ORIGINAL ARTICLE



Evaluation of pharmacokinetic-pharmacodynamic relationships and selection of drug combinations for tuberculosis

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Aims: Despite evidence of the efficacy of anti-tubercular drug regimens in clinical practice, the rationale underpinning the selection of doses and companion drugs for combination therapy remains empirical. Novel methods are needed to optimise the antibacterial activity in combination therapies. A drug-disease modelling framework for rational selection of dose and drug combinations in tuberculosis is presented here.

Methods: A model-based meta-analysis was performed to assess the antibacterial activity of different combinations in infected mice. Data retrieved from the published literature were analysed using a two-state bacterial growth dynamics model, including fast- and slow-growing bacterial populations. The contribution of each drug to the overall antibacterial activity of the combination was parameterised as relative change to the potency of the backbone drug (EC_{50} -F and/or EC_{50} -S). Rifampicin and bedaquiline were selected as paradigm drugs to evaluate the predictive performance of the modelling approach.

Results: Pyrazinamide increased the potency (EC_{50} -F and EC_{50} -S) of rifampicin (RZ) and bedaquiline (BZ) by almost two-fold. By contrast, pretomanid and isoniazid were found to worsen the antibacterial activity of BZ and RZ, respectively. Following extrapolation of *in vivo* pharmacokinetic-pharmacodynamic relationships, the dose of rifampicin showing maximum bactericidal effect in tuberculosis patients was predicted to be 70 mg·kg⁻¹ when given in combination with pyrazinamide.

Conclusions: The use of a drug-disease modelling framework may provide a more robust rationale for extrapolation and selection of dose and companion drugs in humans. Our analysis demonstrates that RZ and BZ should be considered as a backbone therapy in prospective novel combination regimens against tuberculosis.

KEYWORDS

bedaquiline, combination therapy, dose rationale, rifampicin, translational PKPD modelling, tuberculosis

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1 | INTRODUCTION

The development of shorter treatments remains a high priority in tuberculosis (TB) research. Unfortunately, recent efforts to develop novel and shorter combination regimens have been either unsuccessful or cancelled prematurely upon reaching Phase 3.1,2 These results raise an important question regarding the cause of such failures in late clinical development. In fact, historically poor efficacy has been considered the greatest cause of attrition in clinical trials.^{3,4} In this regard, it should be noted that pharmacokinetic-pharmacodynamic (PKPD) relationships have been systematically overlooked or not considered as basis for the translation of preclinical findings. Evolving understanding of pharmacokinetics (PK) and pharmacodynamics (PD) of anti-infectives has provided insight into the importance of achieving efficacious drug levels at the site of action to ensure the desired response (i.e., reducing or preventing relapses) and appropriate dose rationale for patients.⁵ Examples of such advancement are illustrated by the development of antivirals, in particular those used for the treatment of human immunodeficiency virus (HIV).⁶⁻⁸ A similar approach is urgently needed to tackle the slowly increasing epidemics of TB.

While *in vitro* systems, such as the hollow-fibre system model of tuberculosis (HFS-TB), have been proposed for the screening of single drugs and combinations, ⁹ they fail to replicate the physiologic conditions associated with the onset and progression of human infection, during which bacterial growth occurs in the presence of different phenotypes or subpopulations. These subpopulations appear to have different metabolic activity and consequently show different sensitivity to drug effects.

On the other hand, due to their ease of handling, relatively low cost and good predictive accuracy, ¹⁰ murine infection models have been commonly used as *in vivo* models for early evaluation of antitubercular drugs. ¹¹ Even though it has been shown that no mouse model fully recapitulates all relevant aspects of the human disease (e.g. pathophysiology, route of infection, bacillary burden or *M. tuberculosis* strain), ¹² they provide insight into the contribution of the different bacterial phenotypes to the progression of the disease, and can be used as basis for prediction of human doses and extrapolation of the overall response to antibiotics. ¹³

Previously, we have shown how a model-based approach can be used to characterise bacterial growth dynamics, enabling the distinction between disease- and drug-specific properties. ¹⁴ The model was developed based on a hypothesis initially proposed by Mitchison, ¹⁵ who first described the time course of infection in humans based on the existence of different mycobacteria populations with different sensitivity to antibiotics. In fact, Mitchison hypothesised that most tubercle bacilli in the lesions at the start of infection consist of fast-growing bacteria, which, if treated with antibiotics such as isoniazid (INH), are killed rapidly. Other bacilli are slow-growing or semi-dormant and as such require prolonged exposure to drugs such as pyrazinamide (PZA). More recently, these phenotypes have been described using different techniques, which have revealed significant differences in metabolic activity and immunohistology of mycobacteria, but these findings have not been

What is already known about this subject

- More effective use of preclinical data to inform the selection of dose and drug regimens in patients is required to overcome attrition in the development of antitubercular drugs.
- Selection of drug combinations in clinical trials has been based on empirical evidence of efficacy with limited consideration of the underlying pharmacokineticpharmacodynamic relationships.

