1   Moxifloxacin replacement in contemporary tuberculosis drug regimens is ineffective against

2   persistent *Mycobacterium tuberculosis*: Novel insights from the Cornell mouse model

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10   Running title: Moxifloxacin regimens do not kill culture filtrate-dependent *Mycobacterium*

11   *tuberculosis*

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27   Abstract

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29   Tuberculosis (TB) caused by *Mycobacterium tuberculosis* remains a leading killer worldwide,

30   and disease control is hampered by ineffective control of persistent infections. Substitution of

31   moxifloxacin for isoniazid or ethambutol in standard TB regimens reduces treatment duration

32   and relapse rates in animal studies and four-month regimens were not non-inferior in clinical

33   trials. Resuscitation promoting factor (RPF) dependent bacilli have recently been implicated in

34   *M. tuberculosis* persistence. We aimed to investigate the therapeutic effects of moxifloxacin

35   substitution in the standard drug regimen for eradicating colony forming count (CFU) positive

36   and RPF-dependent persistent *M. tuberculosis* using the Cornell murine model. *M. tuberculosis*

37   infected mice were treated with regimens in which either isoniazid or ethambutol were

38   replaced by moxifloxacin to the standard regimen. The efficacy of the regimens was compared

39   to the standard regimen for bacterial CFU count elimination and removal of persistent tubercle

40   bacilli evaluated using culture filtrate (CF) derived from *M. tuberculosis* strain H37Rv. We

41   also measured disease relapse rates. Moxifloxacin-isoniazid substituted regimen achieved total

42   organ CFU count clearance at 11 weeks post-treatment, faster than standard regimen (14

43   weeks), and with a 34% lower relapse rate. Moxifloxacin-ethambutol substituted regimen was

44   similar to standard regimens in these regards. Importantly, neither moxifloxacin-substituted

45   regimens nor the standard regimen could remove CF-dependent persistent bacilli. Evaluation

46   of CF-dependent persistent *M. tuberculosis* requires confirmation in human studies, and has

47   implications in future drug design, testing and clinical applications.

48   Key words: *Mycobacterium tuberculosis*, moxifloxacin, Resuscitation promoting factors,

49   Cornell mouse model

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INTRODUCTION

TB caused by *Mycobacterium tuberculosis* remains a leading cause of mortality worldwide (1). Current combination antimicrobial regimens require a prolonged 6-month treatment period. This long regimen leads to poor patient compliance which gives rise to the emergence of drug resistance and high relapse rates (2). Substitution of moxifloxacin (an 8-methoxy fluoroquinolone) for drugs in the contemporary anti-TB regimen has shown promise for improving treatment efficacy (3, 4). *In vivo*, replacement of isoniazid with moxifloxacin led to shortened treatment duration (5, 6), reduced relapse rates (6, 7) and favourable outcomes in BALB/c and granuloma-forming C3HeB/FeJ mice (8). In the recent REMox-TB trial, shorter (four-month) moxifloxacin-replacement regimens (either for isoniazid or ethambutol) in human clinical trials failed to achieve non-inferiority compared to standard regimens (3, 4), mainly due to higher relapse rates (3, 4, 9). Persistent bacteria that are tolerant to drug therapy may be implicated in the higher disease relapse (9).

M. tuberculosis persistence is the single most important hurdle hampering effective TB disease control (10). *M. tuberculosis* has the ability to survive in a dormant, non-multiplying and persistent state (11-14). These persistent bacteria do not grow on solid or liquid media and are undetectable using the conventional diagnostic methods, however can be resuscitated using resuscitation promoting factors (RPF) which are present in *M. tuberculosis* culture supernatant (15). Recently, we found that using culture filtrate (CF) containing RPF (15), could induce persistent bacteria to recommence multiplication, rendering them detectable once more in mice (16-18). Moreover, these CF-resuscitated tubercle bacilli could be completely eliminated using high-dose rifampicin regimens, shortening treatment duration with no disease relapses (16, 18). In this study, we used the Cornell mouse model (19, 20) to investigate the therapeutic impact of moxifloxacin replacement in standard TB regimen against both CFU count positive and CF-dependent bacteria. We compared the regimens in which either isoniazid or ethambutol were replaced by moxifloxacin to the standard regimen by measurements of the elimination rates of

80   CFU counts, the presence of CF-dependent *M. tuberculosis* in mouse organs and disease

81   relapse rates.

