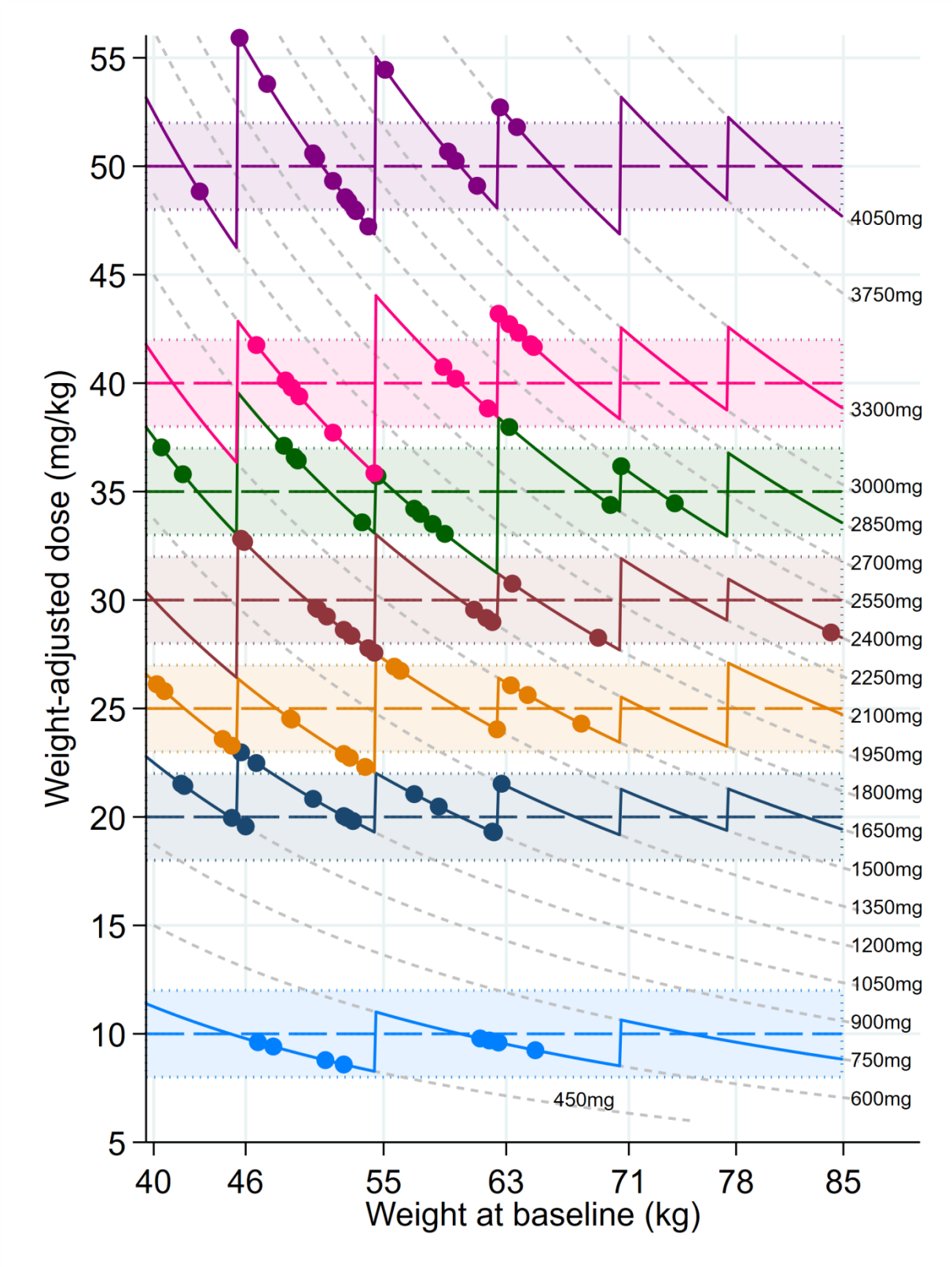
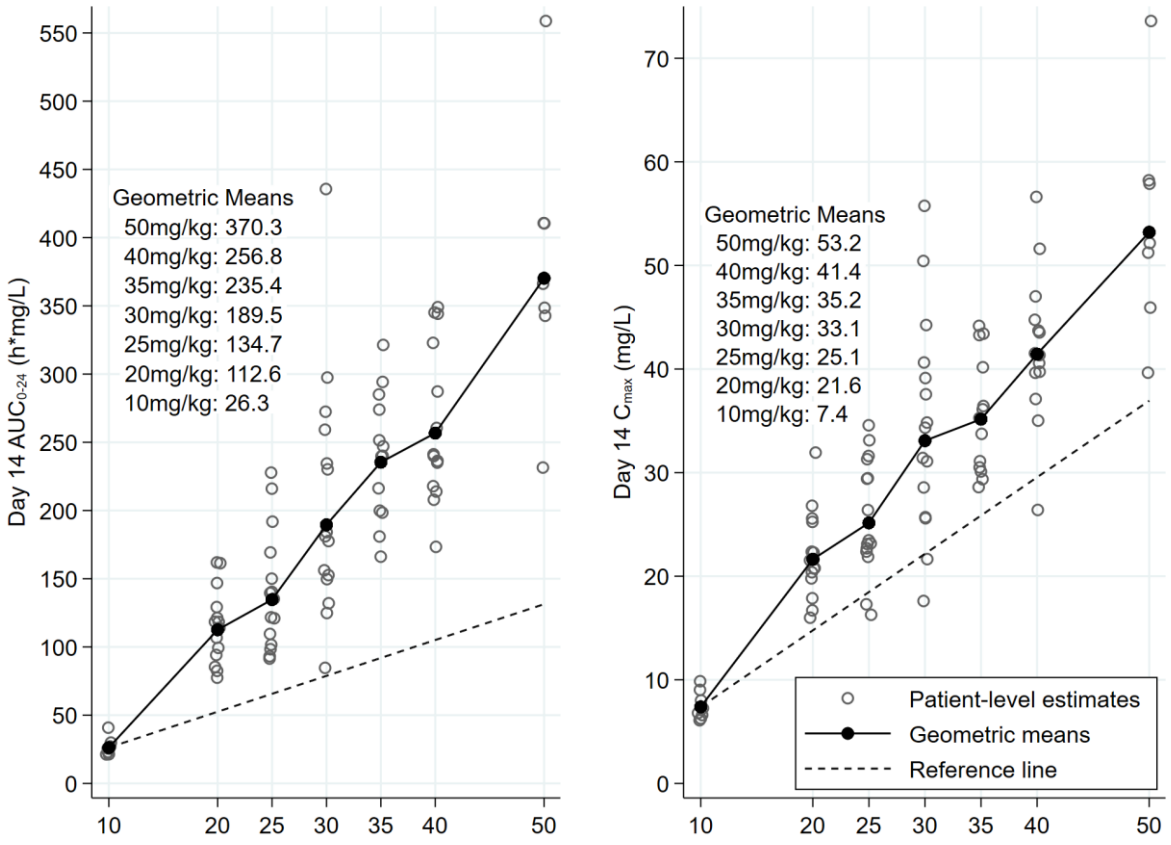