What this study adds

- The use of a bacterial growth dynamics model enabled the characterisation of the contribution of companion drugs to the overall antibacterial activity of rifampicin and bedaquiline.
- Future combinations for the treatment of tuberculosis may be optimised by using pyrazinamide with rifampicin or bedaquiline as backbone therapy.

perceived as a target for the evaluation of novel candidate molecules. ^{16,17} In addition to describing the proportion or fraction of fast- and slow-growing bacteria over time across different experimental protocols, our choice of parameterisation allowed insight into the potential mechanisms of antibacterial activity, as assessed by differences in the potency of drugs on the growth rate of subpopulations of *M. tuberculosis*.

Irrespective of the potential advantages of such an approach for the evaluation of novel antitubercular drugs, a critical step in the development of candidate molecules remains the empiricism based on which companion drugs are selected and how the dose rationale is defined for combination therapy in clinical trials. To date, evidence of efficacy and safety in late clinical development relies on an add-on and replace strategy, whereby currently approved first line standard of care drug combinations are not only used as reference, but their doses and dosing regimens are considered as optimal. This situation is partly explained by the lack of understanding and appropriate methodology to quantify pharmacodynamic drug-drug interactions in preclinical and clinical phases of development.

In *in vivo* protocols based on murine models of tuberculosis infection, the time to negative lung culture (as measured by colony forming unit [CFU] count) along with relapse rate are usually considered the main criteria for the selection of novel combination regimens. ^{18–20} Clearly, the biggest flaw in this approach is that novel regimens are selected without any insight into the underlying PKPD relationships. Moreover, one cannot distinguish the contribution of the individual drugs to the overall efficacy of the combination regimen. In fact,

empirical choices of drug combinations in TB have led to a first-line regimen that is potentially suboptimal due to reported antagonism between **rifampicin** (RIF), **pyrazinamide** (PZA) and isoniazid (INH).^{21,22} Such a scenario contrasts with the evaluation of drug combinations for other therapeutic indications.^{23,24}

The availability of parametric approaches that enable quantification of the contribution of each drug in the combination regimen is essential for a robust dose rationale in humans. Whilst some of the available methods for the evaluation of combinations regimens^{25,26} cannot be readily applied to antitubercular drugs due to experimental requirements, there has been no formal attempt to identify, optimise and rank drug combinations based on a systematic, quantitative evaluation of the underlying PD interactions, as assessed by changes in estimates of potency and/or maximum effect. Using RIF and bedaquiline (BDQ) as paradigm compounds, we aim to demonstrate how a drug-disease model describing bacterial growth dynamics and antitubercular drug effects can be applied as a screening tool for the evaluation of drug combinations and provide the basis for dose and drug selection in humans.

2 | METHODS

2.1 | Data source for the characterisation of pharmacodynamic interactions

Published data describing the antitubercular activity of RIF and BDQ were retrieved from PubMed. Only efficacy data measured as change in CFU count per lung in BALB/c mice infected with *M. tuberculosis* H37Rv strain were included in this meta-analysis to ensure comparable experimental conditions to those used in initial protocols for RIF and BDQ. In addition, given that the sterilising activity of anti-TB drugs cannot be adequately estimated from short experiments, only data from experiments that lasted for at least 8 weeks were considered for the purposes of our investigation. A detailed list of the preclinical protocols and corresponding publications included in current analysis can be found in Supplementary Materials Table S1 and S2 available online. All experimental procotols have been performed in accordance with guidelines for animal research and approved by an ethics committee.

2.2 | Bacterial growth dynamics

Details of the bacterial growth dynamics model have been previously reported elsewhere. The two-state growth approach assumes that fast-(F) and slow-(S) growing subpopulations of *M. tuberculosis* co-exist and each subpopulation can switch from one state to another. In addition, the onset of an infection was assumed to be determined by *M. tuberculosis* in the F state. An overview of the parameterisation of disease- and drug-specific parameters is provided in the Supplementary Materials (Figure S1, Table S3) available online.

2.3 | Parameterisation of pharmacodynamic interactions

Given the requirement to rank combinations during the screening of compounds and the assumption that maximum killing rates can be achieved by selection of the optimal dose of the backbone therapy (RIF or BDQ), we have attempted to parameterise the contribution of companion drugs in terms of a shift in the potency estimates of both F and S subpopulations, as shown in Figure 1.

Based on this approach, drug interaction is described as a discrete covariate (equation 1). Consequently, the identification and ranking of drug combinations can be performed by a stepwise forward inclusion, backward deletion procedure for covariate selection. In principle, a requirement for such evaluation is the availability of concentration-effect curves for each compound as monotherapy, but when different doses of the companion drug are evaluated, each dose level is treated as an independent treatment:

$$EC50_{combination} = EC50_{backbone} \cdot \theta 1_{Drug 1} \cdot \theta 2_{Drug 2} \cdot \theta ..._{Drug ...}$$
 (1)

where $\text{EC50}_{\text{backbone}}$ is the potency of RIF or BDQ as monotherapy and each θ represents the magnitude of shift in $\text{EC50}_{\text{backbone}}$ upon addition of the companion drug at a given dose level. Four possible interactions can be considered during the stepwise covariate selection: 1) combination affects $\text{EC}_{50}\text{-F}$ only, 2) combination affects $\text{EC}_{50}\text{-F}$ and $\text{EC}_{50}\text{-S}$ and 4) combination does not alter potency estimates of the backbone drug. These interactions assume steady-state conditions, during which fluctuations in drug levels due to differences in the pharmacokinetics of each compound do not affect the overall anti-tubercular activity.

