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.

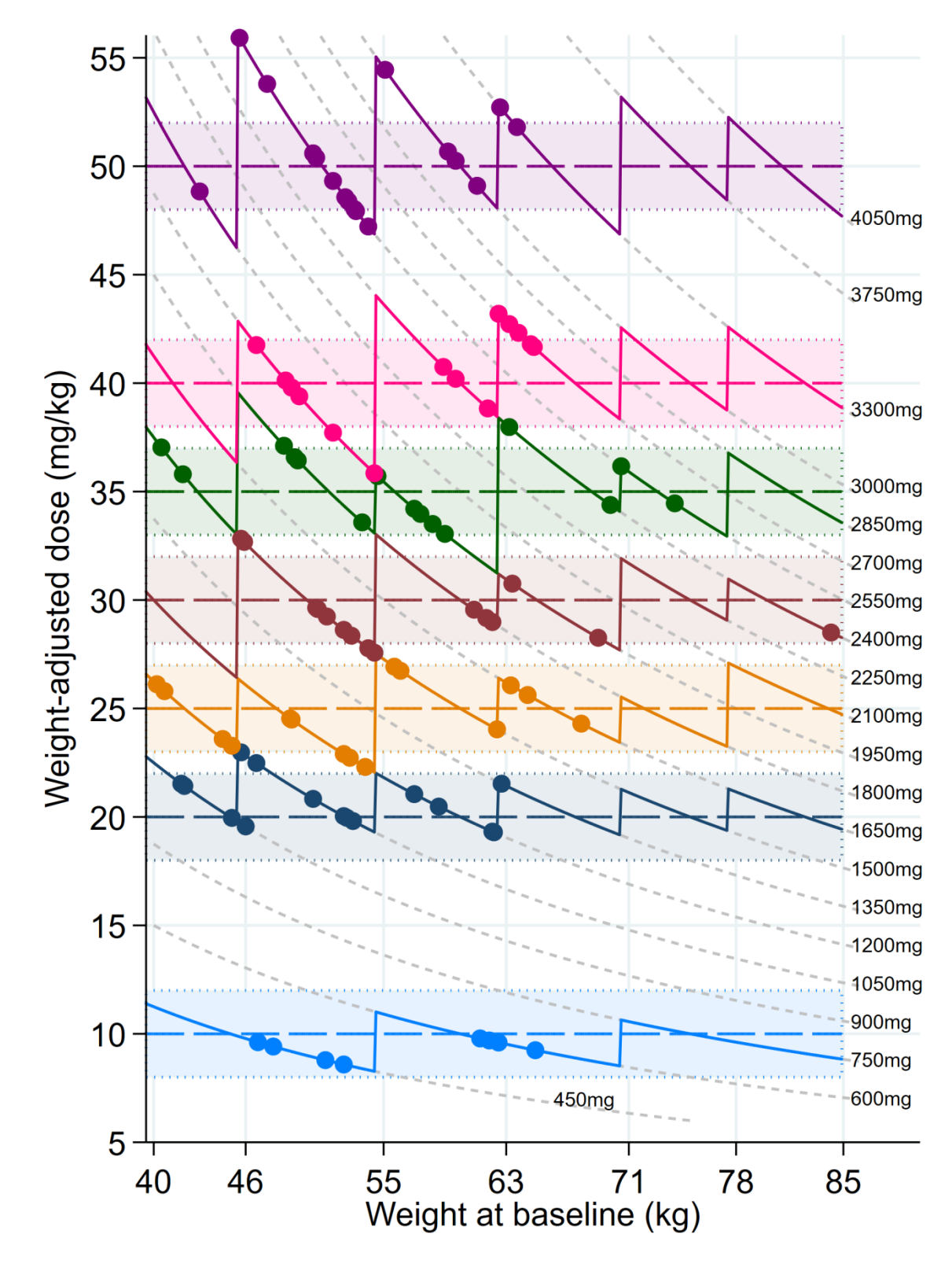
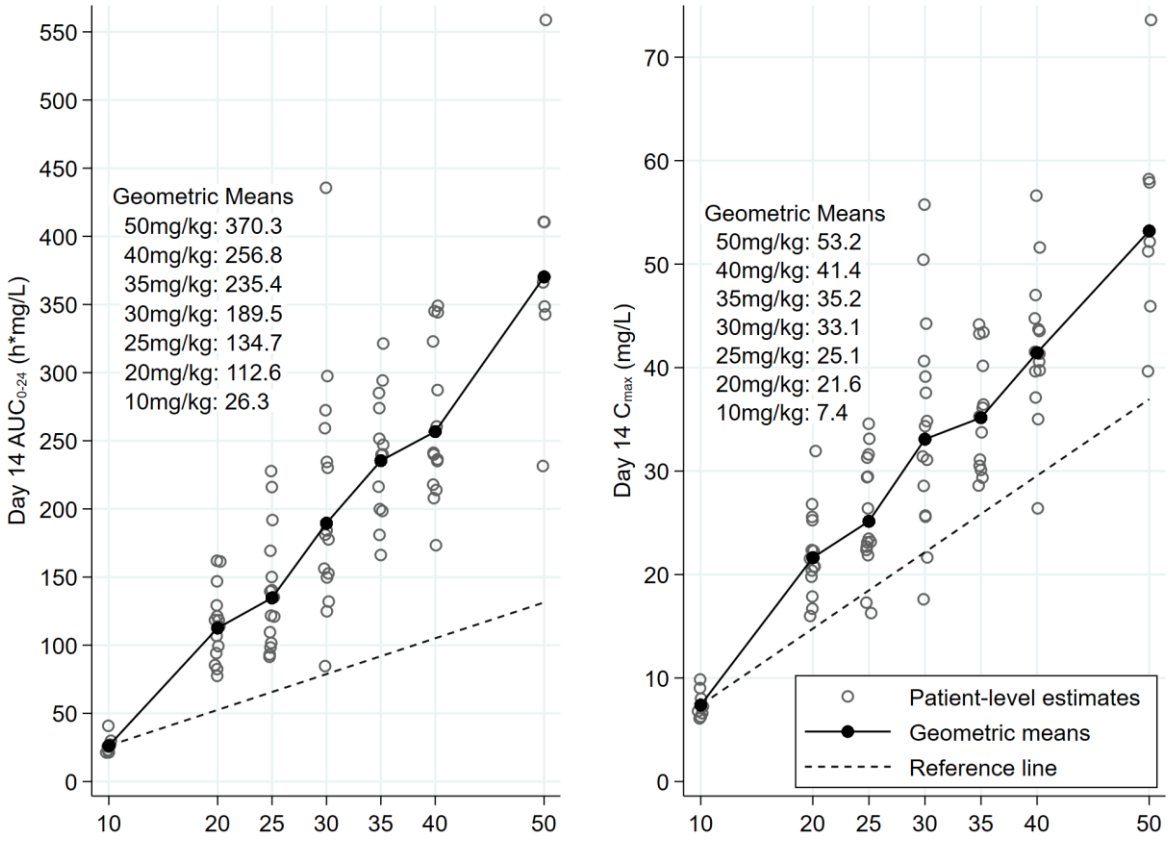