Figure E2| Distribution of exposure to rifampicin ($AUC_{0–24h}$) at day 14 (steady state) in the various rifampicin dose groups.

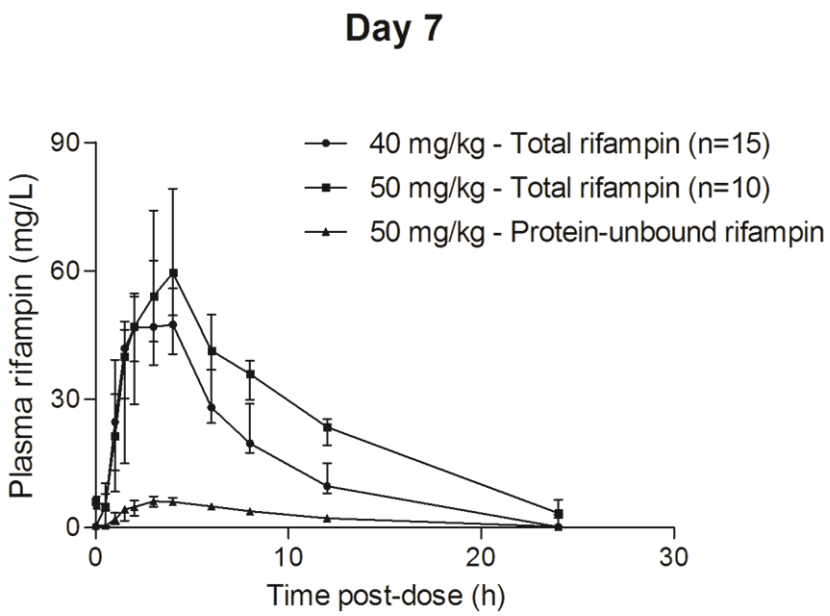


The reference line mimics a linear relationship.