As in a standard covariate analysis, prior to the evaluation of the effect of a companion drug on the potency of the backbone regimen, EC50 $_{\rm backbone}$ estimates are fixed to the values obtained in the previous step. Differences in the minimum value of objective function ($\Delta MVOF$) were used to assess the statistical significance of the covariate effect, i.e. the magnitude of the shift in the potency estimates of the backbone treatment. A significance level of 0.05 was used in this analysis which corresponds to a $\Delta MVOF$ of 3.84 for 1 degree of freedom. The combination with the highest $\Delta MVOF$ was chosen. An external validation procedure was implemented to assess the predictive performance of the modelling approach using published experimental data in which CFU count was measured following administration of various RIF- or BDQ-based combinations in BALB/c mice. Details of the validation steps and experimental protocols are summarised in the Supplementary Materials.

2.4 | Extrapolation of the PKPD relationships from mice to humans

Given the increasing focus on RIF dose optimisation,²⁷ RIF was subsequently selected to illustrate how further understanding of the underlying concentration-effect relationship from preclinical experiments can be utilised to support dose selection in humans. To that purpose,

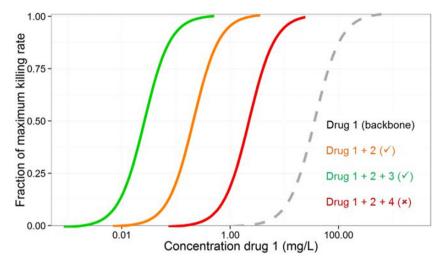


FIGURE 1 Visual representation of the proposed parametric approach for the assessment of drug combinations in tuberculosis. The contribution of companion drugs to antibacterial activity of the combination therapy is treated as a discrete covariate factor on the potency of the backbone drug (grey dashed line). Ranking of compounds and drug combinations is based on the assumption of a shift in EC_{50} estimates for fast- and slow-growing M. tuberculosis subpopulations. For instance, the addition of drug 2 increases the potency of backbone drug 1, as seen by the shift of the concentration–effect relationship to the left (orange line). As such, the combination of drugs 1 and 2 can be considered the new backbone therapy. Further evaluation of a third companion drug reveals that addition of drug 3 results in a further increase in potency (green line), while the addition of a fourth companion drug leads to a reduction in the potency of the backbone therapy (red line). From this analysis, it can be concluded that the combination of drugs 1, 2 and 3 (green line) can be considered as the best regimen. The selection of the doses of companion drugs is based on an initial evaluation of the PKPD relationships of each compound as monotherapy. Moreover, drug exposures as well as their ratio in the combination are assumed to be relatively constant during steady-state conditions

assumptions were made about the role of interspecies differences in PK and PD. Whilst allometric scaling is an accepted technique to describe drug disposition in humans in the absence of clinical data, interspecies differences in factors such as immunocompetence, 28 bacterial load 29 and protein binding 30 can also have a relevant effect on treatment response. We have assumed comparable bacterial load and attempted to correct for the differences in protein binding between mice $(97\%)^{31}$ and humans $(85\%)^{32}$ (equation 2).

$$EC50_{human} = EC50_{mouse} \cdot \frac{Fraction \, unbound \, RIF \, (mice)}{Fraction \, unbound \, RIF \, (human)} \tag{2}$$

Even though our model does not account for the contribution of the immune system, immunocompetence was assumed to have minor implications for drug potency after infection has evolved into symptomatic disease conditions. The normalised RIF concentration-effect relationships were subsequently used as basis for the selection of doses which are likely to be associated with higher or maximum killing rate in humans. The contribution of companion drugs to the antibacterial activity of RIF (in terms of the shift in EC50-F and EC50-S) in humans was assumed to be the same as in mice.

2.5 | Software

WebPlotDigitizer was used to extract CFU vs time profiles (in treated and untreated mice) from the publications.³³ Data analysis was

performed using NONMEM $7.3^{.34}$ Data manipulation and graphical presentations were performed in R version $3.2.5^{.35}$

2.6 | Nomenclature of targets and ligands

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.³⁶

3 | RESULTS

3.1 | Pharmacodynamic interactions: Rifampicin-based combination regimens

Given that most combination regimens were tested in mice infected via high dose aerosol (HDA), RIF potency estimates in the HDA experimental model were used as reference (EC $_{50}$ -F = 3.25 mg·L $^{-1}$; EC $_{50}$ -S = 40.2 mg·L $^{-1}$). The Css,av of RIF, PZA, INH and/or EMB is shown in Supplementary Materials (Table S4). An overview of the effects of each drug on CFU counts and subsequent changes in the RIF potency are summarised in Table 1. The shifts of the RIF EC $_{50}$ -F and EC $_{50}$ -S as a result of addition of the companion drugs are shown in Figure 2.

