Bedaquiline kills persistent *Mycobacterium tuberculosis* with no disease relapse: an *in vivo* model of a potential cure

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Running title: Bedaquiline in the Cornell mouse model

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Abstract

OBJECTIVE: Non-replicating persistent *Mycobacterium tuberculosis* is difficult to kill since they become undetectable using our conventional diagnostic methods and tolerant to anti-TB drugs. Resuscitation promoting factors (RPF) have been used to "wake up" non-replicating persisters, making them easy to be detected. Bedaquiline is a novel bactericidal and sterilizing anti-TB drug with the potential to eradicate RPF-dependent persistent *M. tuberculosis.* We present the first head-to-head comparison between standard anti-TB regimen versus bedaquiline-modified regimen for eradicating RPF-dependent persistent *M. tuberculosis*, using the well-defined Cornell Model.

METHODS: *M. tuberculosis* infected mice were treated with either the standard regimen, rifampicin, isoniazid, pyrazinamide and ethambutol or the same regimen where ethambutol was replaced by bedaquiline for 14 weeks. The efficacy of both drug regimens was measured by cfu count elimination and eradication of persistent bacteria as evaluated using culture filtrate (CF) containing RPF. At the end of treatment, the remaining cfu count-negative mice were administrated with hydrocortisone for 8 weeks. The induced disease relapse rates were determined by the percentage of mice that developed positive *M. tuberculosis* in lungs, spleens or both.

RESULTS: Bedaquiline-containing regimen achieved total organ cfu count clearance at 8 weeks after treatment initiation, faster than the standard regimen (14 weeks). Importantly, the bedaquiline-containing regimen removed CF-dependent persistent bacilli at 8 weeks, leading to no disease relapse.

Conclusions: A bedaquiline regimen eradicated persistent tuberculosis infections and completely prevents disease relapse in mice. These findings offer the potential of a faster cure for tuberculosis with reduced relapse rate.

Introduction

For more than 4000 years, we have known about tuberculosis (TB), [1] yet it is still a leading killer worldwide, responsible for about two million deaths annually. [2] Often thought of as a disease of the developing countries, TB is now making a substantial comeback in the developed world. [3] One of the key reasons for its "hard-to-kill" nature lies in the ability of *M. tuberculosis* to become metabolically inactive, evading detection by diagnostic tests and immune responses. Of additional importance to the patients is that the non-replicating persistent state renders the bacteria tolerant to conventional anti-TB drug therapy. To achieve good long term clinical results, an arduous 6-month course of multi-agent antibiotic therapy is necessary, which is associated with poor patient compliance, leading to high (7 - 13%) relapse rates and drug-resistance. [4] Therefore the ideal drug regimen is one that can eliminate all of the bacterial populations, that is, both the actively replicating cells and the non-replicating persisters.

To kill the persistent bacteria, one must find them first. *M. tuberculosis* persisters are non-replicating, [5, 6] which are present in mouse organs [7] and in human sputum samples [8, 9] and cannot be detected using conventional diagnostic tests such as culture and microscopy. [10, 11] Since most conventional anti-TB drugs were tested for their ability to eliminate *M. tuberculosis* grown on culture agar or in broth, none of these drugs are actually tested for their ability to eliminate persistent bacteria. It is therefore of no surprise that persistent *M. tuberculosis* populations have never been detected. They are tolerant to conventional anti-TB regimens and prolonged treatment duration is needed. [12]

We recently showed in mice that using *M. tuberculosis* culture supernatant filtrates (CF) containing secreted proteins, known as Resuscitation Promoting Factors (RPF), [8] we can stimulate the persistent bacteria which do not grow on agar and liquid media to actively multiply. [7, 13-15] Furthermore, these CF-resuscitated persisters could be completely eliminated using high-dose rifampicin regimens, resulting in shortened treatment duration with no disease

relapses in mice. [7, 15] High-dose rifampicin regimens are not routinely used for TB therapy and have just begun to enter Phase III trials for use in patients.

Bedaquiline is a novel diarylquinoline antimycobacterial agent, approved by the FDA for use in combination regimens against adult multidrug-resistant pulmonary TB. [16] It has both bactericidal and sterilizing activities. [17-24] In this study, we examined the therapeutic effects of bedaquiline-modified regimens for eradicating CF-dependent persistent *M. tuberculosis* using the well-defined Cornell mouse model. [11, 25] We hypothesized that bedaquiline-modified regimen can eliminate CF-dependent *M. tuberculosis* more effectively than the conventional anti-TB regimen.

Materials and methods

Bacterium strain and growth condition

M. tuberculosis strain H37Rv was grown in 7H9 medium supplemented with 10% albumin dextrose complex (ADC; Becton and Dickinson, UK) and containing 0.05% Tween 80 at 37°C without disturbance for 15 days. The culture was subsequently stored at -70°C. To determine the viable counts prior to infection, cfu counting was performed prior to freezing and once again after thawing. Cfu counting was carried out by plating serial 10-fold dilutions of the cultures on 7H11 agar medium supplemented with oleic albumin dextrose complex (OADC, Becton and Dickinson, UK). Colonies were counted after incubation of the plates at 37°C for 3 to 4 weeks and viability was expressed as Log cfu/mL. The cultures were diluted in PBS and used for inoculations in mice.