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83 MATERIALS AND METHODS

84 **Bacterium and growth conditions.** *M. tuberculosis* strain H37Rv was mouse-passaged and

85 grown in 7H9 medium containing 0.05% Tween 80 and supplemented with 10% albumin

86 dextrose complex (ADC; Becton and Dickinson, UK) at 37°C without disturbance for 15

87 days. The culture was stored at -70°C for subsequent animal infection. To determine the viable

88 counts prior to infection, colony forming unit (CFU) counts were performed prior to freezing

89 and once again after thawing. The CFU counting was carried out by plating serial 10-fold

90 dilutions of the cultures on 7H11 agar medium supplemented with oleic albumin dextrose

91 complex (OADC, Becton and Dickinson, UK). Colonies were counted after incubation of

92 the plates at 37°C for 3 to 4 weeks. Viability was expressed as Log CFU/ml. The cultures were

93 subsequently diluted in PBS and used for inoculations in mice.

94 **Cornell mouse model.** Moxifloxacin (M) substitution for either isoniazid (H) or ethambutol

95 (E) in the standard TB drug regimen with rifampicin (R) and pyrazinamide (Z) was tested

96 using the Cornell mouse model (19, 20). The model was conducted using the experimental

97 design and procedure described previously (17).

98 Female BALB/c mice (6 to 8 weeks old, Harlan UK Ltd) were infected intravenously via the

99 tail vein with 1.2×10^5 CFU of mouse-passaged *M. tuberculosis* strain H37Rv per mouse as

100 described previously (16, 17, 20). The animal husbandry guidelines and all animal experiments

101 were performed according to the Animals Scientific Procedures Act, 1986 (an Act of the

102 Parliament of the United Kingdom 1986 c. 14) (Home Office Project licence Number 70/7077)

103 with approval from St George's, University of London ethics committee.

104 As shown in Table 1, control group consisted of 8 infected and untreated mice. The treatment

105 groups each contained 54 mice which were treated orally (0.2 ml) with RHZE, RHZM and

106 RMZE regimens, respectively, 5 days per week for 14 weeks. The dosages for the drugs were

107 R 10 mg/kg, H 25 mg/kg, Z 150 mg/kg, E 100 mg/kg and M 100 mg/kg. Rifampicin was

108 administered 1 hour before the other drugs to avoid drug to drug interactions.

109   For assessment of treatment efficacy, a sample of 4 mice was sacrificed at 2, 4, and 6 weeks

110   and 8 mice were sacrificed at 8, 11 and 14 weeks of treatment (Table 1). Mouse lungs and

111   spleens were transferred into 2 ml tubes each containing 1 ml sterile distilled water and 2 mm

112   diameter glass beads, followed by homogenizing using a reciprocal shaker (Thermo Hybaid

113   Ltd) for 40 seconds at 6.5 speed. The CFU counts from each lung and spleen were performed

114   using serial dilutions of the homogenates to plate on 7H11 agar plates.

115   At 11 and 14 weeks of treatment, the entire organ homogenates from the 8 mice were aliquoted

116   equally into three tubes which were used 1. The CFU counting by plating out the organ

117   homogenate suspension on 6 selective 7H11 agar plates. 2. culturing in 5 ml of selective

118   Kirchner liquid medium (21) by the addition of polymyxin B 200 U/ml, carbenicillin 100

119   mg/l, trimethoprim 20 mg/l and amphotericin B 10 mg/l (Selectatab, Mast Diagnostica GmbH)

120   for 4 weeks with subsequent sub-culturing of the entire culture onto Löwenstein-Jensen slopes

121   for a further 4 weeks and 3. resuscitation of persistent bacteria by culture filtrate. Kirchner

122   liquid medium was used to isolate different species of mycobacteria from human specimens.

123   Mitchison *et al* (21) showed that liquid Kirchner medium, made selective by the addition of the

124   antimicrobials, was more effective in the isolation of mycobacteria than other media tested.

125   Culture negative organs were defined as no colonies grown on 7H11 agar plates and no growth

126   in selective Kirchner liquid medium following inoculation on Löwenstein-Jensen slopes.

127   Immediately after termination of 14 weeks of chemotherapy, the remaining mice were

128   administered 0.5 mg/mouse of hydrocortisone acetate by daily oral administration for 8 weeks

129   to suppress host immunity, followed by CFU counting from lungs and spleens to determine

130   disease relapse.