The potency of RIF increased upon addition of INH, PZA or EMB. For a two-drug combination regimen, PZA was deemed to be the best companion drug for RIF (increase in RIF EC_{50} -F and EC_{50} -S by almost

TABLE 1 Estimated contribution of isoniazid (H), pyrazinamide (Z) and/or ethambutol (E) to the overall antibacterial activity of the combination therapy, as assessed by the changes in the potency of rifampicin (R) given in combination with different companion drugs

	Ranking	Regimen	EC ₅₀ -F in mg·L ⁻¹	EC_{50} -S in $mg \cdot L^{-1}$
Two-drug regimen				
	1	RZ	1.7 (0.516)	20.7 (0.516)
	2	RH50	2.5 (0.76)	30.6 (0.76)
	3	RH12.5	2.6 (0.785)	31.6 (0.785)
	4	RH3.125	3 (0.919)	36.9 (0.919)
	5	R	3.25 (reference)	40.2 (reference)
	6	RE	0.7 (0.214)	77.6 (1.93)
Three-drug regimen				
	1	RZ	1.7 (reference)	20.7 (reference)
	2	RZE	1.7 (1)	20.7 (1)
	3	RZH1.56	1.7 (1.01)	21 (1.01)
	4	RZH3.125	1.8 (1.06)	22 (1.06)
	5	RZH6.25	1.9 (1.13)	23.4 (1.13)
	6	RZH12.5	2.1 (1.23)	25.5 (1.23)
	7	RZH50	2.1 (1.28)	26.6 (1.28)
	8	RZH25	2.2 (1.32)	27.4 (1.32)
Four-drug regimen				
	1	RZE	1.7 (reference)	20.7 (reference)
	2	RZEH10	2.3 (1.39)	28.8 (1.39)

Numbers between parentheses represent the estimated shift in potency of the backbone regimen; EC_{50} -F = potency against fast-growing population; EC_{50} -S = potency against slow-growing population. The following doses were administered unless stated otherwise: R = 10 mg·kg⁻¹; Z = 150 mg·kg⁻¹; E = 100 mg·kg⁻¹.

two-fold to 1.7 and 20.7 mg·L $^{-1}$, respectively). The addition of INH to RIF (RH) led to a smaller increase in potency for both populations (0.76–0.92-fold), while EMB (RE) had a mixed effect (change of EC $_{50}$ F and EC $_{50}$ -S by respectively 0.21- and 1.93-fold). Moreover, it should be highlighted that combining INH to RZ worsened the antibacterial activity of RZ in a dose dependent manner (1.01–1.32-fold). A similar effect was observed when INH was added to RZE.

Results of the external validation of the PKPD model for each combination regimen are summarised in the Supplementary Materials. In brief, the parameter estimates obtained from the PKPD model describing the interaction between RIF and PZA were predictive of the antibacterial activity of the regimen in other experiments (Figure 3). Similar results were observed for experiments in which infection was induced by HDA or IV infection route (Figures S2–S5, Supplementary Materials). No external validation could be performed for RE due to lack of experimental data. As such, only model diagnostic plots (VPCs) are shown for this regimen (Figure S6, Supplementary Materials).

3.2 | Pharmacodynamic interactions: Bedaquiline-based combination regimens

An overview of the Css,av of BDQ, PZA, PMD, LZD and/or MXF is shown in Supplementary Materials Table S5. The estimated

effects of each drug on BDQ potency are summarised in Table 2, whereas the shifts in the potency of BDQ (EC₅₀-F and EC₅₀-S) as a result of the addition of companion drugs are depicted in Figure 4. From the evaluated two-drug regimens, the addition of PZA (BZ) was once again the best companion drug for BDQ, indicated by a 0.55-fold increase in in potency, which varied from 0.19 to 0.11 mg·L⁻¹ for the fast growing subpopulation, and from 3.0 to 1.7 mg·L⁻¹ for the slow growing subpopulation. Overall, these results suggest that PZA together with RIF or BDQ should be considered as standard backbone treatment for novel regimens. The addition of LZD (BL) did not change the activity of BDQ, while the addition of PMD (BPa) was found to reduce the potency estimates for the fast- and slow-growing subpopulations by 1.72-fold, to 0.33 (from 0.19) and 5.2 (from 3.0) mg·L⁻¹, respectively. MXF was found to yield minor improvement to BZ (0.9-fold increase in both EC₅₀ values; EC₅₀- $F = 0.1 \text{ mg} \cdot L^{-1}$; EC_{50} - $S = 1.5 \text{ mg} \cdot L^{-1}$). By contrast, addition of PMD to either BZ or BZM resulted in worsening of the overall antibacterial activity, as indicated by the decrease in potency estimates (Table 2).