Figure E2| Distribution of exposure to rifampicin ($AUC_{0–24h}$) at day 14 (steady state) in the various rifampicin dose groups.

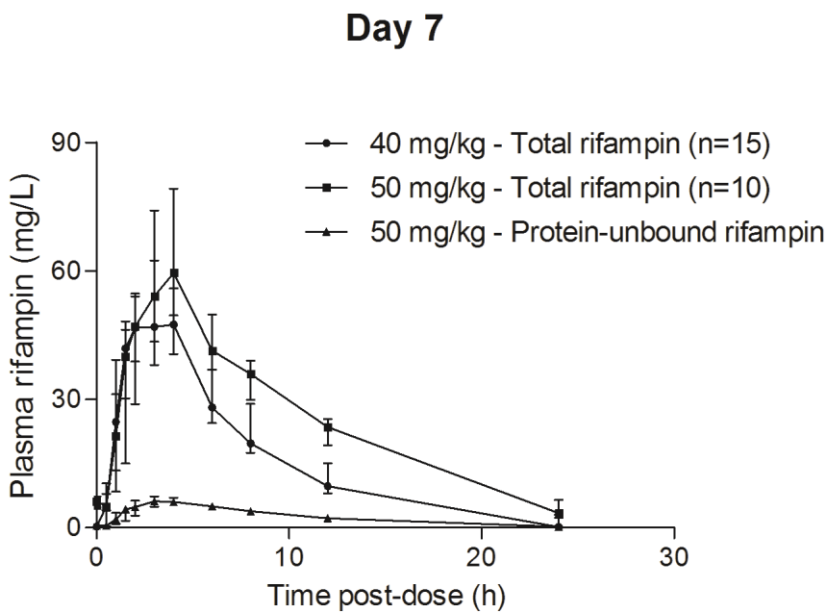


The reference line mimics a linear relationship.