Antimicrobial Agents

Bedaquiline was provided by Janssen Pharmaceutica (Beerse, Belgium), while the other drugs used were human medicines: isoniazid from Morningside Healthcare UK, rifampicin from Sanofi-Aventis France, pyrazinamide from Genus Pharmaceuticals UK and ethambutol from Morningside Healthcare UK.

Cornell mouse model

The standard TB drug regimen (isoniazid, rifampicin, pyrazinamide and ethambutol) and bedaquiline substitution for ethambutol in the standard TB drug regimen (Bedaquiline-containing regimen) were tested using the Cornell mouse model. [11, 25] The model was conducted using the experimental design and procedure described previously. [7, 13-15] Briefly, a total of 118 BALB/c mice (Female, Harlan UK Ltd) was infected intravenously via the tail vein with 1.2×10^5 cfu of mouse-passaged *M. tuberculosis* strain H37Rv per mouse. At 3 weeks after infection, treatment was given to the mice for 14 weeks by daily oral administration (0.2 mL) for 5 days per week with daily prepared drug suspension at the dosages of rifampicin 10 mg/kg, isoniazid 25 mg/kg, pyrazinamide 150 mg/kg, ethambutol 100 mg/kg or bedaquiline 25 mg/kg. For assessment of treatment efficacy, 4 mice were sacrificed at 0, 2, 4, and 6 weeks, 6 mice was sacrificed at 8 weeks and 8 mice were used at 11 and 14 weeks of post treatment to monitor cfu counts. Importantly, sampling time points for viable counting were always on Monday before the start of the 5 day drug dosing schedule to avoid drug carryover. It has been shown that after a signal oral dose of bedaquiline (25 mg/kg), at 72 hours, the drug levels were reduced to approximately 1 and 0.3 µg/g per lung and spleen, respectively. [26] There was no accumulation of the drug in the tissue, [26] indicating that there was not enough drug remaining in the organs to inhibit bacterial growth. The organ homogenates from the 14^{th} week for the standard regimen and from the 6^{th} week to the 14^{th} week for the bedaquiline - containing regimen were cultured in selective Kirchner liquid medium by the addition of polymyxin B 200 U/mL, carbenicillin 100 mg/L, trimethoprim 20 mg/L and amphotericin B 10 mg/L (Selectatab, Mast Diagnostica GmbH) for 4 weeks with subsequent sub-culturing onto selective Löwenstein-Jensen slopes for a further 4 weeks to conform organ cfu count clearance. Immediately after termination of 14 weeks of chemotherapy, the remaining mice were administered hydrocortisone acetate (0.5 mg/mouse) by daily oral dosing for 8 weeks to

suppress host immune response. Disease relapse rates for the treatments with the standard and bedaquiline-containing regimens were determined by the percentage of mice that developed positive *M. tuberculosis* cfu counts in lungs, spleens or both.

Organ drug carryover was examined. The suspension of tissue homogenates was collected after viable cell counting by centrifugation at 14,000 rpm for 5 minutes. *M. tuberculosis* cells were removed by filtration using 0.2 μm filter (Sartorius) twice. The cell-free suspension of tissue homogenates was diluted 10-fold in 7H9 medium followed by addition of *M. tuberculosis* to the final concentration of 10^7, 10^6 and 10^5 cfu/mL, respectively. Controls were 7H9 only which were incubated with the same numbers of *M. tuberculosis*. The cultures were incubated at 37°C for 20 days, viability was examined by cfu counting at 0, 4, 10 and 20 days.

The animal husbandry guidelines and animal experiments were performed according to the Animals Scientific Procedures Act, 1986 (an Act of the Parliament of the United Kingdom 1986 c. 14) (Home Office Project licence Number 7008492) with approval from St George's, University of London ethics committee.

Resuscitation of *M. tuberculosis* in mouse lungs and spleens

For resuscitation of *M. tuberculosis* in mouse organs, culture filtrates containing RPFs were prepared as described previously. [8, 13] *M. tuberculosis* H37Rv was grown in 7H9 medium for 15 to 20 days until an optical density of 1 to 1.5 was reached. The culture supernatants were collected by centrifugation at 3600 rpm for 15 minutes and sterilised by filtration with 0.2 μm filter (Sartorius) twice. The culture filtrates were made selective by addition of the Selectatab (Mast Diagnostica GmbH) and immediately used for broth counting of the most probable number (MPN) of the bacilli. Broth counting of lungs and spleens was performed as serial 10-fold dilutions in triplicate in which 0.5 mL of tissue homogenates were added to 4.5 mL of the culture filtrates. At 10-day intervals over a 2-month period of incubation at 37°C, the broth cultures were examined for visible turbidity changes. Growth of *M. tuberculosis* in turbid tubes

was confirmed by colonial morphology on 7H11 agar plates. The MPN of viable bacilli was then estimated from the patterns of positive and negative tubes. [27] The absence of microorganisms other than mycobacteria from turbid tubes was confirmed by plating on blood agar medium (Oxoid) and Sabouraud dextrose agar (Oxoid). In order to assess the sterility of culture filtrates free of *M. tuberculosis*, tubes containing culture filtrates were incubated at 37°C for 2 months to ensure the absence of *M. tuberculosis* in the culture filtrates.