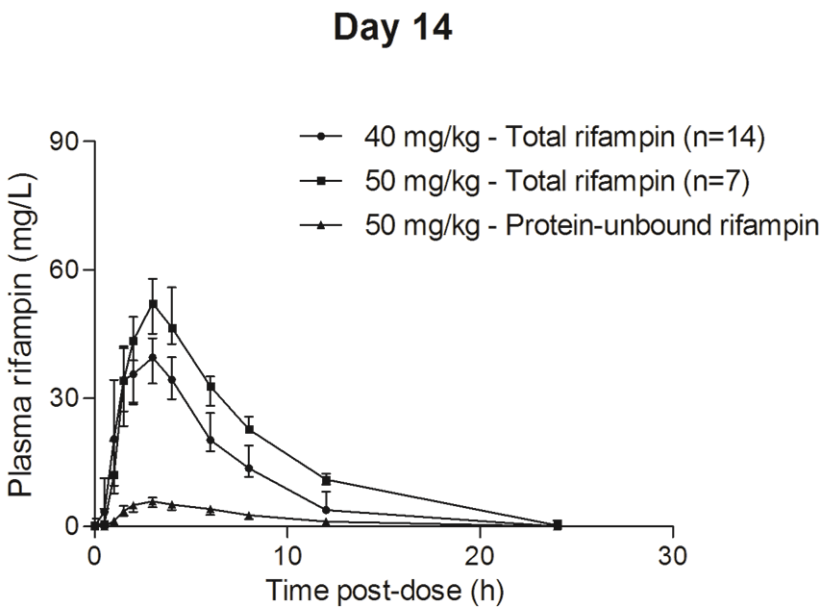
AUC = area under the plasma concentration-time curve; C_{max} = peak plasma concentration.

Figure E3| Pharmacokinetic profiles at day 7 (A) and 14 (B) after start of daily 40 mg/kg and 50 mg/kg rifampicin.

A)



B)

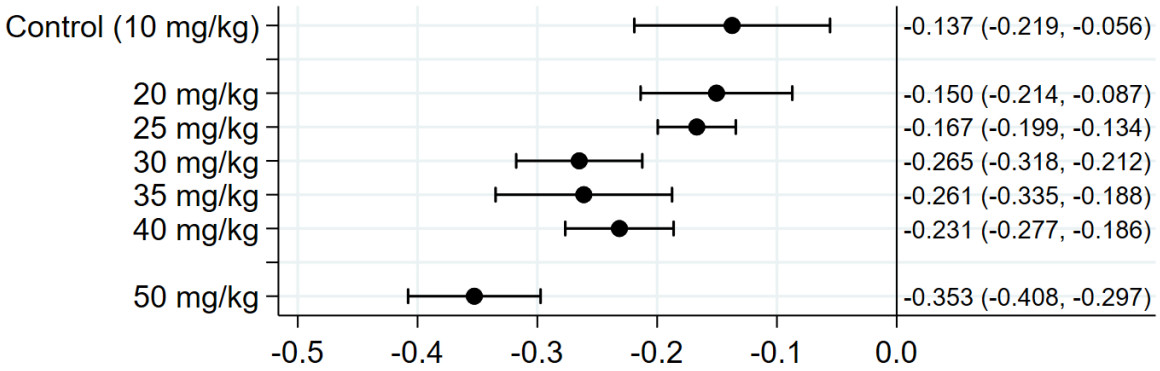


Concentrations per time point are presented as median ± interquartile range.

Figure E4 | Early bactericidal activity (EBA) of rifampicin based on CFU (A) and TTP (B) for patients with cultures after day 5.

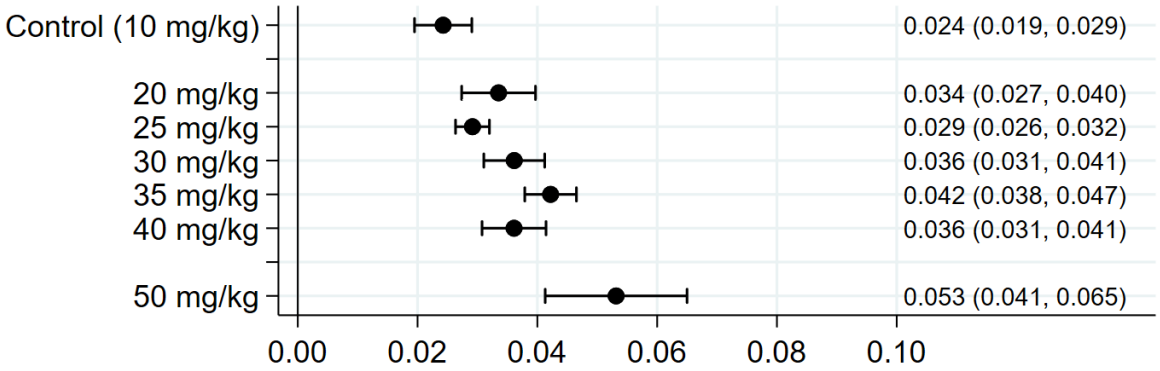
Data from all patients were included with the exception of data from one patient from each of 20mg/kg and 50mg/kg group that had consistent negative cultures at baseline and throughout, and from six patients in the 50mg/kg group without any cultures after day 5.