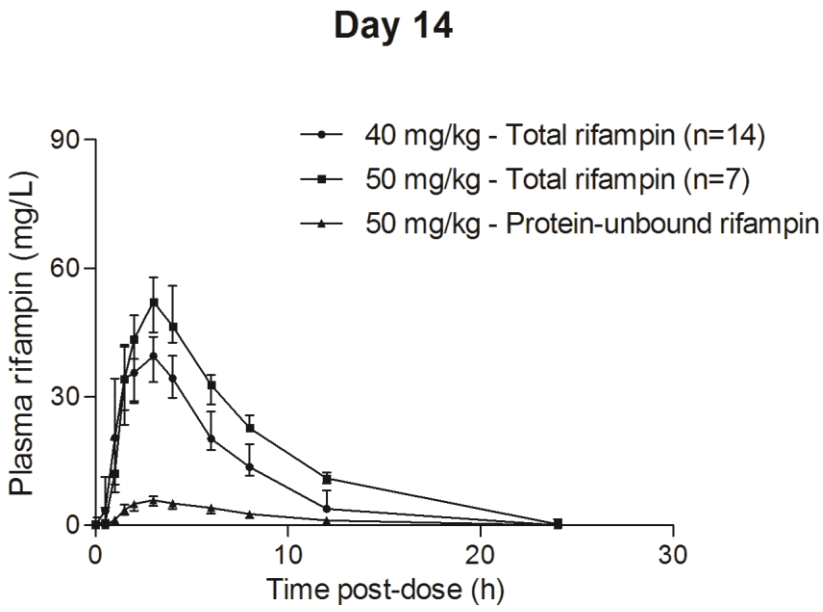
AUC = area under the plasma concentration-time curve; C_{max} = peak plasma concentration.

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)

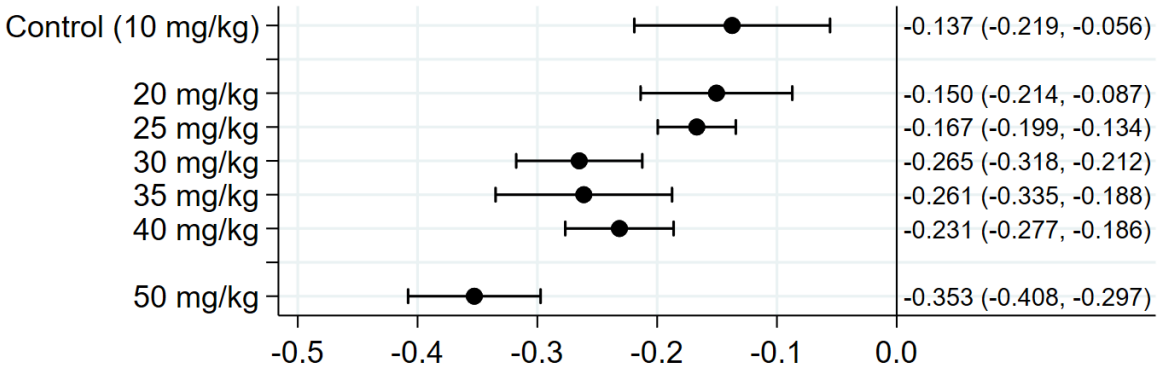


Concentrations per time point are presented as median ± interquartile range.

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

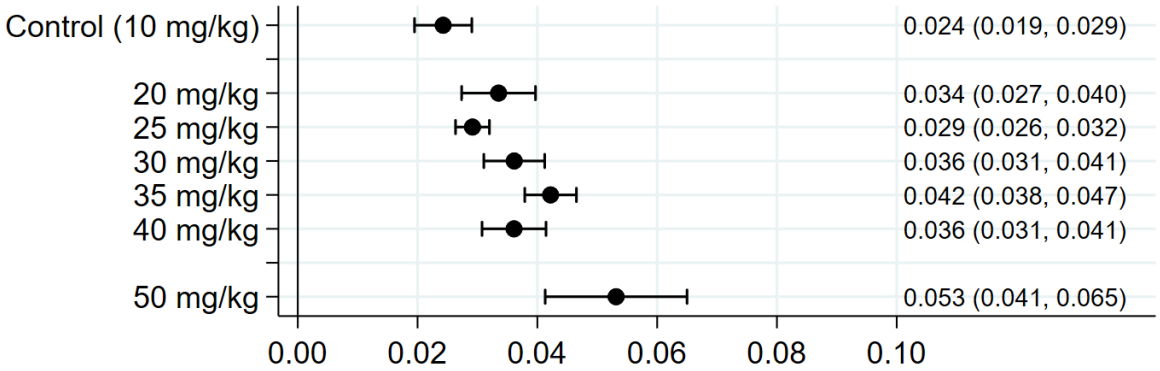
Data from all patients were included with the exception of data from one patient from each of 20mg/kg and 50mg/kg group that had consistent negative cultures at baseline and throughout, and from six patients in the 50mg/kg group without any cultures after day 5.

