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Evaluation of Moxifloxacin-Containing Regimens in Pathologically Distinct Murine Tuberculosis Models

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In the recently concluded REMox-TB trial, two 4-month moxifloxacin-containing regimens did not meet the criteria for noninferiority compared to the current 6-month first-line regimen to treat tuberculosis (TB). Despite the disappointing result, this phase 3 clinical trial provides a rare opportunity to gauge the predictive accuracy of the nonclinical models used to support regimen development. In parallel with the REMox-TB trial, we compared the efficacy of the same three regimens against chronic TB infection in the commonly used BALB/c mouse strain and in C3HeB/FeJ mice, which have attracted recent interest as a nonclinical efficacy model because they develop caseous lung lesions which may better resemble human TB. In long-term treatment experiments at two institutions, using low-dose aerosol infection models with 6- to 8-week incubation periods in both mouse strains, control mice received rifampin, isoniazid, pyrazinamide, and ethambutol (RHZE), and test mice received the same regimen with moxifloxacin replacing isoniazid (RMZE) or ethambutol (RHZM). Outcome measures were lung CFU counts during treatment and relapse after various durations of treatment. At both institutions and in both mouse strains, RMZE and RHZM reduced by approximately 1 month and 0 to 1 month, respectively, the treatment duration needed to produce the same relapse rate as RHZE. These results demonstrating generally similar treatment-shortening effects of the moxifloxacin-containing regimens in each mouse strain, with effect sizes consistent with the REMox-TB trial results, reinforce the predictive value of murine models for TB regimen development.

Animal models provide a critical bridge between *in vitro* studies and human clinical trials. Mice have served as models for evaluating the efficacy of new antituberculosis drugs and regimens for more than 50 years. The treatment-shortening potential of rifampin (RIF) and pyrazinamide (PZA) was forecast in mice, as was the lack of such sterilizing activity for streptomycin, isoniazid (INH), ethambutol (EMB) and second-line agents developed up until the 1990s (1–3). However, interspecies differences in the spectrum of lung pathology caused by *Mycobacterium tuberculosis* infection have fueled concerns that mouse models may not accurately represent the efficacy of new antituberculosis drugs or regimens (4). Specifically, it is well known that commonly used mouse strains infected with *M. tuberculosis* do not develop caseous necrosis and cavitation, which are pathological hallmarks of pulmonary tuberculosis (TB) in humans. If such lesions affect drug exposures at the site of infection or if a microenvironmental condition associated with caseous necrosis (e.g., hypoxia, nutrient depletion, and altered pH) is a determinant of bacterial persistence or drug activation or activity, then the sterilizing activity of new TB drug candidates could be misjudged in mice. Recently, C3HeB/FeJ mice have attracted interest because, in contrast to commonly used mouse strains, they develop caseating granulomas, caseous pneumonia, and even cavities after low-dose aerosol infection with *M. tuberculosis* (5–11). By providing more human-like pathology while preserving the economy of murine models, these mice may provide a promising alternative to conventional mouse strains and larger animal species for nonclinical efficacy studies.

Experiments conducted a decade ago in BALB/c mice first identified moxifloxacin (MXF) as a candidate to replace INH or EMB in the first-line regimen for TB (12, 13). Specifically, substitution of MXF in place of INH reduced the duration of treatment needed to prevent relapse in mice by at least 1 month, a result which was supported by additional experiments at two different laboratories using another *M. tuberculosis* strain (Erdman), differing aerosol infectious doses, or intravenous rather than aerosol infection (14). The addition of MXF to the RIF-INH-PZA combination, which approximates the substitution of MXF for the bacteriostatic agent EMB, also increases the bactericidal activity of the regimen in mice (13, 14). However, the potential of using MXF in place of EMB to shorten the duration of treatment needed to prevent relapse has not been carefully examined in mice. Subsequent phase 2/3 clinical trials have demonstrated a modestly higher rate of sputum culture conversion when MXF is substituted for INH or EMB (15–21). However, as recently demonstrated in the phase 3 REMox-TB trial, the benefit of substituting MXF for INH or EMB is not sufficiently large to enable a reduction of the treatment duration from 6 to 4 months without increasing the number of unfavorable outcomes, including relapse (16).