A) 14-day EBA with 95% confidence intervals, log_{10}CFU/ml/day



The slight change in CFU estimates for 10 mg/kg and 20 mg/kg compared to previously (6) are because of data corrections (4 CFU cultures were recorded as negative while they were actually missing).

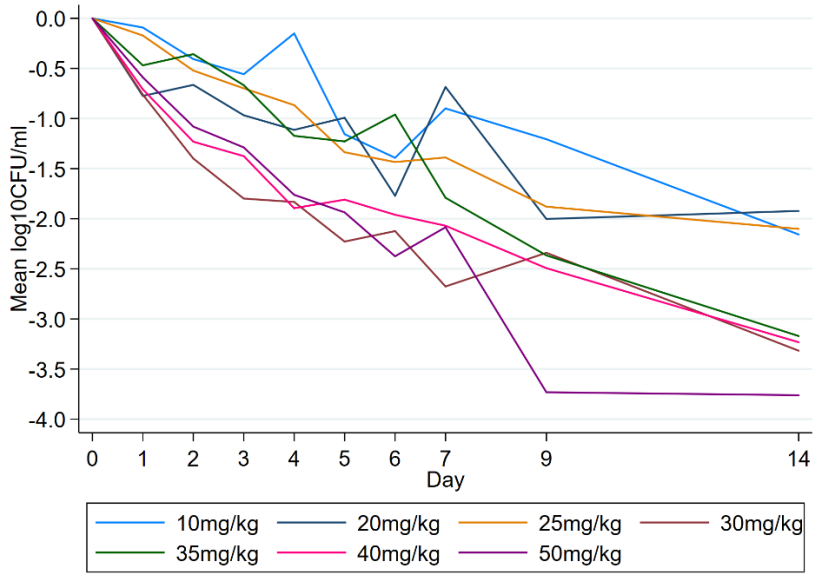
B) 14-day EBA with 95% confidence intervals, log_{10}TTP/day



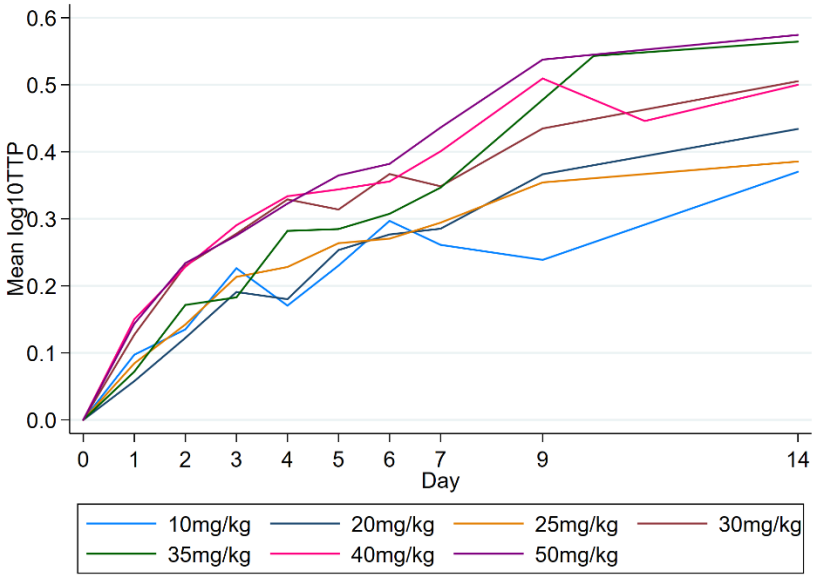
CFU = colony-forming units; TTP = time to positivity

Figure E5 | Fitted estimates of differences from mean baseline log10 CFU (A) and log10 TTP per millilitre (B) by visit and treatment arm. Data from all patients included with the exception of data from one patient from each of 20mg/kg and 50mg/kg group that had consistent negative cultures at baseline and throughout.

A)