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).

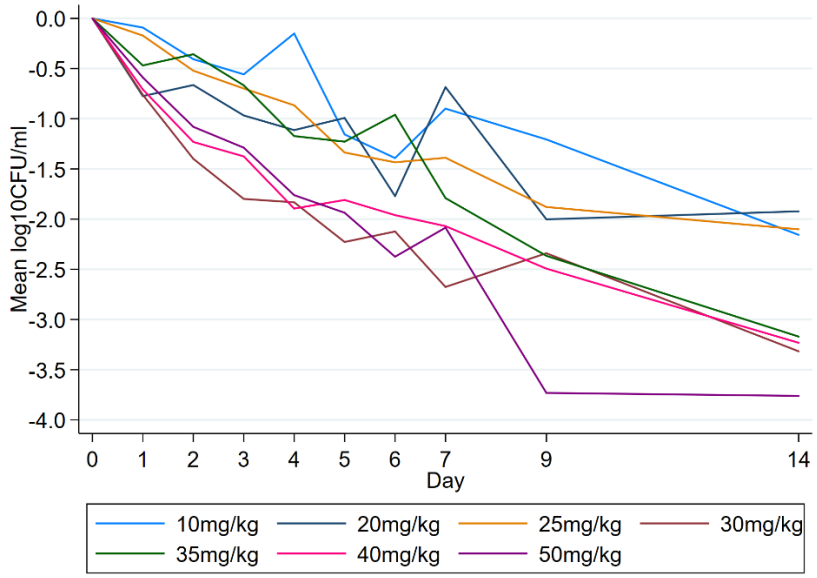
B) 14-day EBA with 95% confidence intervals, log_{10}TTP/day



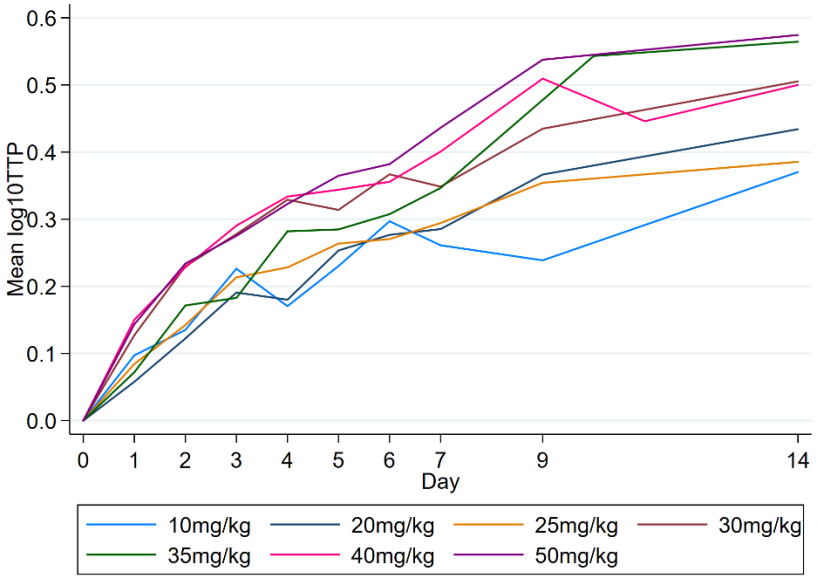
CFU = colony-forming units; TTP = time to positivity

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)