Statistical analysis

A simple model for mono-exponential bacterial growth and elimination [28] was fitted to the profiles of cfu versus time obtained experimentally, which was demonstrated previously. [13-15] Parameter estimation of elimination rate constants was carried out using the nonlinear least-squares optimization function "lsqnonlin" as part of the "pracma" package in the R statistical software language (v 3.4.2) with an objective function weighted by 1/(predicted value)^2. Standard errors of parameter estimates were calculated using the method described previously [29] with the Jacobian of model parameter sensitivities estimated using a numerical central difference method. The datasets comprised from multiple individual subject animals were treated as a naïve pool for data analysis purposes [30] rather than using the average of the data at each time-point. The significance of differences between model parameter estimates under different therapies was examined with pairwise Z-tests, where P values <0.05 would be considered significant. The significance of differences between the relapse rates was determined with pairwise Fisher's exact tests, with P values <0.05 considered significant.

RESULTS

Bedaquiline-containing regimen more than doubles TB kill rate and completely prevents disease relapse in mice

We investigated the effects of the standard and bedaquiline-containing regimens on the rate of bacterial cfu elimination and relapse in the Cornell mouse model. No mice died during the experimental period (6 months) from either of the two groups.

After 3 weeks, the bacterial cfu counts reached a peak with mean cfu counts of log 7.54 in the lungs and 6.99 in the spleens (Table 1). After initiation of the treatment, the bedaquiline-containing drug regimen demonstrated rapid bactericidal activity, leading to 99% kill at 1.2 weeks. In contrast, the same feat took more than twice as long using standard drug regimen (3 weeks for 99% kill). Using the bedaquiline-containing regimen, complete kill (100% bacterial elimination with undetectable cfu counts) in the lungs and spleens was achieved after 8 and 6 weeks of treatment, respectively. Again, the same feat took much longer using the standard regimen (complete kill in 14 weeks for both the lungs and the spleen; Table 1). Incubation of the cell-free suspension of tissue homogenates with *M. tuberculosis* showed no inhibition of growth compared with the control (data not shown) indicating there was no drug carry-over from tissue homogenates to the subsequent cultures.

The enhanced bactericidal activity with the bedaquiline-containing regimen was further confirmed using mono-exponential bacterial elimination rate constants (Fig 1, and Table 2) where the exponential rate constants (logarithmic base 10) for net bacterial elimination during treatment ($k_{net_with_drug}$) for the standard regimen versus bedaquline-containing regimens were -0.46 versus -0.75 in the lungs and -0.46 versus -1.22 in the spleens, respectively (Table 2). These values indicate that compared to a standard regimen, bedaquiline-containing regimen produced a significant increase in bacterial elimination in both the lungs and the spleens.

Remarkably, after 8 weeks of immunosuppression with hydrocortisone, mice treated with the bedaquiline-containing regimen had no (0%) disease relapse, while mice treated with the standard regimen had a high (90%) relapse rate (Table 3).

Determination of post-treatment levels of CF-dependent bacteria

To investigate the effect of bedaquiline-containing regimens on the post-treatment level of persistent bacteria through CF-induced resuscitation, lung and spleen homogenates at 0, 2, 4, 6, 8, 11 and 14 weeks after initiation of treatment for the standard and bedaquiline-containing regimens were incubated with the culture filtrates containing RPF. At the beginning of the treatment, the MPN counts were 1 log higher than cfu counts in both the lungs and the spleens (Figure 2). For the standard regimen, there was a stepwise reduction of both cfu counts and MPN counts in both the lungs and the spleens with approximately two-log difference between cfu counts and MPN counts (Figure 2A and 2B). At 14 weeks of treatment in both the cfu count free lungs and spleens, high levels of CF-resuscitated bacilli (2.4 logs) still remained. For mice which were treated with the Bedaquiline-containing regimen, there were 2 logs more CF-dependent persister counts than the cfu counts at 2 weeks. This was followed by a significant reduction of persister counts at 4 and 6 weeks (Figure 2C), there were only a mean of 0.17 (1/6 lungs) log of MPN in cfu count-free mouse lungs at 8 weeks (Figure 2C). At 11 and 14 weeks post-treatment, both organs were free of CF-dependent bacilli. There were significant reductions of RPF-dependent bacilli in the spleens with a 0.78 log of persisters fall at 6 weeks post treatment and complete sterilization of the spleens was achieved at 8 weeks (Figure 2D).

DISCUSSION

This is the first study to elucidate the therapeutic efficacy of bedaquiline replacement of ethambutol against CF-dependent *M. tuberculosis* persisters. The main findings are: (i) the bedaquiline-containing regimen rapidly eliminates TB in mice, at more than double the rate achievable using the standard regimen; (ii) the bedaquiline-containing regimen completely prevents disease relapse, and (iii) the bedaquiline-containing regimen removed CF-dependent persistent bacteria even at early stages of treatment. These promising findings represent an important step in a paradigm shift to directly target persisters in the treatment of tuberculosis.

The Cornell model combined with CF-dependent persister measurement.