External validation of the PKPD models could be performed only for BPa, BZPa and BZM given that no other data sets were available for the other combinations. PKPD models of BPa and BZPa were predictive for the external validation experiments

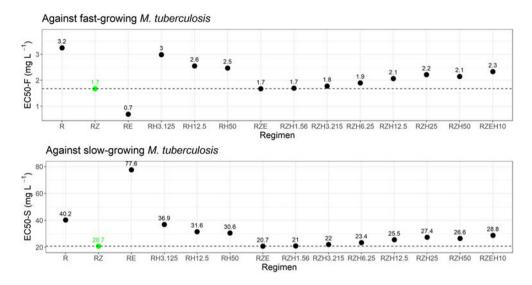


FIGURE 2 Change in the potency estimates of rifampicin (R) against the fast- (EC₅₀-F) and slow-growing (EC₅₀-S) subpopulations when given with isoniazid (H), pyrazinamide (Z) and/or ethambutol (E) in different combinations. The most potent rifampicin-based regimen is shown in green and marked with the horizontal dashed line. The following doses were administered unless stated otherwise: $R = 10 \text{ mg} \cdot \text{kg}^{-1}$; $E = 100 \text{ mg} \cdot \text{kg}^{-1}$

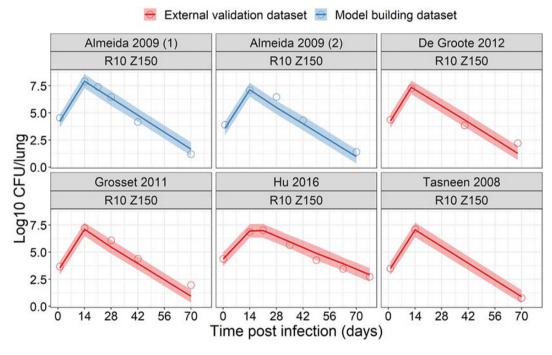


FIGURE 3 Visual predictive check and external validation of the PKPD model describing the effects of combination therapy with rifampicin (R) and pyrazinamide (Z). Solid lines and shaded area represent the median and 90% confidence interval of the predicted log_{10} colony forming unit (CFU)/lung over time. Open circles represent the observed log_{10} CFU/lung. Numbers between parentheses represent the experiment number in the original publication. Numbers behind R or Z in each panel represent the doses in log_{10} Sources of experimental data are summarised in Table S1

(Figures S7–S9, Supplementary Materials), while BZM overpredicted the activity of the regimen in the external validation data set. We could not assess the performance of the approach for BZ, BL, BPaMZ. As such, only model diagnostic plots (VPCs) were shown for these regimens (Figures S10–S12, Supplementary Materials).

3.3 | Extrapolation to humans

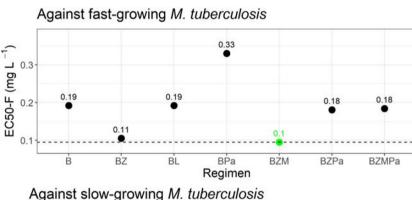
After taking into account the difference in plasma protein binding, EC₅₀-F and EC₅₀-S estimates for RIF in patients were predicted to be, respectively, 0.65 $\text{mg}\cdot\text{L}^{-1}$ and 8.04 $\text{mg}\cdot\text{L}^{-1}$ when given as monotherapy. Predicted values for potency were 0.34 $\text{mg}\cdot\text{L}^{-1}$ and

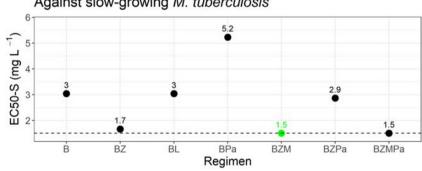
TABLE 2 Estimated contribution of pretomanid (Pa), pyrazinamide (Z), moxifloxacin (M) and/or linezolid (L) to the overall antibacterial activity of the combination therapy, as assessed by the changes in the potency of bedaquiline (B) given in combination with different companion drugs

	Ranking	Regimen	EC_{50} -F in $mg \cdot L^{-1}$	EC ₅₀ -S in mg·L ⁻¹
Three-drug regimen				
	1	BZ	0.11 (0.548)	1.7 (0.548)
	2	В	0.19 (reference)	3 (reference)
	3	BL	0.19 (1)	3 (1)
	4	BPa	0.33 (1.72)	5.2 (1.72)
Three-drug regimen				
	1	BZM	0.1 (0.903)	1.5 (0.903)
	2	BZ	0.11 (reference)	1.7 (reference)
	3	BZPa	0.18 (1.72)	2.9 (1.72)
Four-drug regimen				
	1	BZM	0.1 (reference)	1.5 (reference)
	2	BPaMZ	0.18 (1.94)	1.5 (1)

Numbers between parentheses represent the estimated shift in potency of the backbone regimen; EC_{50} -F = potency against fast-growing subpopulation; EC_{50} -S = potency against slow-growing subpopulation. The following doses were administered: 25 mg·kg⁻¹ (B), 50–100 mg·kg⁻¹ (Pa), 150 mg·kg⁻¹ (Z), 100 mg·kg⁻¹ (M and L).

FIGURE 4 Change in the potency estimates of bedaquiline against the fast- (EC_{50} -F) and slow-growing (EC_{50} -S) subpopulations following the addition of pyrazinamide (Z), pretomanid (Pa), moxifloxacin (M) and/or linezolid (L) in different combinations. The most potent bedaquiline-based regimen is shown in green and marked with the horizontal dashed line





 $4.15~\text{mg}\cdot\text{L}^{-1}$ if given with PZA alone, or $0.47~\text{mg}\cdot\text{L}^{-1}$ and $5.77~\text{mg}\cdot\text{L}^{-1}$ when combined with PZA, INH and EMB.