The performance of large phase 3 trials such as REMox-TB

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provides a rare opportunity to evaluate the correlation between outcomes in humans and nonclinical efficacy models on the basis of a preferred endpoint such as relapse. In an effort to determine whether C3HeB/FeJ mice add predictive value to conventional mouse strains as a model for nonclinical efficacy testing, we compared the response to treatment with the three regimens studied in the REMox-TB trial in BALB/c and C3HeB/FeJ mice at two institutions: the Johns Hopkins University (JHU) and Colorado State University (CSU). In both models and at both institutions, the small magnitude of the treatment-shortening effect of substituting MXF for INH or EMB was consistent with the inability of these substitutions to shorten treatment by 2 months in the REMox-TB trial.

MATERIALS AND METHODS

***M. tuberculosis* strain.** For the experiment performed at JHU, *M. tuberculosis* H37Rv was passaged in mice, grown in Middlebrook 7H9 broth (Fisher Scientific) supplemented with 10% oleic acid-albumin-dextrose-catalase with 0.05% Tween 80 (Sigma-Aldrich) to an optical density of 0.80, and frozen in titered 1-ml aliquots at −80°C.

In the experiment at CSU, *M. tuberculosis* Erdman (TMCC 107) was used. The bacteria were originally grown as a pellicle to generate low-passage-number seed lots. Working stocks were generated by growing to mid-log phase in Proskauer-Beck medium containing 0.05% Tween 80 (Sigma-Aldrich, St. Louis, MO) in three passages, enumerated by serial dilution on 7H11 agar plates, divided into 1.5-ml aliquots, and stored at −80°C until use.

Antimicrobials. RIF, INH, and EMB were purchased from Sigma-Aldrich. PZA was purchased from Acros Organics. MXF was generously provided by Bayer. All drugs were prepared weekly in distilled water. All antibiotic solutions were stored at 4°C.

Aerosol infection. At JHU, 145 BALB/c (Charles River Laboratories) and 145 C3HeB/FeJ mice (Jackson Laboratory), each 4 to 6 weeks old, were aerosol infected using a Middlebrook inhalation exposure system (Glas-Col) using dilutions of a titered stock of *M. tuberculosis*. At CSU, 190 mice, 15 to 17 weeks old, of each strain were infected in a similar fashion. Two aerosol infection runs were performed for each mouse strain. All animal procedures were approved by the respective Animal Care and Use Committees at JHU and CSU.

Chemotherapy. Mice were block randomized by infection run into treatment groups, and treatment was initiated 42 days after infection at JHU and 56 days after infection at CSU. Drugs were administered 5 days per week, by oral gavage. The drug doses (in mg/kg) were as follows: RIF, 10; INH, 10; PZA, 150; MXF, 100; and EMB, 100 (13, 22). At JHU, control mice received 2 months of RIF-INH-PZA-EMB, followed by 2 months of RIF-INH. To assess the potential role of MXF in first-line regimens, EMB was replaced with MXF in the first test regimen and INH was replaced with MXF in the second test regimen, as shown in Table 1. The test mice received 2 months of RIF-INH-PZA-MXF, followed by 1 month of RIF-INH-MXF or 2 months of RIF-MXF-PZA-EMB, followed in turn by 1 month of RIF-MXF. At CSU, the same four drugs in each regimen were administered throughout the experiment.

Assessment of treatment efficacy. Baseline lung CFU counts were determined the day after aerosol infection (D-42 at JHU; D-56 at CSU) and at treatment initiation (D0). Treatment efficacy was assessed on the basis of lung CFU counts determined during treatment and the proportion of mice with culture-positive relapse after completing treatment of various durations. CFU counts were compared after 4 and 8 weeks of treatment at JHU and after 4, 8, 12, and 16 weeks of treatment at CSU. Relapse was defined by the recovery of ≥1 CFU after plating the entire lung homogenate and was assessed by holding cohorts of 15 mice for 12 additional weeks after completion of 8, 12, and 16 weeks of treatment. Plates were incubated for 28 days at 37°C before the final CFU counts were determined.