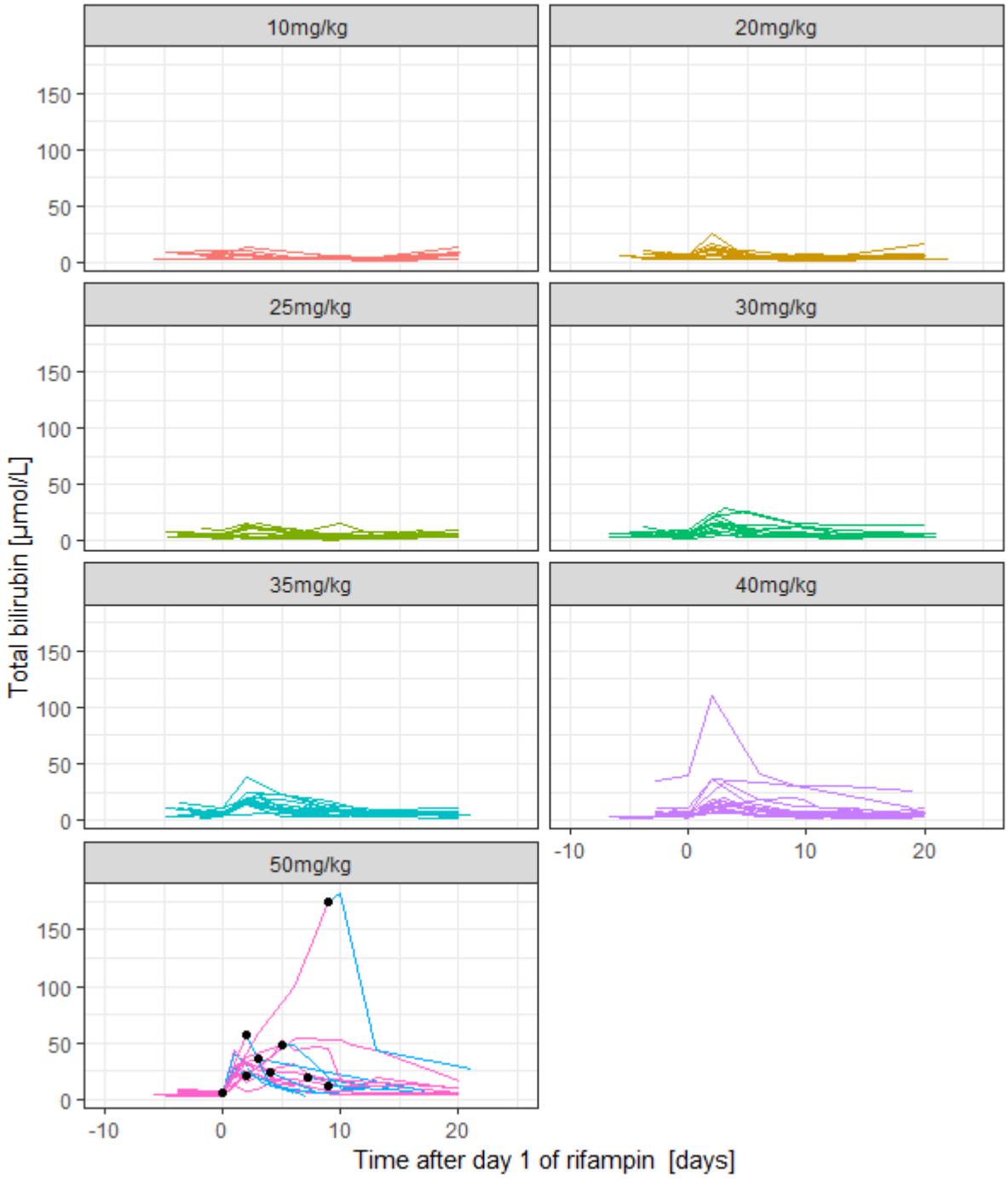


B)



CFU = colony-forming units; TTP = time to positivity

Figure E6 | Total serum bilirubin over day on rifampicin treatment per dose group (n=100).



The black dots in the 50 mg/kg group represents when patients interrupted rifampicin treatment and the blue lines represent measurements after rifampicin was stopped.

Figure E7 | Rifampicin total exposures ($AUC_{0\text{-}24h}$) plotted against total ALT in the HIGHRIF1 study (n=93).