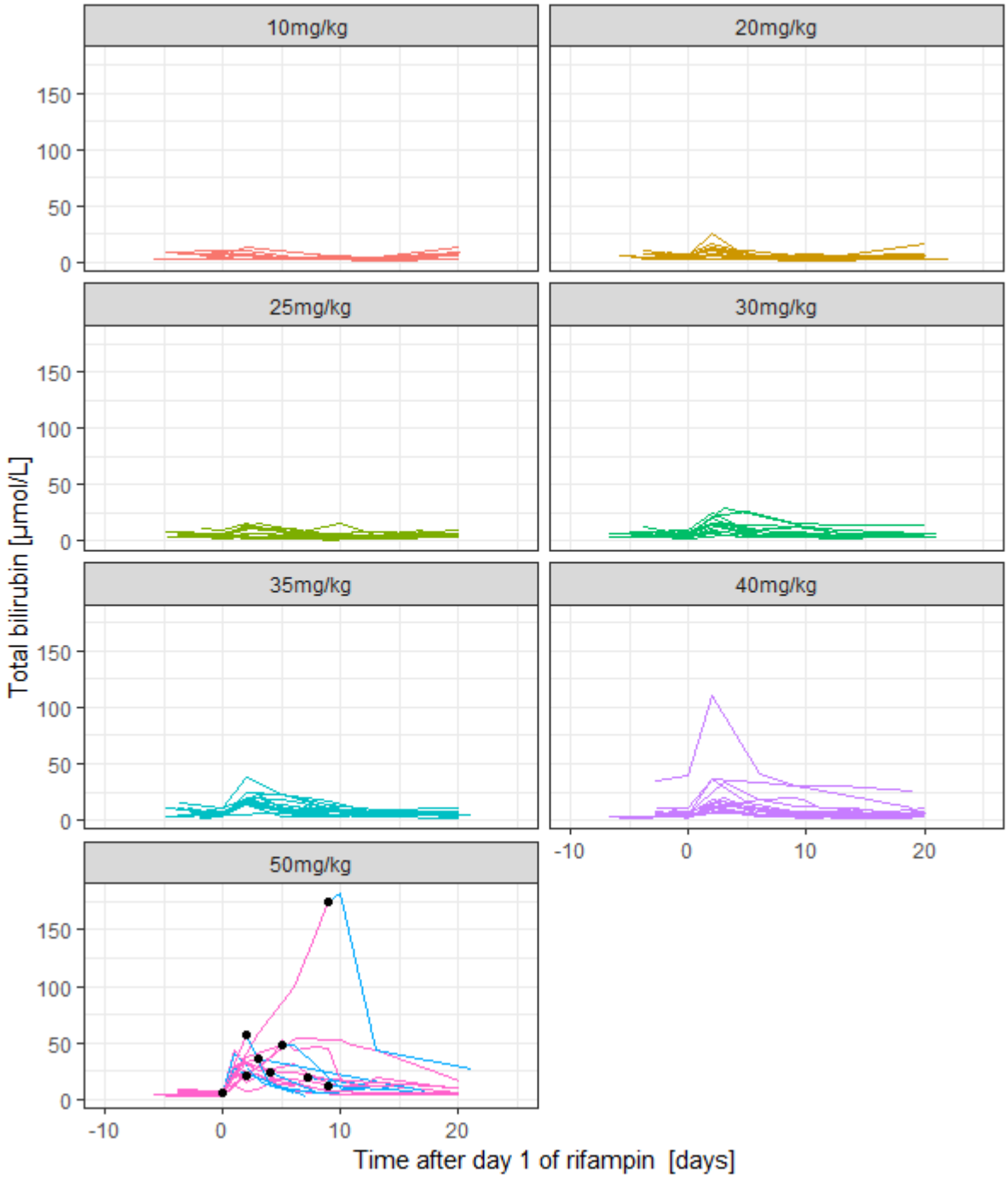


B)



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).