Assuming that efficacy is achieved at 90% of RIF maximum killing rate when given as monotherapy, Figure 5 shows that an average RIF concentration at steady-state (Css,av) of 5.85 mg·L⁻¹ is needed to achieve the desired drug effect against the fast-growing subpopulation, whereas a Css,av of 72.36 mg·L⁻¹ is required for the slow-growing subpopulation.

In combination with PZA, the target efficacy against the fast and slow-growing subpopulation can be obtained at $3.02 \text{ mg} \cdot \text{L}^{-1}$ and $37.34 \text{ mg} \cdot \text{L}^{-1}$, respectively (Figure 5), allowing for RIF dose reduction

when given together with PZA. By contrast, higher RIF Css,av appears to be required when RZ is given together with INH and EMB (from 3.02 to 4.2 $\text{mg} \cdot \text{L}^{-1}$ and from 37.34 to 51.9 $\text{mg} \cdot \text{L}^{-1}$), which pharmacologically indicates an antagonistic interaction between RZ and INH.

Based on the aforementioned EC₅₀ values, it is also possible to predict the relationship between RIF steady-state AUC_{0-24} and efficacy levels in TB patients (Table 3), when given as RZ or RZEH. RIF has been evaluated up to 35 mg·kg⁻¹ in TB patients, with reported mean AUC_{0-24} of 170 mg·L⁻¹·h (range: 103–266).²⁷ In fact, extrapolation from *in vivo* EC₅₀ estimates suggest that RIF 35 mg·kg⁻¹ yields 90–95% of its maximum effect against the fast-growing

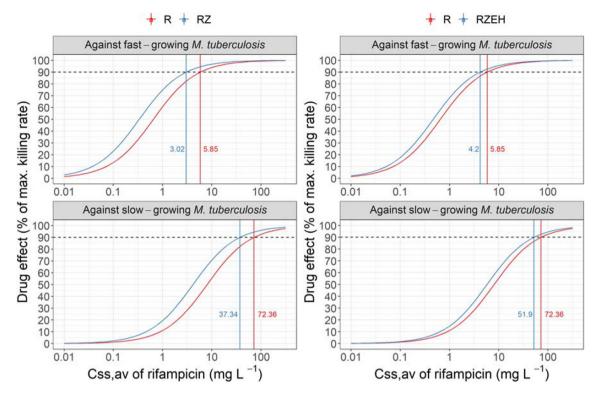


FIGURE 5 Predicted concentration-effect relationship of rifampicin (R) and the contribution of pyrazinamide (Z) (left panel) and pyrazinamide-ethambutol-isoniazid (ZEH) (right panel) to its antibacterial activity. Numbers along the vertical lines represent the average concentration at steady-state of rifampicin that is associated with 90% of the maximum killing rate (i.e. EC_{90} -F and EC_{90} -S), either as monotherapy or in combination. *In vivo* EC_{50} of rifampicin was corrected for interspecies differences in protein binding. This resulted in the following predicted rifampicin EC_{50} -F and EC_{50} -S values in humans: 0.65 mg·L⁻¹ and 8.04 mg·L⁻¹ (monotherapy), 0.34 mg·L⁻¹ and 4.15 mg·L⁻¹ (RZ), or 0.47 mg·L⁻¹ and 5.77 mg·L⁻¹ (RZEH)

population, while its effect against the slow-growing population is around 50–60% of the maximum killing rate (when given as RZEH). Removal of INH (e.g. RZ or RZE) was predicted to improve RIF activity against the slow-growing population to 60–70% of the maximum killing rate (corresponding AUC_{0-24} range between 149.3 and 232.3 mg·L⁻¹·h).

Whereas AUC_{0-24} levels that can produce 90–95% of the maximum killing rate against the slow-growing population (>1,000 mg·L⁻¹·h) may not be achieved due to safety concerns, our analysis indicates that current first-line regimen can be improved by focusing on finding alternative companion drugs for an RZ-based regimen. In addition, it may be worth considering further evaluation of RIF doses that correspond to 80% of the maximum killing rate (i.e. predicted AUC_{0-24} of approximately 400 mg·L⁻¹·h in patients). This exposure is likely to be achieved after oral administration of 70 mg·kg⁻¹.

4 | DISCUSSION

Despite evidence of the efficacy of combination regimens in clinical trials, it is not acceptable to continue to evaluate novel candidates and dosing regimens based on trial and error. The choice for drugs in a combination therapy should be based on their individual

contribution to the overall microbial clearance of the regimen, taking into account the different subpopulations of *M. tuberculosis* and the underlying concentration–effect relationship of the combination therapy.