As a testbed, the Cornell model is a reliable surrogate for human TB infections, modelling TB treatment as well as disease relapses. [11, 25] It has been used to determine the pharmacodynamics of TB drug regimens and successfully brought TB regimens from critical preclinical evaluation to the clinical bedside. [31] Previously, we demonstrated that it is feasible to use CF-dependent persistent bacteria which are present in mouse organs in the Cornell model as an evaluation tool for measuring the efficacy of drug regimens which target persistent *M. tuberculosis*. [7, 13-15] Most interestingly, the step-wise reduction of RPF-dependent persister counts coincided with the reduction of cfu counts during the treatment with standard drug regimen, always with a two-log difference between cfu counts and MPN counts. However, at end of treatment, the cfu count free organs still contained considerable numbers of CF-dependent persistent bacteria. This repeatedly demonstrated that the current drug regimen failed to remove CF-dependent persisters in the Cornell model. [7, 13-15] The significance of this is that regimens which are tested in the Cornell model without CF resuscitation are likely to overestimate the power of the regimen in the reduction of relapse. This may be the reason for the recent unsuccessful clinical trials which aimed to shorten the duration of TB therapy. [32, 33]

Bedaquiline – targeting persisters

Bedaquiline is a "new-kid-on-the-block" and armed with FDA approval, has been shown as an effective anti-TB drug. [17, 23, 24, 34, 35] Bedaquiline substitution in current anti-TB regimens is also effective in mouse models of latent TB.[21-23, 26] . Our results showed that bedaquiline-containing regimens could eliminate CF-dependent persistent bacteria, which provides a mechanistic insight into its efficacy against latent TB in previous studies. More importantly, this study confirms the ability of bedaquiline to target persisters with the potential to not only shorten the duration of TB chemotherapy but also reduce disease relapse.

In contrast to current standard drug regimens which fail to kill CF-dependent persisters when organ become apparent bacterial free (cfu count free),[7, 13-15] the bedaquiline regimen treatment group, demonstrated a two-log difference between cfu counts and MPN counts only at 2 week post treatment and the difference was significantly reduced at 4 weeks and 6 weeks (Figure 2C and 2D), This indicated that CF-dependent persisters were rapidly killed at an early stage of treatment, mostly in less than 4 weeks.

Bedaquiline – zero disease relapse

The findings that the bedaquiline-containing regimen could completely prevent any occurrence of disease relapse in mice is astounding, particularly considering the standard regimen was associated with a 90% relapse rate. The likely explanation for this finding is that CF-dependent persisters are a major determinant of TB disease relapse in this model. [7, 13-15] Our findings both strengthens the notion that bedaquiline may target the persisters, thus reducing disease relapse, or, more important from a mechanistic viewpoint, bedaquiline actually kills CF-dependent bacilli, leading to their near complete elimination at an early stage in the treatment period. The mode of bedaquiline action is to inhibit bacterial ATP synthesis [34] which is an essential metabolic activity of replicating bacilli. Interestingly, it has been reported that bedaquiline kills *in vitro* non-replicating persistent *M. tuberculosis* [35] which retain residual ATP synthase enzymatic activity. This is consistent with our previous finding that non-replicating persisters of *M. tuberculosis in vitro* and in mice is metabolically active containing a residual level of transcriptional activity. [36] The specific molecular interactions between CF-dependent persisters and bedaquiline, such as membrane cascade activation, are currently unclear and deserve investigation to further our understanding of this novel drug regimen.

Bedaquiline – aggressive TB elimination

Bedaquiline substitution in current anti-TB regimens in other laboratories achieved organ cfu-count clearance in mice after 2 months of treatment. [24, 26] We clearly demonstrated that the drug

regimen with bedaquiline in combination with rifampicin, isoniazid and pyrazinamide significantly increased the cfu count elimination rate compared to the standard regimen. The bedaquiline regimen achieved cfu count free organs at 8 weeks in lungs and at 6 weeks in spleens; this early bactericidal activity is consistent with previous findings. [24, 26] The incorporation of the Cornell model with an established mono-exponential bacterial growth and elimination model, as well as the CF resuscitation method means that we could capture the bactericidal and sterilizing activities of the drug regimen over the entire treatment duration. By measured organ cfu counts at two- to three-week intervals over the entire course of treatment, we achieved better temporal assessment of drug efficacy during treatment. This study demonstrated the excellent efficacy of a bedaquiline-containing regimen in mice. To make the transition into the clinical arena, our findings for targeting CF-dependent persisters need to be confirmed in human studies and in larger multi-centre clinical trials. In clinical practice, the quartet of rifampicin, isoniazid, pyrazinamide and ethambutol have been the cornerstone of anti-TB treatment for many decades. Anti-TB therapy typically requires 6 months to achieve clinical disease clearance and to minimize relapses. Prolonged antibiotic therapy is the antithesis of good therapeutic compliance, and the resultant emergence of multidrug resistant TB only complicates the already-difficult task of global disease control. No current non-investigational regimen has been proven to consistently reduce the duration of treatment to less than 6 months, our results demonstrate that bedaquiline may possess most of these features in mice.