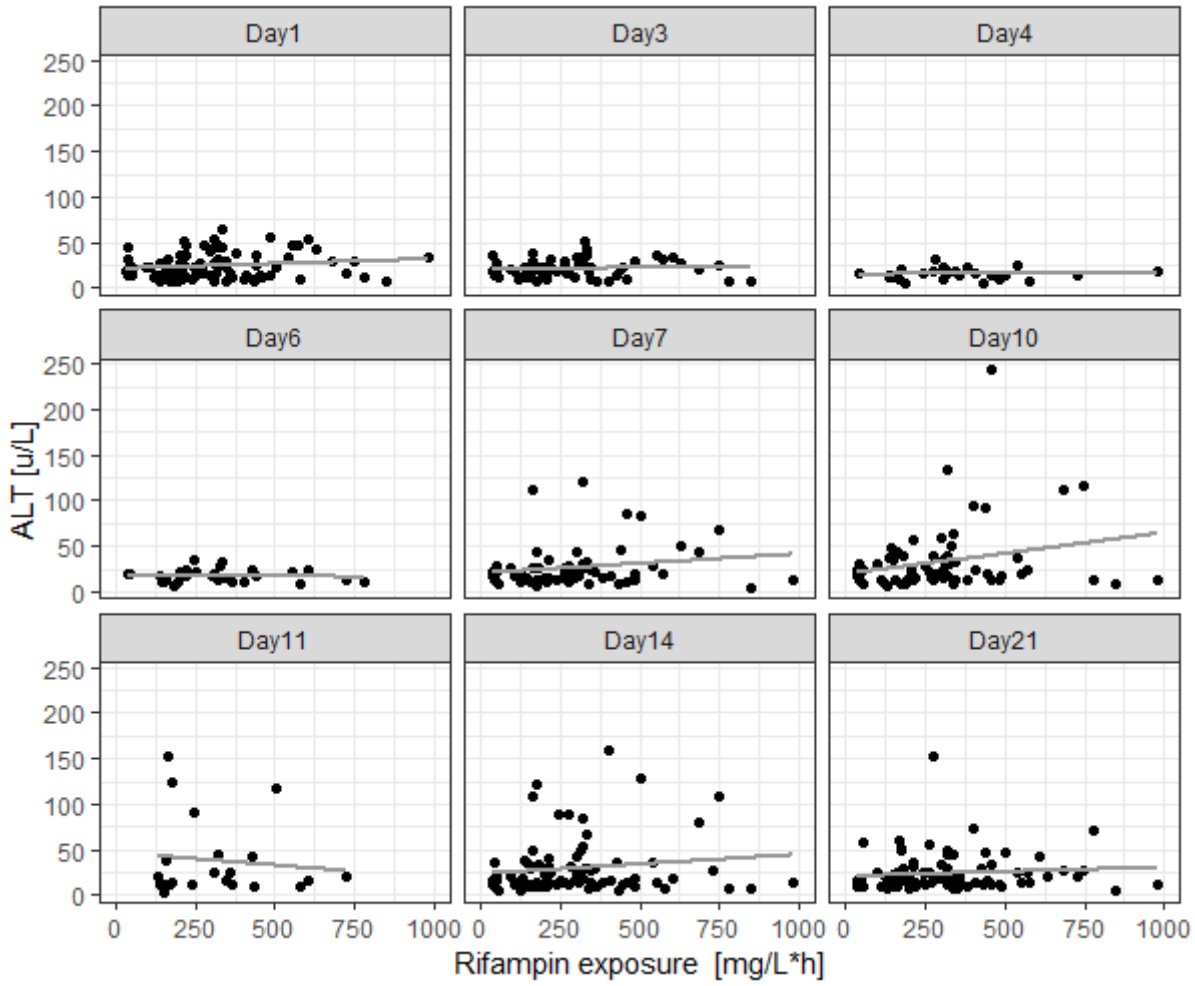


Figure E8 | Rifampicin total exposures ($AUC_{0\text{-}24h}$) plotted against total AST in the HIGHRIF1 study (n=93).