Whilst the use of modelling has become common practice across different therapeutic areas as a tool for the assessment of the PKPD relationships of individual drugs and consequently as basis for the dose rationale, the implementation of such concepts is fraught with challenges when considering drug combinations. In fact, there has been limited attention to the characterisation of in vivo PD antibiotic drug interactions in preclinical models. Methods that are currently available for assessing combination therapies^{25,26} are often applicable to no more than two or three drugs. Moreover, the joint antibacterial activity of a combination is often quantified by comparing the observed and expected effects using a reference model, which relies on different assumptions regarding the nature or mechanism of the interaction, i.e. that the combination will be synergistic. These models fail to: (i) account for multiple bacterial subpopulations with differing susceptibilities; (ii) quantify or interpret the explicit interaction (synergy/antagonism) mechanisms; and (iii) eventually discriminate or accommodate the impact of spontaneous mutations.³⁷ Attempts to address such limitations require data-rich experiments arising from factorial designs, so that the full concentration-effect curve is characterised for each drug separately and in combination. In practice,

TABLE 3 Predicted rifampicin AUC_{0-24} that is required to achieve a predefined bactericidal activity (i.e. efficacy target) in tuberculosis patients, as measured by the percentage (%) of maximum killing rate obtained from *in vivo* experiments when given together with pyrazinamide (RZ) or pyrazinamide, isoniazid and ethambutol (RZEH). Extrapolation from mice to humans was performed taking into account the following factors: 1) known disposition characteristics in humans, 2) interspecies differences in protein binding to reflect the difference in rifampicin free plasma concentrations between humans and mice (rifampicin $EC_{50,human}$ calculated from $EC_{50,mice} \cdot fu_{mice}/fu_{human}$ with $fu_{mice} = 0.03^{22}$ and $fu_{human} = 0.15$). Predicted concentrations associated with 50% of the maximum killing rate were: 8.2 and 99.6 mg·L⁻¹·h (RZ), or 11.3 and 138.5 mg·L⁻¹·h (RZEH), for the fast- and slow-growing subpopulations, respectively. Calculations were based on the assumption of a once daily dosing regimen (with 24 hours dosing interval)

	Rifampicin AUC ₀₋₂₄ (in mg·L ⁻¹ ·h)				
	Against fast-growing bacterial population		Against slow-growing bacterial population		
% of estimated maximum killing rate	RZ	RZEH	RZ	RZEH	
10	1	1.2	11	15.4	
20	1.9	2.9	25	34.6	
30	3.4	4.8	42.7	59.3	
40	5.3	7.4	66.5	92.2	
50	8.2	11.3	99.6	138.5	
60	12	16.8	149.3	207.6	
70	18.7	26.2	232.3	323	
80	32.2	44.6	398.2	553.7	
90	72.5	100.8	896.2	1245.6	
95	152.9	212.6	1891.7	2629.7	

this is very unlikely to occur given the severe underfunding of TB research and drug development.³⁸

Given the practical limitations in terms of data requirement for the existing PD interaction models, 39-41 we have proposed a pragmatic and yet semi-mechanistic method for the evaluation and selection of drug combinations. The parameterisation of PD interactions based on potency estimates offers an efficient way to explore drug combinations. Moreover, dosing recommendations for new regimens as well as the choice of companion drugs can be made taking into account the impact of treatment on slow-growing subpopulation. Our working assumption requires that backbone drugs are tested at dose levels which are close to the maximum effect a compound can exert on bacterial clearance. In practice, this assumption allows for appropriate ranking of compounds to be used in a combination taking into consideration the PKPD relationships of each drug as monotherapy. Even though it was not possible to derive a normalised ratio of the overall antitubercular effect on fast- and slow-growing bacteria, by treating pharmacodynamic interactions as discrete changes in potency of the backbone treatment, dose selection of each companion drug is based on exposure levels associated with the overall antibacterial activity.

From a drug development perspective, it is worth mentioning that our approach highlights the benefits of the integration of PK and PD data from various experiments. We have quantified the effects of INH, PZA and EMB on the apparent potency of RIF, as well as the effects of PMD, LZD, MXF and/or PZA on the apparent potency of BDQ in a systematic manner. As illustrated for both drugs, these findings can be used to further explore the dose rationale for drug combinations in humans.

Another important feature of our analysis is the insight into the potential mechanisms of interaction, in that shifts in parameter estimates may reveal antagonistic activity. We acknowledge that claims about the semi-mechanistic nature of the proposed PKPD model cannot be made with the limited data evaluated so far. However, such an insight offers an important step towards reduction in attrition in clinical development. Moreover, it can be anticipated that extrapolations including PK and PD variability will further enhance the quality of decisions regarding the ranking and selection of the companion drugs for combination therapy.⁴²

4.1 | Predicted interactions and efficacy of drug combinations in clinical trials

While the benefit of adding PZA to RIF was clearly demonstrated by increased potency, we found that EMB does not improve the activity of RZ, while INH reduces the apparent potency of RIF in RZ in a dose-dependent manner. Although others have attributed antagonism between INH and RZ to be *M. tuberculosis* strain-specific, ⁴³ such antagonism has been reported in patients by others.^{22,44} Recently, we have used the hollow-fibre system in conjunction with staining and imaging techniques to evaluate the effects of drug combinations.⁴⁵ Despite the different *in vitro* conditions, these findings seem to explain the apparent antagonism between INH and RIF. When used as monotherapy, RIF was found to produce phenotypical cell death, whereas INH was associated with cell injury alone. By contrast, addition of INH to RIF increased the proportion of injured cells over time at the cost of decreased probability of both alive and dead cells, which

reduced bacillary killing over time. Such interaction needs to be replicated in vivo, but these data strengthen the proposition to remove INH from any RZ-based therapy.