It has been showed that bedaquiline accumulated at high level in the infected organs such as lungs in mice [26] which caused a concern that there might be drug carryover followed by the subsequent microbiological analysis on agar plates or broth. In our study, there was no bedaquiline carryover to either inhibit or kill *M. tuberculosis* in the cultures.

Limitations and future directions

Although bedaquiline is already approved by the FDA with known side effects profiles, [37, 38] its use is currently limited to drug-resistant TB. [39] The major problem to add bedaquiline into the current drug regimen is the drug interaction with rifamycins such as rifampicin or rifapentine. [24, 40, 41] It has been shown that co-administration of bedaquiline with rifampicin or rifapentine significantly reduce bedaquiline exposure in patients. [40, 41] In mice combination of bedaquiline with pyrazinamide was synergistic [24] and bedaquiline replacement of rifampicin was more effective than rifampicin, isoniazid and pyrazinamide combined regimen [24] indicating that rifampicin blunted the effectiveness of bedaquiline. Building bedaquiline into the current clinical regimen is the important next step with careful drug component-adjustment. However, our study showed that substitution of bedaquiline to ethambutol was more potent than the standard regimen indicating that the level of bedaquiline was sufficient to shorten the treatment duration, thus may expose patients to less side effects overall compared to the prolonged 6-month therapy with standard regimen, while offering better efficacy against persisters and reduced disease relapse. In this study, bedaquiline was used at a dose of 25 mg/kg, which have been shown in previous studies to give rise to a Cmax from 1.1 to 1.3 mg/L, and AUC from 18.5 to 19.4 mg.hour/L.[26] When this dose of bedaquiline (25 mg/kg) was added to the standard regimen, significant organ cfu count clearance was observed,[26] as confirmed by our results. While we know the minimum effective dose of bedaquiline for preventing gross lung lesions (6.5 mg/kg) and for bactericidal activities (12.5 mg/kg);[26] the minimum effective dose for eliminating CF-dependent persisters remains unknown. This is an area for future work.

It has not escaped our notice that bedaquiline-based regimens could also be effective against latent sub-clinical tuberculosis because persistent bacteria can live in people for 70 years or more. If bedaquiline could kill persistent bacteria in otherwise healthy people (one third of the world's population carrying TB) this could reduce disease relapse in TB carriers which is a major source of the disease world-wide.

Conclusion

A bedaquiline antimicrobial regimen eradicates persistent tuberculosis infections and completely prevents disease relapse in mice. The findings offer the potential of a new cure for tuberculosis with reduced relapse rate.

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438

439 Figure legends

440 Figure 1. Treatment profiles of *M. tuberculosis* H37Rv with standard and bedaquiline-
441 containing regimens in the Cornell mouse model. a. Elimination of cfu counts in lungs. b.
442 Elimination of cfu counts in spleens. RHZE, standard regimen (R, rifampicin. H, isoniazid. Z,
443 pyrazinamide. E, ethambutol. RHZB, bedaquiline-containing regimen (B, bedaquiline). The
444 solid arrow indicates the treatment starting at 3 weeks of post infection. The empty arrow
445 indicates starting steroid treatment after the termination of 14 week therapy.

Figure 2. Post-treatment levels of RPF-dependent persisters with standard and bedaquiline-containing regimens in the Cornell mouse model. Lungs (a) and spleens (b) treated with standard regimen. Lungs (c) and spleens (d) treated with bedaquiline-containing regimen.

Table 1. Organ cfu counts before and after treatment with the standard (RHZE) and bedaquiline-containing (RHZB) regimens

Time of infection and treatment	Mean Log cfu per lung ± SD			Mean Log cfu per spleen ± SD		
	Control	RHZE[a]	RHZB[b]	Control	RHZE[a]	RHZB[b]
D0[c]	4.80 ± 0.040			5.29 ± 0.002		
D21[d]	7.54 ± 0.002			6.99 ± 0.007		
2W[e]		6.09 ± 0.002	4.35 ± 0.051		5.43 ± 0.014	4.24 ± 0.003
4W		4.80 ± 0.009	1.67 ± 0.417		4.05 ± 0.001	1.05 ± 0.692
6W		4.07 ± 0.025	1.53 ± 0.679		3.47 ± 0.068	0
8W		3.02 ± 0.003	0		2.45 ± 0.054	0
11W		1.35 ± 0.457	0		1.10 ± 0.251	0
14W		0	0		0	0

a. Standard regimen, R, rifampicin. H, isoniazid. Z, pyrazinamide. E, ethambutol. b. RHZB, bedaquiline-containing regimen, B, bedaquiline. c. 2 hours post-infection. d. 21 days post-infection at the time when treatment was started. e. week 2 post-treatment.