131   **Resuscitation of *M. tuberculosis* in mouse lungs and spleens.** For resuscitation of *M.*

132   *tuberculosis* grown in mouse organs, culture filtrates containing RPFs were used as described

133   previously (15-17).

134   *M. tuberculosis* H37Rv was grown in 7H9 medium without disturbance at 37°C for 15 to 20

135   days until an optical density of 1 to 1.5 was reached. The culture supernatants were collected

136   by centrifugation at 3000 g for 15 minutes and sterilized by double filtration with 0.2 µm filters

137   (Sartorius). The sterilized culture filtrates were made selective by the addition of polymyxin B

138   200 U/ml, carbenicillin 100 mg/l, trimethoprim 20 mg/l and amphotericin B 10 mg/l

139   (Selectatab, Mast Diagnostica GmbH) and immediately used for broth dilution to count the

140   most probable number (MPN) of the bacilli (22).

141   Broth counting of lungs and spleens was performed as serial 10-fold dilutions in which 0.5 ml

142   of tissue homogenates were added to 4.5 ml of the culture filtrates. At 10-day intervals over a

143   2-month period of incubation at 37°C, the broth cultures were examined for visible turbidity

144   changes. Growth of *M. tuberculosis* in turbid tubes was confirmed by colonial morphology on

145   7H11 agar plates. The MPN of viable bacilli was then estimated from the patterns of positive

146   and negative tubes accosting to the method of US Food and Drug Administration (22). The

147   absence of microorganisms other than mycobacteria from turbid tubes was confirmed by

148   plating on blood agar medium (Oxoid) and Sabouraud dextrose agar (Oxoid). In order to assess

149   the sterility of culture filtrates free of *M. tuberculosis*, tubes containing culture filtrates were

150   incubated at 37°C for 2 months to ensure the absence of *M. tuberculosis* in the culture filtrates.

151   **Statistical analysis.** A simple model for mono-exponential bacterial growth and elimination

152   (17, 23) was fitted to the profiles of CFU vs. time obtained experimentally. As simultaneously

153   occurring exponential replication and death rates cannot be differentiated with this type of data,

154   a "knet" exponential rate constant was estimated separately before treatment began

155   ("knet_no_drug" where it would take a net positive value) and during treatment

156   ("knet_with_drug" where it would take a net negative value). During therapy, knet is a 1st order

157   elimination rate constant which can be interpreted as the slope of the modelled line fit through

158   the logarithmic-transform of the data (with units in these data of wk^{-1}). Parameter estimation

159   was carried out with nonlinear regression using the nonlinear least squares optimisation

160   function "lsqnonlin" as part of the "pracma" package in the R statistical software language,

161   with an objective function weighted by 1/(predicted value)^2. Standard errors of parameter

162   estimates were calculated using the method described previously (24) with the Jacobian of

163   model parameter sensitivities estimated using a numerical central difference method. The

164   datasets comprised from multiple individual subject animals were treated as a naïve pool for

165   data analysis purposes (25) rather than using the average of the data at each time-point. The

166   significance of differences between model parameter estimates under different therapies was

167   examined with pairwise Z-tests incorporating a Bonferroni correction of 3, where P values

168   <0.017 would be considered significant. The significance of differences between the relapse

169   rates was determined with pairwise Fisher's exact tests also with a Bonferroni correction of 3,

170   with P values <0.017 considered significant.

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172 RESULTS

173 **Treatment with moxifloxacin containing regimens in the Cornell mouse model.** In the

174 Cornell model, after three weeks of infection, mean CFU counts in the organs reached log 7.54

175 in lungs and 6.99 in spleens (Table 2).

176 When we investigated the substitution of moxifloxacin for either isoniazid or ethambutol in the

177 current drug regimen on the rate of bacterial CFU elimination, we found that the early

178 bactericidal activities were similar amongst the three drug regimens, which were 99% kill at

179 2.2 weeks for moxifloxacin replacing isoniazid regimen (RMZE), 2.7 weeks for standard

180 regimen (RHZE) and 3 weeks for moxifloxacin replacing ethambutol regimen (RHZM).

181 Treatment with RMZE increased the rate of bacterial elimination showing undetectable CFU

182 counts at 11 weeks compared to 14 weeks for the standard regimen and moxifloxacin replacing

183 ethambutol regimen (Table 2).