Given that the early bactericidal activity (EBA) of RZ has never been tested nor compared to RZH in TB patients before, additional EBA clinical studies may be required to confirm these findings. Our analysis also provides a quantitative basis for the use of higher doses of RIF. Assuming no tolerability or safety issues, RIF exposure levels associated with a dose of 70 mg·kg⁻¹ are likely to reach higher bactericidal activity in both subpopulations.

The powerful sterilising activity of PZA was also observed when added to BDQ, suggesting its critical role in the treatment of TB. We found that addition of LZD does not improve the activity of BDQ, while MXF had minor improvement on the activity of BZ. In contrast, PMD was found to consistently worsen the activity of BDQ, whether given as monotherapy or in combination with other drugs (e.g. BZ, BZM). Despite the lack of experimental data in vivo, the antagonism between BDO and PMD has been reported before. 46,47 In TB patients. the addition of PMD was not found to improve the EBA between day 7 and day 14 (EBA₇₋₁₄) of BDQ (from 0.123 to 0.114, respectively).⁴⁸ In contrast, an improvement was observed when PZA was added (from 0.123 to 0.152). Moreover, the EBA of BZ between day 2 and day 14 appears to be better as compared to BPa (0.143 vs 0.114 respectively). Finally, the addition of PMD to BZ does not yield an improvement in EBA₇₋₁₄ (from 0.152 to 0.146).⁴⁹ Overall, these results indicate that whilst the use of PMD as companion drug may contribute to a lower relapse rate. 50 it does not appear to improve the overall antimicrobial activity of the combination.

4.2 | Limitations

Our analysis has several limitations. First, we have had to assume that PK variability has minor impact on the PD interactions. The variability in PK on the observed treatment effect could not be systematically quantified due to the fact that PK samples were not collected in most experiments. On the other hand, PK interaction between antitubercular drugs is generally considered of little clinical importance and was therefore not addressed in the present study. 51-53 Second, we have assumed that PKPD relationships in BALB/c mice are representative of the concentration-effect relationships in humans, where different pathological conditions determine the course of infection. In a separate analysis, scaling for interspecies differences in system-specific properties (e.g. bacterial load and growth dynamics) was indeed required to accurately predict early bactericidal activity in patients from mouse (unpublished data).

Third, the ranking of drug combinations was based on 8-week efficacy, which may have limited predictive value for the evaluation of long-term treatment response (i.e. relapse). For example, it is possible that the addition of EMB to RZ may yield lower relapse as compared to RZ alone, despite no improvement observed in the first 8 weeks of treatment. Irrespective of such a limitation, the proposed approach still provides a more robust selection of a set of candidate

regimens that could be subsequently evaluated in murine relapse experiments prior to progression into the clinical phase. It should also be noted that the main purpose of antimicrobial combination therapy is often to block the emergence of drug resistance.⁵⁶ Although identified here as the most synergistic combination to be used as backbone therapy with novel molecules, a regimen consisting of RZ and BZ alone may not be as effective as RZEH or BPaMZ in preventing relapse or drug resistance.

Fourth, the parameterisation of drug interactions as discrete covariates on the potency of the backbone drug requires the assumption of steady state conditions since the start of the treatment. Such an assumption prevents the immediate application of the approach for drugs with long half-life, such as clofazimine.⁵⁷ In addition, it is worth mentioning that only the optimal exposure of the backbone drug (e.g. RIF and BDQ) could be identified. This is a consequence of current murine experimental designs, wherein companion drugs are administered using single "standard" dose levels alone (e.g. 150 mg·kg⁻¹ for PZA). Additional experiments designed to explore the optimal exposure of each companion drug, in conjunction with the proposed model-based framework would be required to ensure optimal dose selection. Lastly, whilst RIF doses up to 35 mg·kg⁻¹ have been tolerated in patients (mean $AUC_{0-24} = 170 \text{ mg} \cdot L^{-1} \cdot h$), ²⁷ there is a risk that drug levels associated with a 70 mg·kg⁻¹ dose may lead to increased adverse events. A dose escalation study to inform the therapeutic window of RIF is hence urgently needed. Consequently, our analysis could be used as the basis for an initial 14-day EBA study to investigate the short-term efficacy and safety of RIF doses >35-70 mg·kg⁻¹. This could be followed up by a longer EBA study (e.g. 1 month) to assess the long-term safety and efficacy of selected RIF doses based on the results from the preceding EBA study. We envisage that INH, PZA and EMB should not contribute to additional toxicity as no dose increase is proposed for these drugs.

In short, our analysis demonstrates that RZ and BZ should be considered as a backbone therapy in prospective novel combination regimens against TB. The use of a model-based approach provides an excellent platform for describing the magnitude of PD interactions and overall antibacterial activity following combination therapy while taking into account the mechanisms of different antibiotics and susceptibility of the different bacterial populations. It is anticipated that estimates of the apparent potency along with information on maximum bacterial clearance can form the basis for more effective screening of novel compounds and design of more informative study protocols in early clinical phases of antitubercular drug development.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

MM performed the analysis. ODP and MM developed the research protocol, reviewed the results and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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