Table 2. Elimination constant rates of the standard (RHZE) and bedaquiline-containing (RHZB) regimens

Treatment group	Elimination rate constant (wk-1)			
	Lungs[a]		Spleens[b]	
	Estimate	%RSE	Estimate	%RSE
RHZE	-0.46	3.2	-0.46	4.8
RHZB	-0.75	19.8	-1.22	2.79

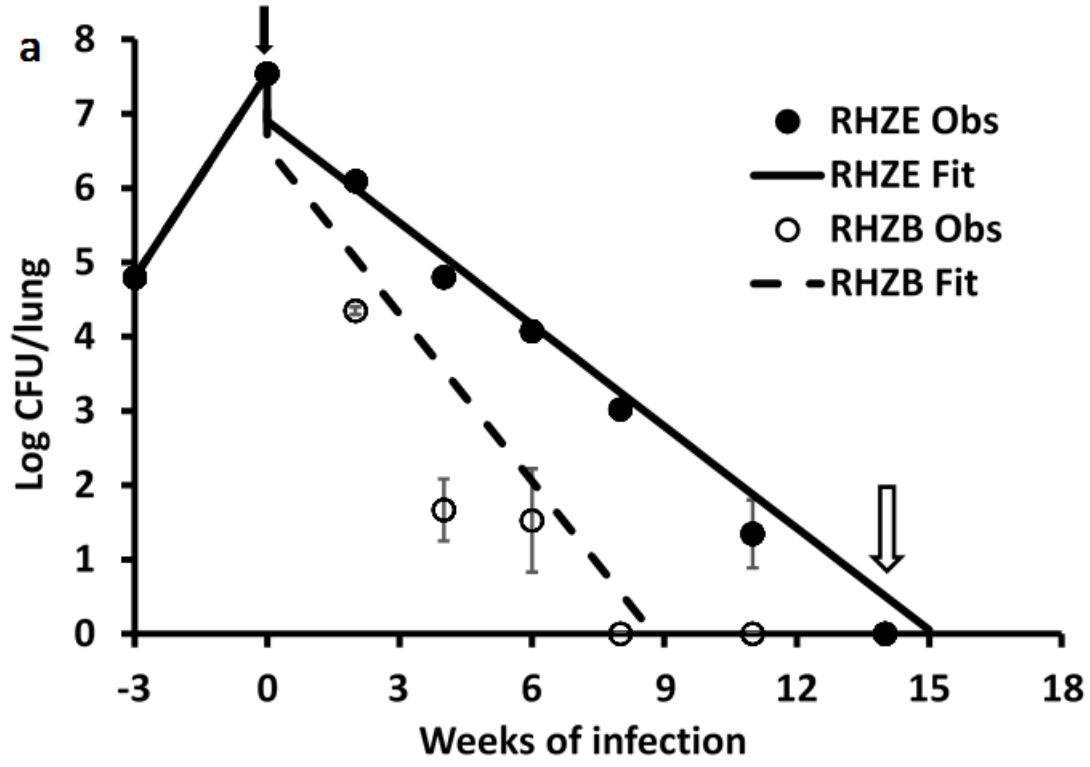
a. P = 0.05 RHZE versus RHZB. b. P = 0.002 RHZE versus RHZB.

Table 3. Relapse rate of the mice after treatment with the standard (RHZE) and bedaquiline-containing (RHZB) regimens

Treatment[a] group	Total mice	Mice with positive organs	Mice with negative organs	Relapse rate %
RHZE	21	19	2	90
RHZB	21	0	21	0

a. Treatment for 14 weeks followed by 8 weeks of hydrocortisone treatment
P = <0.001 RHZE versus RHZB