184 These observations coincided with bactericidal activities as assessed using the mono-

185 exponential bacterial elimination rate constants (Fig 1, and Table 3) where the exponential rate

186 constants (logarithmic base 10) for net bacterial elimination during treatment ($k_{net\_with\_drug}$) for

187 standard, moxifloxacin replacing ethambutol and moxifloxacin replacing isoniazid regimens

188 were -0.46, -0.50 and -0.65, in lungs and -0.46, -0.46 and -0.60 in spleens, respectively (Table

189 3). The higher the absolute value of this elimination rate constant (i.e. the steeper the slope of

190 the elimination on the logarithmic scale with units of wk^{-1}), the quicker the exponential

191 elimination rate of CFU counts in the organs These values indicate therefore that compared to

192 the standard therapy, substitution of moxifloxacin for isoniazid gives a significant increase in

193 bacterial elimination in both lungs and spleens while substitution of moxifloxacin for

194 ethambutol makes a statistically indistinguishable difference.

195 In the CFU count free organs, no tubercle bacilli were recovered which was determined by

196 negative cultures of the organ homogenates in selective Kirchner broth for 4 weeks followed

197 by growth on Löwenstein–Jensen medium.

198 **Post-treatment level of CF-resuscitated MPN in the Cornell model.** In order to investigate

199 the effect of moxifloxacin containing regimens on the post-treatment level of persistent bacilli

200 through CF-induced resuscitation, lung and spleen homogenates at the weeks of treatment

201 when CFU counts reached zero for each of the regimens were incubated with culture filtrates.

202 As shown in Table 4, after 14 weeks of treatment with RHZE and RHZM, high levels of CF-

203 resuscitated bacilli remained in both lungs and spleens. For RMZE treatment, at 11 weeks post-

204 treatment, although CFU counts were zero, there were average 2.96 and 3.01 log of CF-

205 resuscitated MPN of bacilli per lung and spleen, respectively. At 14 weeks of treatment, there

206 were still 2-log MPN of the bacilli present (Table 4). The numbers of CF-dependent bacteria at

207 14 weeks amongst the three treatment groups were not significantly different ($p > 0.05$, $n = 8$).

208 **Relapse rate of treatment with the moxifloxacin containing regimens in the Cornell**

209 **model.** After 8 weeks of high dosage steroid immunosuppression, disease relapse rates for the

210 treatment with the three drug regimens were determined by the percentage of mice that

211 developed positive *M. tuberculosis* cultures (CFU counts) in lungs, spleens or both. As shown

212 in Table 5, treatment with the standard regimen RHZE gave rise to positive organs in 19 out of

213 21 mice (90% relapse rate) and RHZM led to 95% relapse rate after 14 weeks of treatment. In

214 contrast, treatment with RMZE resulted in 59% of relapse ($P = 0.03$ vs. RHZE, $P = 0.009$ vs.

215 RHZM, $P < 0.017$ significant at 0.05 level after Bonferroni correction for 3 pairwise

216 comparisons).

217 We also measured CF-dependent bacilli using culture filtrates in the organs which

218 showed CFU count negative after 8 weeks of steroid treatment. As shown in Table 5,

219 the negative organs in each treatment group contained high numbers of CF-dependent

220 cells. There are average 3.3, 3.51 and 3.05 logs of MPN per organ in the groups of

221 RHZE, RHZM and RMZE, respectively.

222

223   DISCUSSION

224   This is the first study, using the reliable Cornell mouse model, to characterize the therapeutic

225   efficacy of moxifloxacin-replacement *in vivo* against CF-dependent *M. tuberculosis* persistent

226   cells. Compared to standard TB regimen, we found that moxifloxacin replacement for

227   isoniazid: (i) failed to remove CF-dependent bacilli, despite having (ii) faster organ CFU count

228   elimination rates and (iii) lower disease relapse rates. In contrast, moxifloxacin replacement for

229   ethambutol failed to demonstrate any therapeutic benefits compared to the standard regimen.

230   These results of CF resuscitation need to be confirmed in human studies, but may provide a

231   novel mechanistic explanation for the results of moxifloxacin-replacement regimens in clinical

232   trials. Findings in this study also have important future implications in TB novel drug design,

233   diagnostic testing and clinical applications.