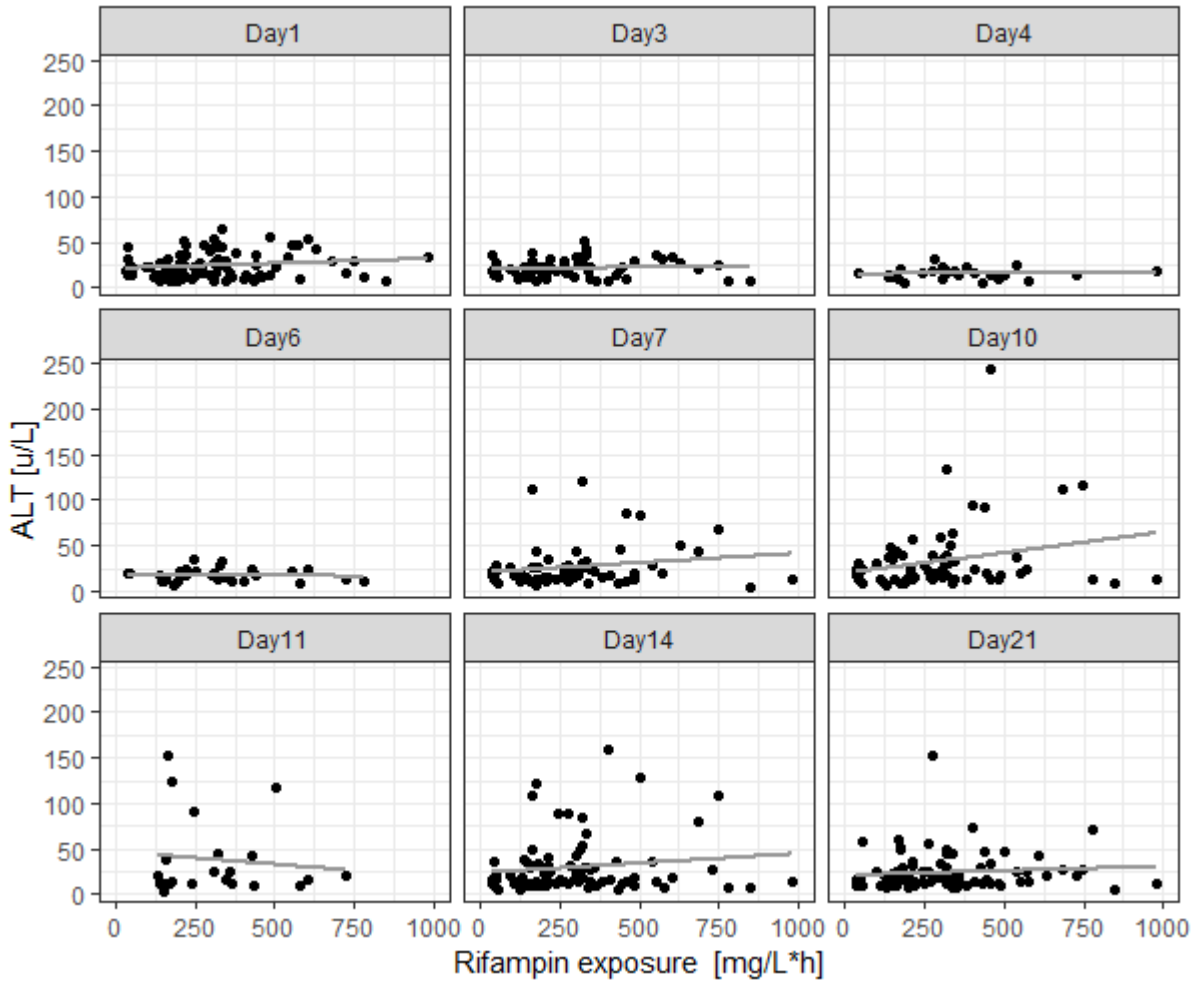


Figure E8 | Rifampicin total exposures ($AUC_{0\text{-}24h}$) plotted against total AST in the HIGHRIF1 study (n=93).