Figure 1


a
Log CFU/lung
Weeks of infection
RHZE Obs
RHZE Fit
RHZB Obs
RHZB Fit

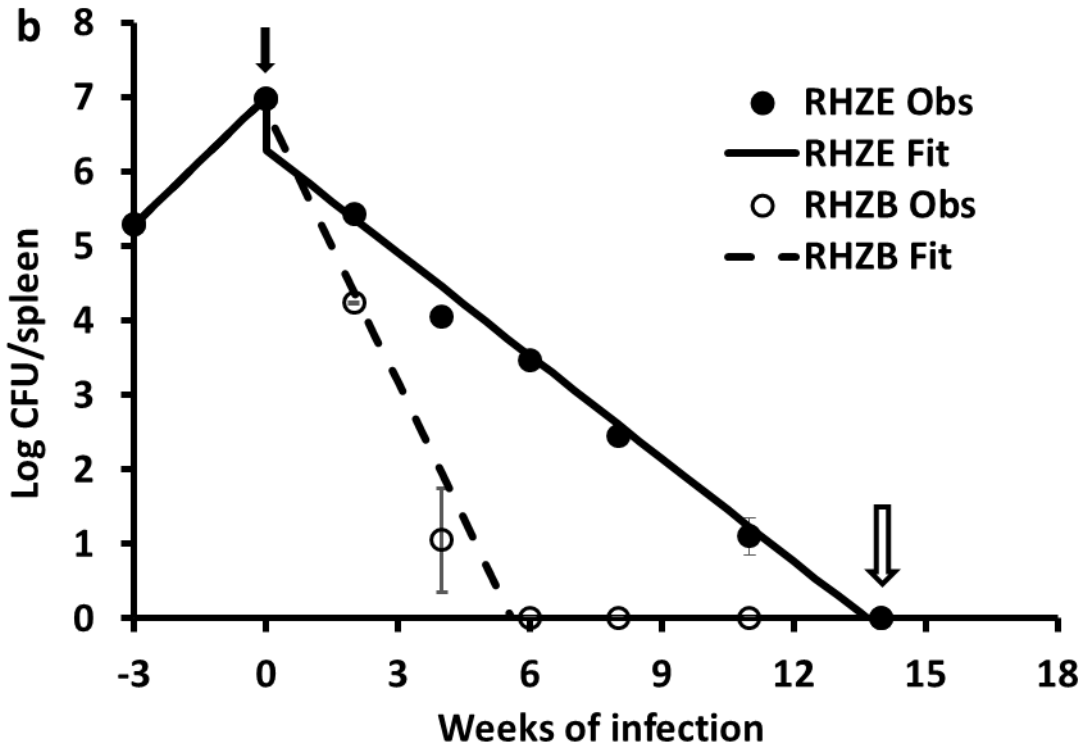
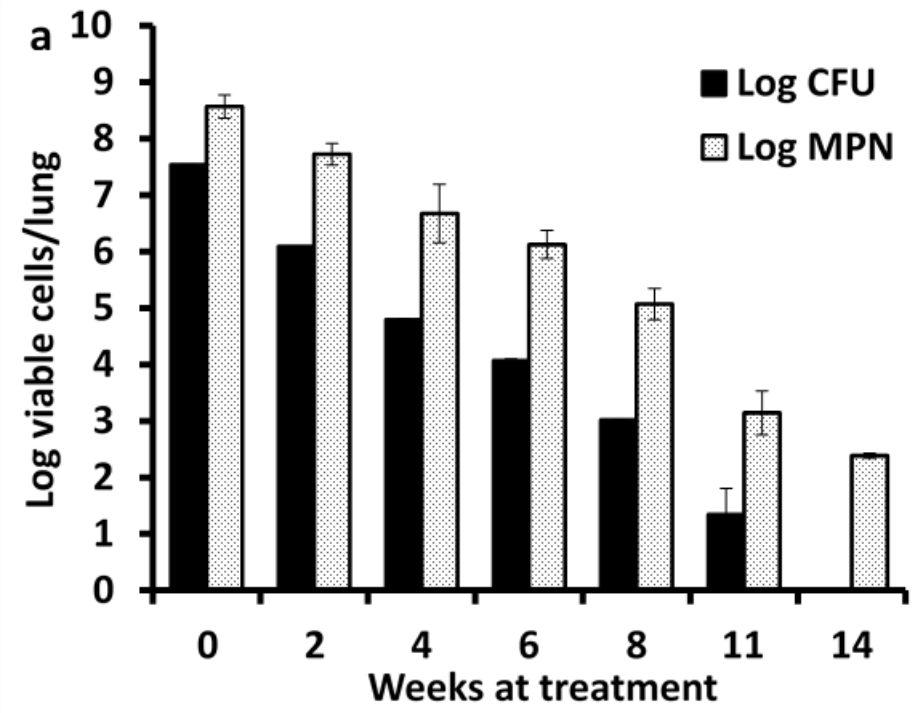

b
Log CFU/spleen
Weeks of infection
RHZE Obs
RHZE Fit
RHZB Obs
RHZB Fit

Figure 2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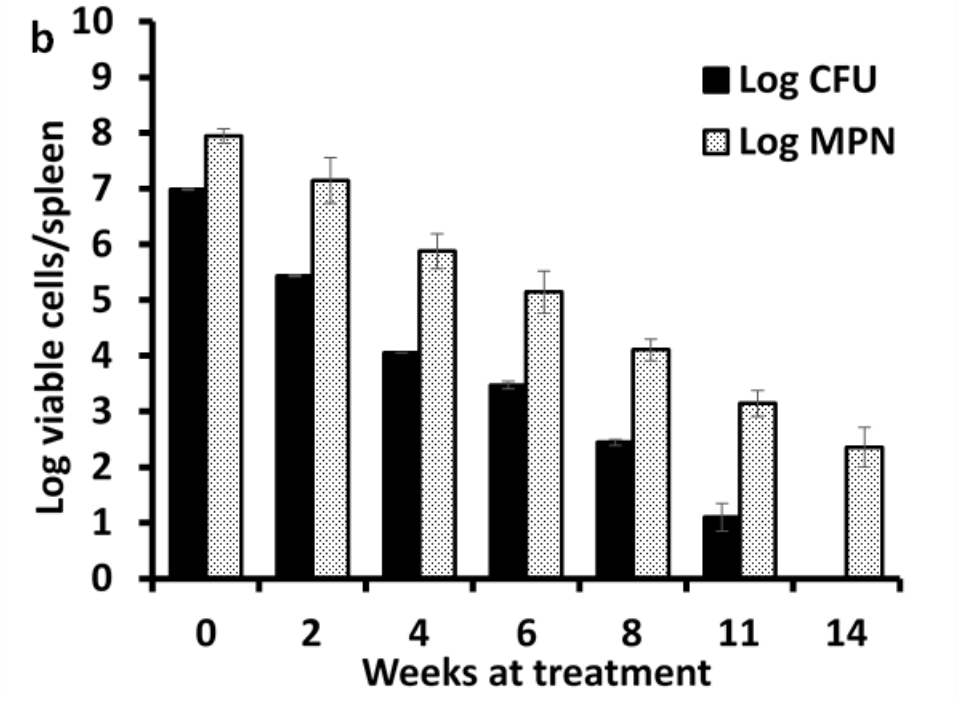
Log viable cells/lung
10
9
8
7
6
5
4
3
2
1
0
Log CFU
Log MPN
0
2
4
6
8
11
14
Weeks at treatment