234   **Moxifloxacin-replaced drug regimens are ineffective against CF-dependent tubercle**

235   **bacilli.** The greater therapeutic efficacy and lower relapse rates achieved with moxifloxacin-

236   isoniazid replacement regimen, as compared to the standard regimen, in this study is consistent

237   with previous reports (5-8). De Groote *et al* demonstrated that moxifloxacin replacing isoniazid

238   in the standard regimen gave rise to 63% disease relapse (6). In other studies, Nuermberger and

239   colleagues showed that the same drug regimen produced a lower disease relapse at 33.3% (7).

240   Late studies using two pathologically distinct murine tuberculosis models demonstrated very

241   similar low disease relapses (8). Our study confirmed this interesting observation showing 56%

242   relapse, indicating the consistency of the drug regimen in different mouse models.

243   The underlining mechanisms that moxifloxacin replacing isoniazid was more efficacious than

244   the standard or moxifloxacin replacing ethambutol regimens were unknown. It has been shown

245   previously that when mice were treated with rifampicin-isoniazid-pyrazinamide, rifampicin-

246   isoniazid or rifampicin-pyrazinamide for 6 months, rifampicin-pyrazinamide treated group

247   demonstrated significantly lower relapse rates than the other two groups containing isoniazid

248 (26), suggesting that isoniazid antagonized the actions of rifampicin-pyrazinamide (26). It is

249 possible that replacement of isoniazid with moxifloxacin eliminated the antagonistic drug

250 interaction leading to the rapid organ CFU count clearance in mice.

251 The use of CFU counts as an end point reflects the clinical observations in patients to a large

252 degree, which is related to clinical endpoints such as sputum culture conversion in patients (3,

253 27, 28). The improved efficacy with moxifloxacin replacement for isoniazid compared to the

254 standard regimen reflected the clinical outcome in patients to some extent, for example, the

255 moxifloxacin-isoniazid substitution regimen showed an effective bactericidal activity which

256 was able to kill CFU count positive bacilli faster than the standard regimen, leading to the

257 higher sputum culture conversion rate in humans (3, 27, 28).

258 Despite the improved performance, moxifloxacin-isoniazid replaced regimen was ineffective

259 against CF-dependent bacterial cells, which has not been demonstrated previously. At 11

260 weeks and the end of the antibiotic therapy, despite the elimination of CFU count positive

261 bacilli, considerable MPN of CF-dependent bacilli remained in all the mice, which were

262 similar to those treated with the standard drug regimen or the moxifloxacin-ethambutol

263 replaced regimen. This indicated that although the moxifloxacin-isoniazid replaced regimen

264 was more bactericidal than the standard regimen, the drug regimen failed to show improved

265 sterilizing activity against persistent bacteria. After 8 weeks of immunosuppression with

266 steroid, there were 90% of standard regimen treated mice and 95% of moxifloxacin

267 replacement for ethambutol treated mice with CF-dependent cells which became CFU count

268 positive again. This high disease relapse rate can only be feasibly explained by the reactivation

269 of CF-dependent cells since the mice were CFU count-zero before immunosuppression. In

270 addition to a lower relapse rate, mice treated with moxifloxacin replacement for isoniazid

271 regimen contained similar MPN counts in their negative organs to the other two groups. This

272 indicated that certain drug regimens such as moxifloxacin replacement of isoniazid may induce

273 heterogeneously more diverse bacterial populations, therefore not all CF-responding bacteria

274   regain their ability to form colonies on agar plates at the time we determined relapse, showing a

275   lower disease relapse. Further studies are warranted on the induction of heterogeneity of bacterial

276   populations using different anti-TB drug regimens.

277   **Clinical trials and animal studies: different testbeds, same mechanism**. None of the two

278   moxifloxacin-replacement phase III clinical trials have demonstrated non-inferiority to

279   standard regimens for treatment duration and disease relapses (3, 4); the underlying mechanism

280   is unclear. An important finding of the REMoxTB trial is that there was a proportion of

281   patients who showed sputum conversion quickly but continued to relapse after treatment with

282   all three drug regimens (9). Our experiments suggest that the high relapse rates may have been

283   due to CF-dependent bacilli (9) which, till now, have remained undetectable using

284   conventional culture methods, including those used in the clinical trials (3, 4, 9). Persistent

285   bacteria are established causes of prolonged chemotherapy and disease relapse (10). It has been

286   repeatedly shown that in the Cornell mouse model, high relapse rate after treatment with the

287   standard drug regimen was due to the presence of CF-dependent persistent bacteria (16, 17).