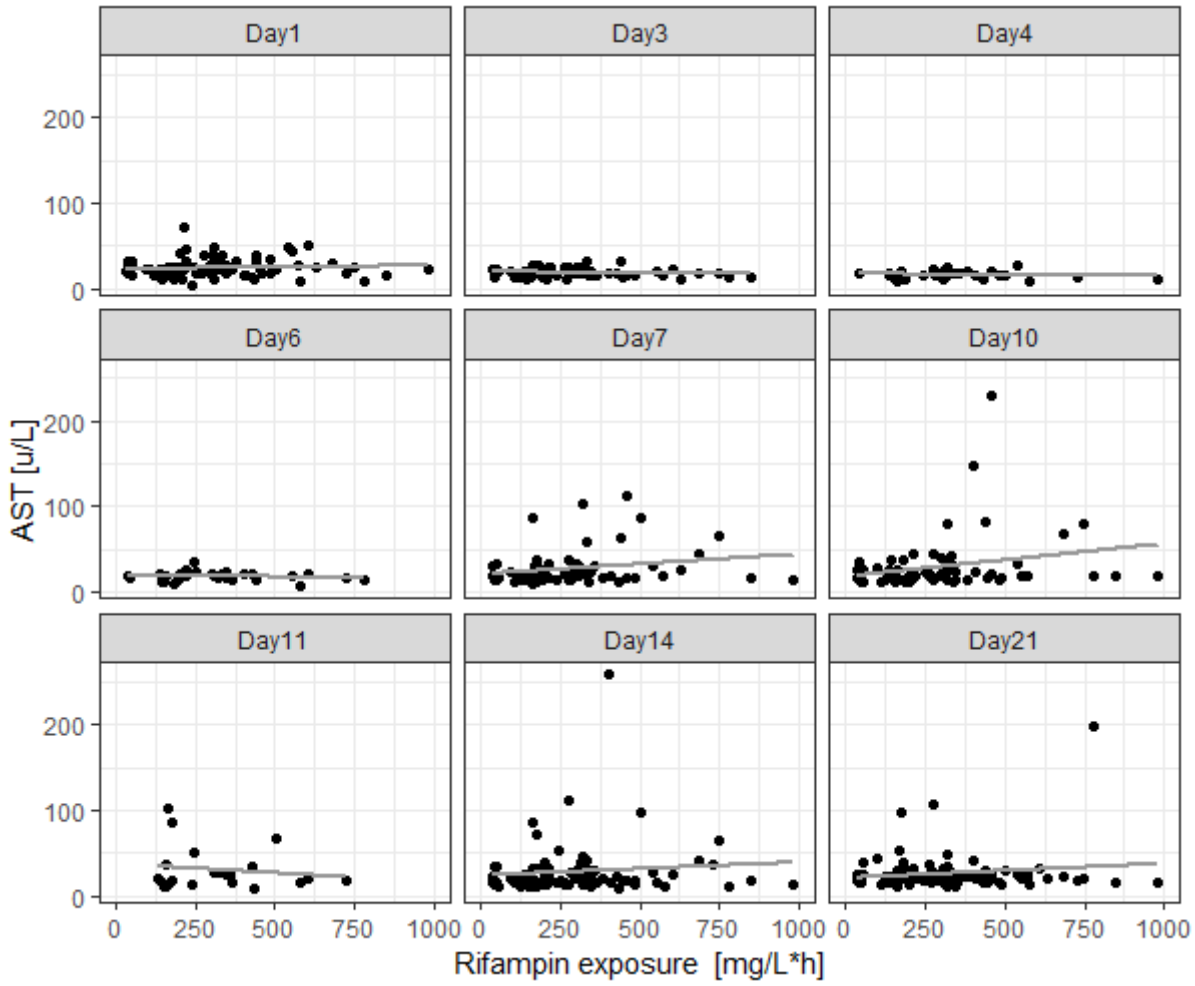
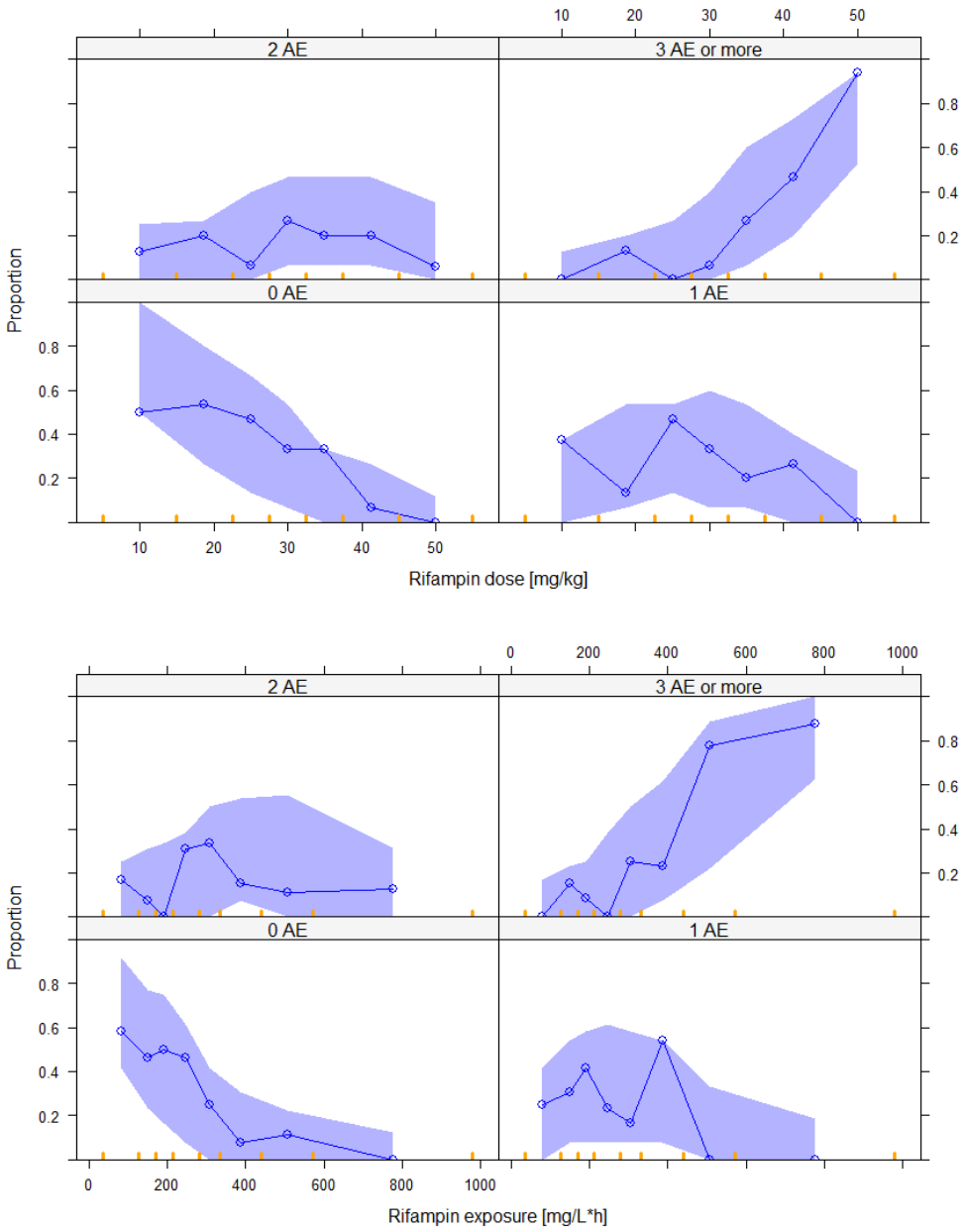
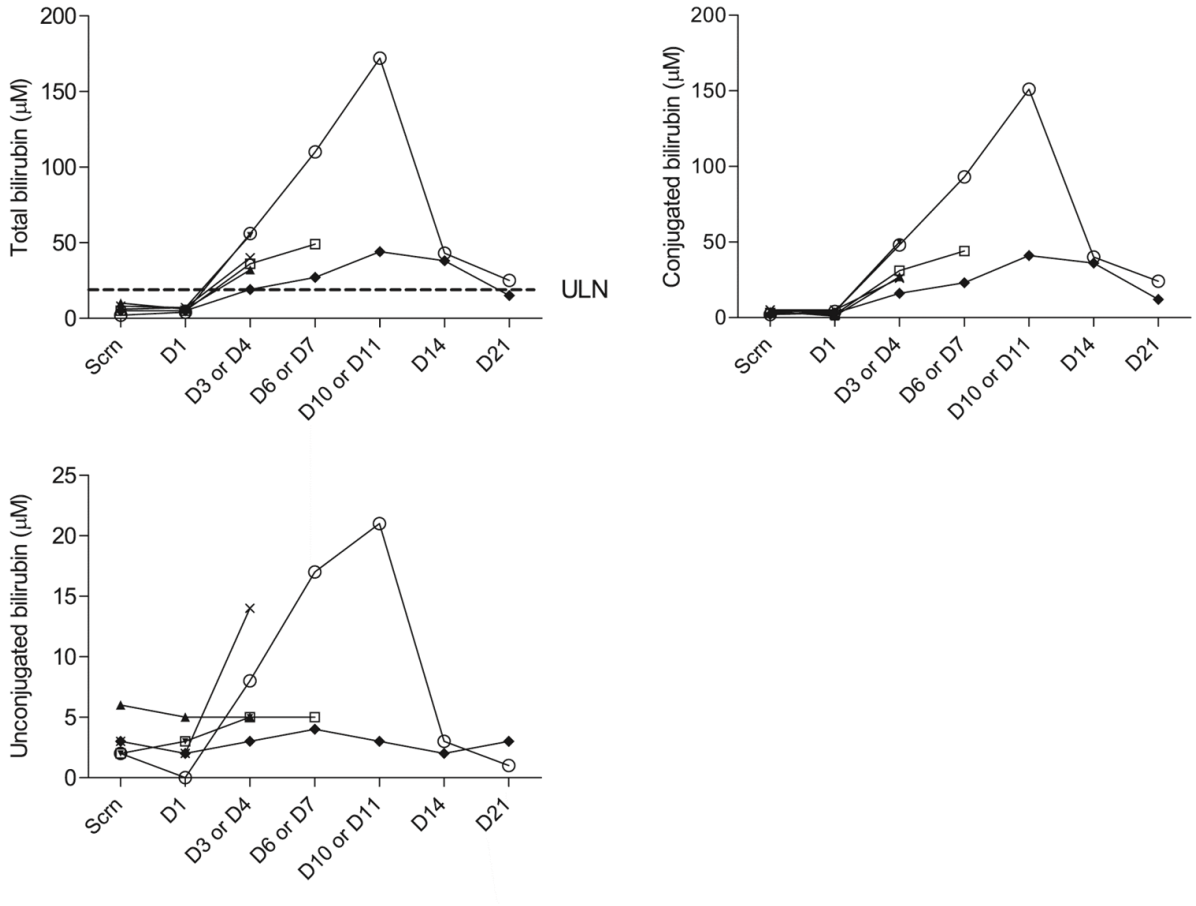


Figure E9 | Visual predictive checks demonstrating the goodness-of-fit of the dose-tolerability (upper panel) and exposure-tolerability (lower panel) ordered categorical models.



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