b
Log viable cells/spleen
10
9
8
7
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3
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Log CFU
Log MPN
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2
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6
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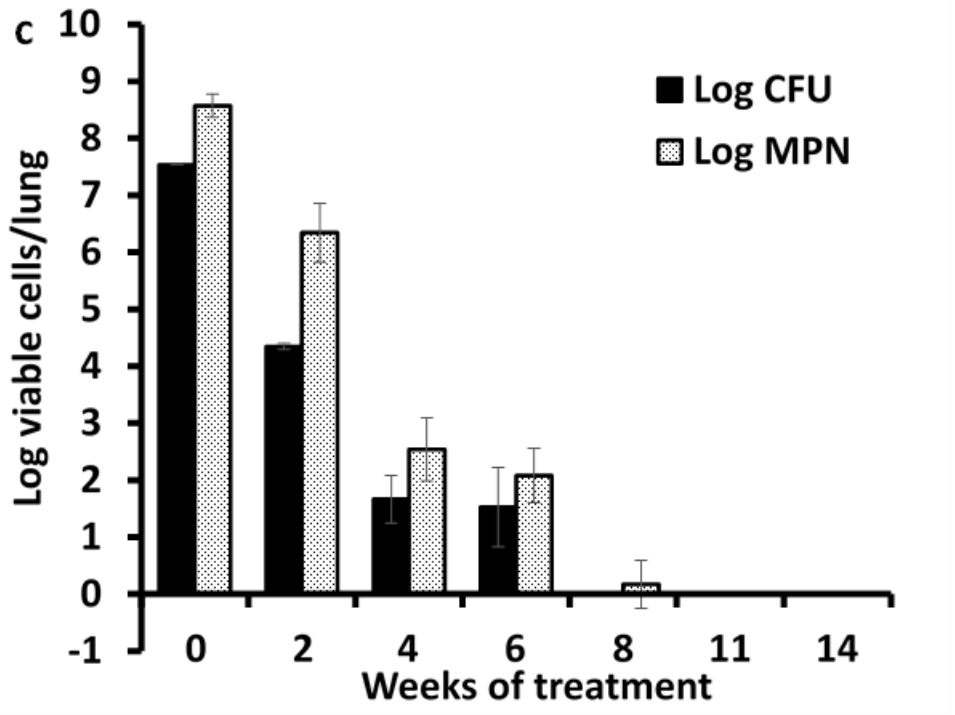
11
14
Weeks at treatment


c
Log viable cells/lung
10
9
8
7
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-1
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2
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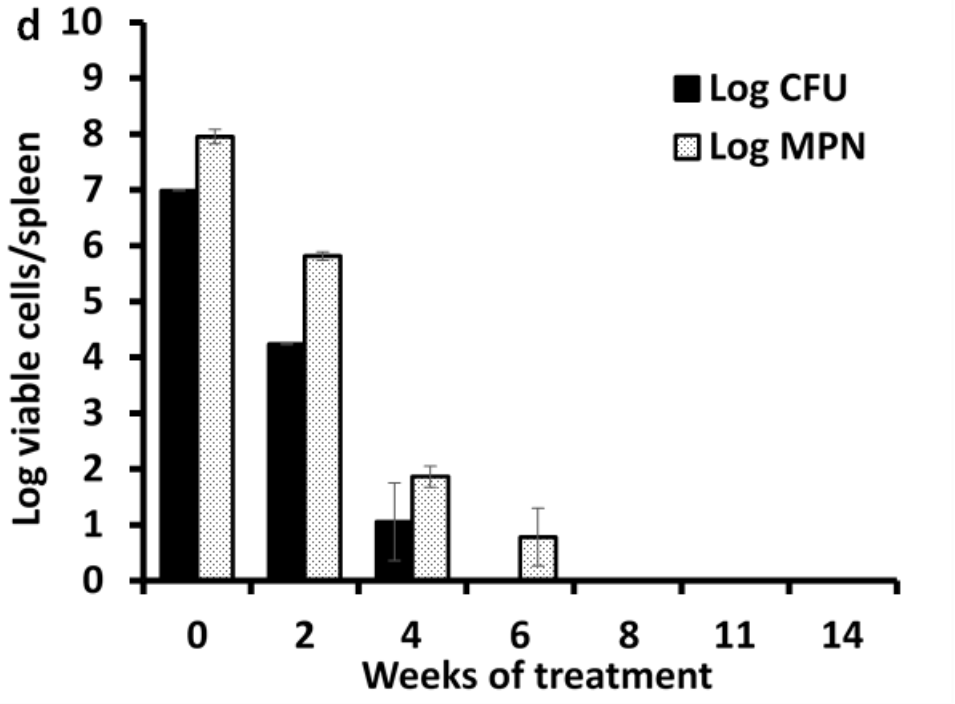
11
14
Weeks of treatment
Log CFU
Log MPN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Log viable cells/spleen
10
9
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0
2
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6
8
11
14
Weeks of treatment
Log CFU
Log MPN