288   Recently, we showed that drug regimens containing high doses of rifampicin (30 mg/kg or

289   higher) could eliminate CF-dependent persistent bacilli, which led to shortened treatment

290   duration from 14 weeks to just 6 weeks, without disease relapses (16, 18). The lesson learnt

291   from the REMoxTB trial is that more rapid culture conversion in the short moxifloxacin

292   containing regimens may not allow for shortened regimens due to the presence of persistent

293   bacteria (9). A previous study also showed that early bactericidal activities of certain novel

294   drug regimens were not necessarily predictive of any sterilizing effects (29). This may be

295   attributed to the inability of the drug regimens to eliminate the persistent bacilli which were

296   undetectable using the traditional microbiological methods. Therefore, in addition to the

297   conventional microbiological methods, evaluation of anti-TB regimens by assessing their

298   efficacies in eliminating RPF-dependent *M. tuberculosis* is important for providing a

299 comprehensive profile of novel drug regimens before proceeding to human clinical trials. The

300 combined data sets on CFU counts, broth growth and persister counts will strengthen any

301 claims to be made on a regimen, which will ultimately increase the confidence of advancing it

302 into humans.

303 **Clinical trials and animal studies: different testbeds and important considerations.** The

304 interpretation of the mouse results of moxifloxacin-replacement regimens compared with the

305 results in clinical trials requires careful consideration. In the previous animal studies for

306 example the burden of persistent bacteria had not been detected and assessed (5, 7) largely due

307 to the undetectable feature of the persisters (16, 17). Importantly, there are clear differences in

308 the pathophysiology of TB between humans and mice. Patients with active TB have persisters

309 residing in a milieu of different pathogenic states, including necrotic/caseating lesions, central

310 liquefactive lesions, open cavities, closed fibrotic granulomas (30, 31). Indeed, a patient over

311 time may develop a combination of these lesions (10). Consequently, these heterogeneous

312 persistent bacteria co-exist with fast growing bacteria at the time of the commencing antibiotic

313 treatment (10). In contrast, mice do not form granuloma structures after *M. tuberculosis*

314 infection (30) and persistent bacteria are generally low in absolute number (similar to the CFU

315 counts) at the beginning of the treatment (16). In addition, an important study has shown that

316 moxifloxacin does not diffuse into caseating lesions, which may also lead to reduced sterilizing

317 activities against persisters in human TB (32).

318 Treatment of TB persisters is complex and future clinical trials require careful consideration of

319 mechanistic *in vivo* studies, which can elucidate further insights into potential therapeutic

320 targets. The study reported here represents an important step in the right direction by showing

321 that RPF-dependent persisters may be a novel and important clinical therapeutic target.

322 In conclusion, moxifloxacin substitution in contemporary drug regimens was ineffective

323 against resuscitation promoting factor dependent persistent *M. tuberculosis*, despite having

324 favorable therapeutic efficacy against actively multiplying bacteria *in vivo*.

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459 Figure 1. Treatment profiles of *M. tuberculosis* H37Rv with regimens RHZE, RMZE and

460 RHZM in the Cornell mouse model. A. Elimination of CFU counts in lungs. B. Elimination of

461 CFU counts in spleens. The solid arrow indicates the treatment starting at 3 weeks of post

462 infection. The empty arrow indicates starting steroid treatment after the termination of 14 week

463 therapy.

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Table 1. Mouse tuberculosis experimental design

Treatment groups[a]	Total No. of mice[b]	D0	D21	2W	4W	6W	8W	11W	14W	22W[c]
Control	8	4	4							
RHZE	54			4	4	4	8	8	8	22
RHZM	54			4	4	4	8	8	8	22
RMZE	54			4	4	4	8	8	8	22

[a] Mice were intravenously infected at day 0. Treatment commenced at 21 days. Dosages for each drug were as follows: R 10 mg/kg, H 25 mg/kg, Z 150 mg/kg, E 100 mg/kg and M 100 mg/kg.
[b] Total mice were infected and treated excluding natural death of the mice during the course of treatment
[c] 8 weeks of hydrocortisone treatment post 14 weeks of treatment