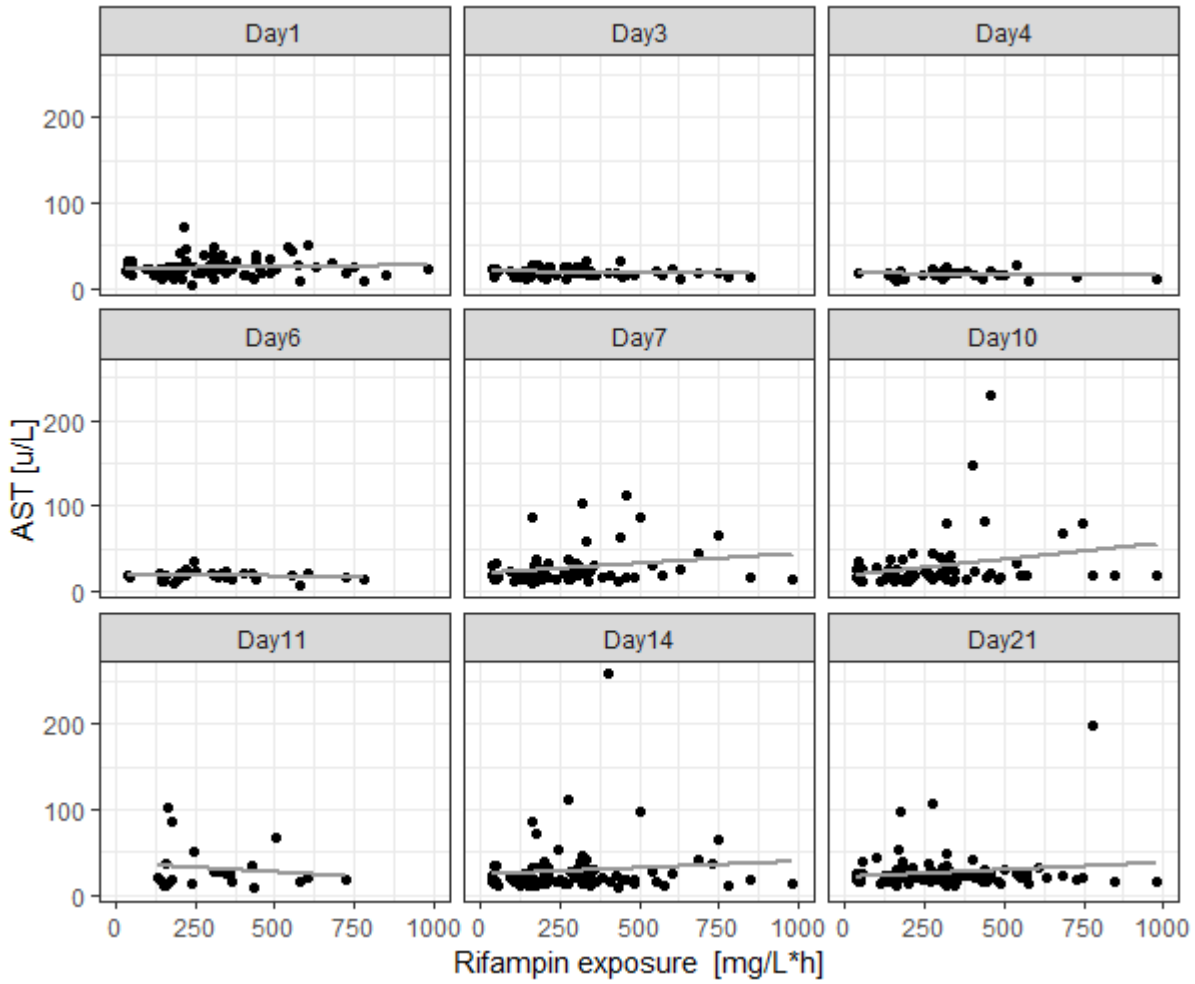
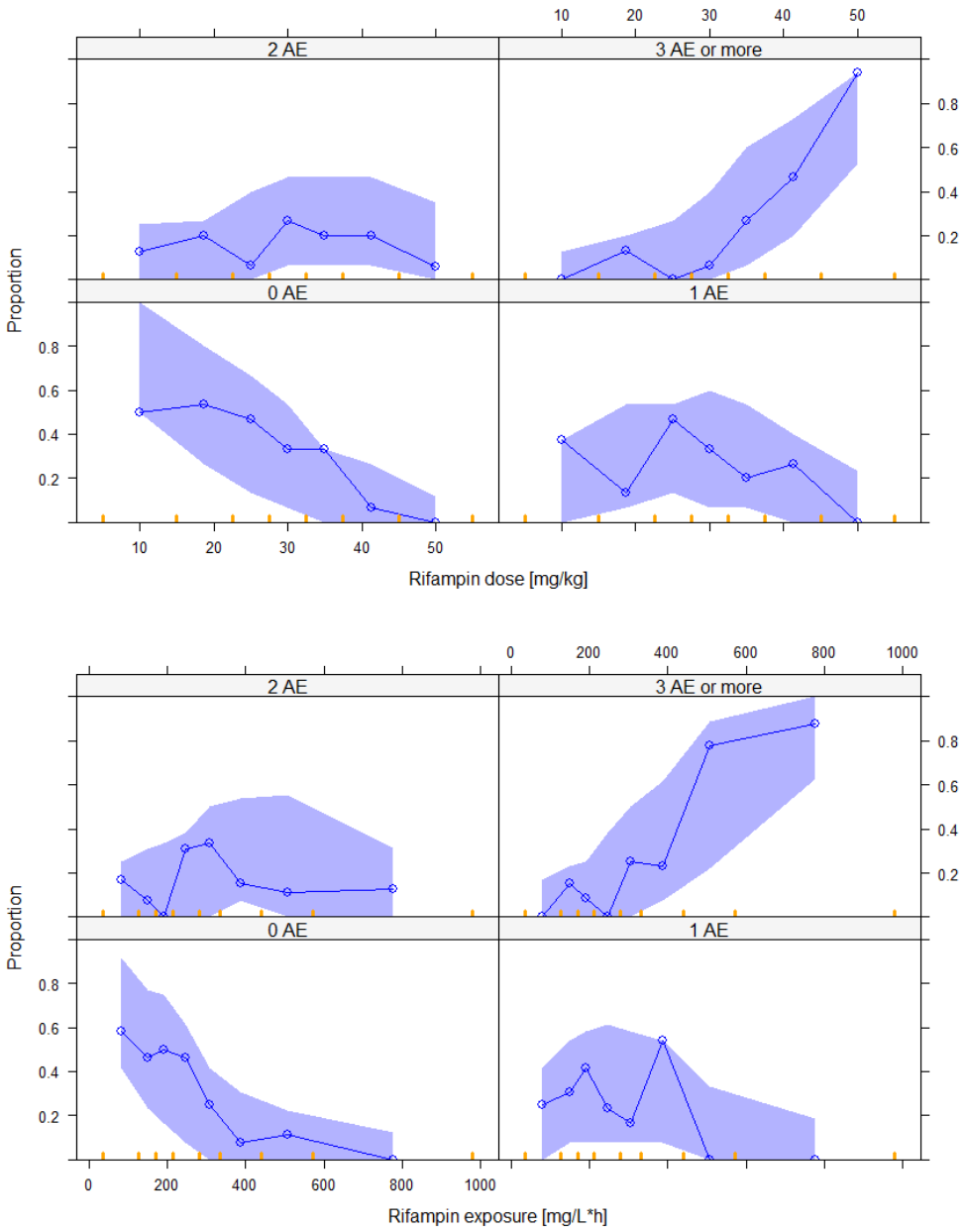
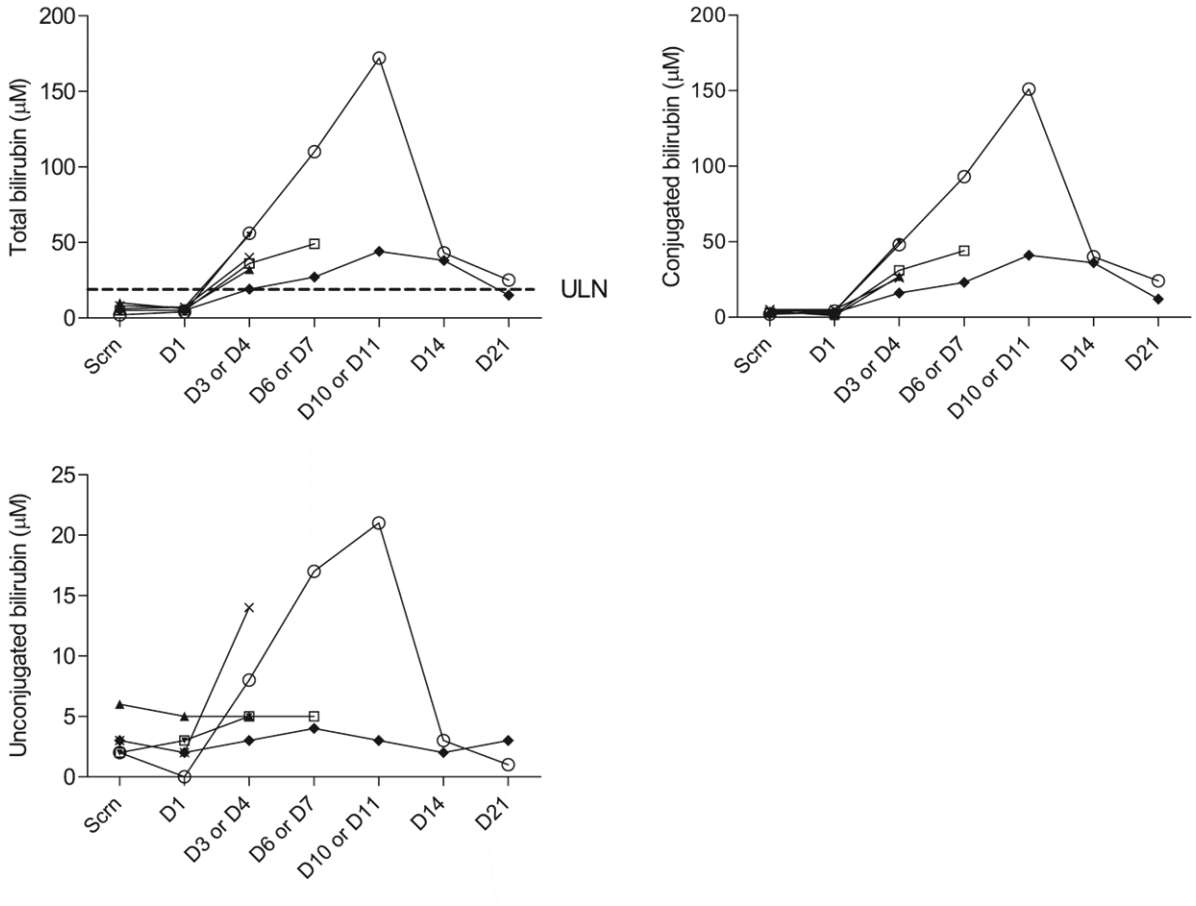


Figure E9 | Visual predictive checks demonstrating the goodness-of-fit of the dose-tolerability (upper panel) and exposure-tolerability (lower panel) ordered categorical models.



The blue rings represent the observed proportion of patients with a given number of tolerability-related adverse events (AEs), the blue lines connect these proportions and the shaded areas are the 95% confidence intervals for the model-predicted proportions. Rifampicin exposure is $AUC_{0\text{-}24h}$ at day 7.

Figure E10 | Total, conjugated and unconjugated bilirubin in the first six patients of the 50 mg/kg cohort during scheduled safety visits.



Scr = screening; D1 = day 1 of treatment; ULN = Upper Limit of Normal