Table 2. Organ CFU counts before and after treatment with experimental regimens

Time of infection and treatment	Mean Log CFU per lung ± SD				Mean Log CFU per spleen ± SD			
	Control	RHZE	RHZM	RMZE	Control	RHZE	RHZM	RMZE
D0[a]	4.80 ± 0.14				5.29 ± 0.03			
D21[b]	7.54 ± 0.03				6.99 ± 0.06			
2W[c]		6.09 ± 0.03	5.92 ± 0.14	5.70 ± 0.12		5.43 ± 0.08	5.54 ± 0.12	4.77 ± 0.10
4W		4.80 ± 0.07	4.52 ± 0.28	3.61 ± 0.16		4.05 ± 0.02	4.49 ± 0.08	3.31 ± 0.21
6W		4.08 ± 0.11	4.00 ± 0.09	2.53 ± 0.29		3.49 ± 0.16	3.81 ± 0.06	2.27 ± 0.20
8W		3.00 ± 0	3.25 ± 0.48	2.00 ± 0		2.44 ± 0.22	3.24 ± 0.13	1.57 ± 0.20
11W[d]		2.00 ± 0	2.00 ± 0	0		1.19 ± 0.29	2.00 ± 0	0
14W[d]		0	0	0		0	0	0

a. 2 hours post-infection. b. 21 days post-infection. c. week 2 post-treatment. d. CFU counts were derived from one third of tissue homogenate and limit detection was 3 CFU/organ.

Table 3. Elimination constant rates of different treatment groups

| Treatment group | Elimination rate constant (wk-1)[a] | | | |
| | Lungs[b] | | Spleens[c] | |
	alpha	%RSE	alpha	%RSE
RHZE	-0.46	3.20	-0.46	4.76
RHZM	-0.50	8.57	-0.46	6.54
RMZE	-0.65	10.96	-0.57	6.40

a. Elimination rate constant equivalent to "knet_with_drug"
b. P = 0.008 RMZE vs. RHZE, P = 0.065 RMZE vs. RHZM, P = 0.384 RHZE vs. RHZM
c. P = 0.018 RMZE vs. RHZE, P = 0.011 RMZE vs. RHZM, P = 0.943 RHZE vs. RHZM
P < 0.017 significant at 0.05 level after Bonferroni correction for 3 pairwise comparisons.

Table 4. MPN of *M. tuberculosis* H37Rv in CFU count negative mouse lungs and spleens after treatment with different drug regimens

Treatment regimen	MPN/lung[a]				MPN/spleen[b]			
	11 week	95% confidence limits	14 week	95% confidence limits	11 week	95% confidence limits	14 week	95% confidence limits
RHZE	-		2.50 ± 0.19	2.35 - 2.67	-		2.56 ± 0.16	2.44 - 2.70
RHZM	-		2.55 ± 0.14	2.44 - 2.67	-		2.60 ± 0.09	2.52 - 2.69
RMZE	2.96 ± 0.15	2.86 - 3.08	2.30 ± 0.23	2.15 - 2.50	3.01 ± 0.14	2.92 - 3.11	2.35 ± 0.16	2.27 - 2.45

[a]determined by MPN of the diluted lung homogenates (n=8) with the culture filtrates. [b]determined by MPN of the diluted spleen homogenates (n=8) with the culture filtrates.
The CFU count zero organs showed no growth in Kirchner liquid medium following inoculation on Löwenstein-Jensen slopes.
Broth counts were derived from one third of tissue homogenate and calculated to represent the MPN of entire organ. The limit of detection was 30 MPN/organ.
-, Colony count positive and MPN counts not performed organs. The limit of detection was 3 CFU/organ.

Table 5. Relapse rates of mice after treatment with different drug regimens

Positive culture from	RHZE	RHZM	RMZE
Spleen only	3	5	4
Lung only	0	2	5
Both organs	16	13	4
Neither organs	2	1	9
Total mice	21	21	22
Relapse rate pn/N (%)[a]	19/21 (90)	20/21 (95)	13/22 (59)
MPN in CFU count negative organs	3.30 ± 0.13	3.51 ± 0.11	3.05 ± 0.18

a, Relapse rates include all lungs or spleens or both organs positive for bacilli. N, total
number of mice. pn, number of mice with CFU count positive organs.
$P = 0.03$ RMZE vs. RHZE, $P = 0.009$ RMZE vs. RHZM, $P = 1$ RHZE vs. RHZM
$P < 0.017$ significant at 0.05 level after Bonferroni correction for 3 pairwise comparisons