TABLE 1 Scheme of the experiments

Treatment[a]	Mouse strain	No. of mice assessed for lung CFU count (relapse) at various time points[b]					
		D-42 or D-56	D0	M1	M2	M3	M4
RIF-INH-PZA-EMB	BALB/c	4	4	5	5 (15)	(15)	(15)
	C3HeB/FeJ	4	4	5	5 (15)	(15)	(15)
RIF-INH-PZA-MXF	BALB/c			5	5 (15)	(15)	
	C3HeB/FeJ			5	5 (15)	(15)	
RIF-MXF-PZA-EMB	BALB/c			5	5 (15)	(15)	
	C3HeB/FeJ			5	5 (15)	(15)	

[a] Drug doses (in mg/kg) were as follows: RIF, 10; PZA, 150; INH, 10; EMB, 100; and MXF, 100. PZA and EMB were given for the first 2 months only at JHU; no drugs were discontinued at CSU.

[b] Time points are shown in days prior to treatment (D-42 [JHU], D-56 [CSU], or D0) or months (e.g., 2 months [M2]) of treatment. "(15)" indicates that the mice were held for 3 months after the completion of treatment at the indicated time point. CFU counts were also assessed at M3 and M4 at CSU.

Statistical analysis. Lung CFU counts were $\log_{10}$ transformed before analysis. Group mean CFU counts were compared using one-way analysis of variance with Dunnett's post-test. The proportions of mice relapsing were compared using the Fisher exact test. Analyses were performed using Prism 5 (GraphPad Software, San Diego, CA). All measures of statistical variation are expressed as standard deviations (SD).

RESULTS

Lung CFU counts during treatment. Lung CFU counts are presented in Table 2. At JHU, the mean lung CFU counts (± the SD) at the start of treatment (D0) were 7.45 ± 0.40 and 8.83 ± 0.55 for BALB/c and C3HeB/FeJ mice, respectively. At CSU, the mean lung CFU counts at day 0 were 5.62 ± 0.25 in BALB/c mice and 6.70 ± 1.21 in C3HeB/FeJ mice.

In BALB/c mice at JHU, RIF-INH-PZA-EMB treatment reduced the mean lung CFU count by approximately 3.5 and 5.5 $\log_{10}$ after treatment for 1 and 2 months, respectively, a finding consistent with the declines of 3.33 and 5.62 $\log_{10}$, or culture negativity, observed at CSU. Substitution of MXF for EMB at JHU produced a small additional reduction of ~0.3 $\log_{10}$ CFU, which was statistically significant after 1 month ($P < 0.05$) but not 2 months of treatment. Substitution of MXF for INH reduced the lung CFU counts by an additional 0.75 and 1.1 $\log_{10}$ after 1 and 2 months, respectively ($P < 0.001$). At CSU the substitution of MXF for EMB or INH produced CFU reductions of approximately 0.9 or 0.4 $\log_{10}$, respectively, after 1 month of treatment, and most mice were rendered culture negative by 2 months, making further comparisons difficult.

In C3HeB/FeJ mice, RIF-INH-PZA-EMB reduced the mean lung CFU count by approximately 5.5 and 7.75 $\log_{10}$ after treatment for 1 and 2 months, respectively, at JHU. At CSU, where the mean CFU count at day 0 was 2.2 $\log_{10}$ lower, the reduction was approximately 4.7 and 6.2 $\log_{10}$ after treatment for 1 and 2 months. At JHU, substitution of MXF for EMB or INH each produced a small additional reduction of ~0.5 $\log_{10}$, which was statistically significant after 1 month ($P < 0.05$) but not after 2 months of treatment. In contrast, at CSU, substitution of MXF for EMB or INH appeared to be less effective than treatment with the standard regimen at month 1, whereas nearly all mice were rendered culture negative by month 2.

TABLE 2 Lung CFU counts in BALB/c and C3HeB/FeJ mice at the beginning of treatment (day 0) and after 1, 2, 3, or 4 months of treatment with the indicated regimens

Mouse strain and treatment	Log_{10} CFU ± SD at various time points[a]							
	JHU			CSU				
	D0	M1	M2	D0	M1	M2	M3	M4
BALB/c	7.46 ± 0.40			5.62 ± 0.25				
RIF-INH-PZA-EMB		3.87 ± 0.05	1.98 ± 0.26		2.29 ± 0.26	0.00 ± 0.00	0.07 ± 0.16	0.00 ± 0.00
RIF-INH-PZA-MXF		3.62 ± 0.24	1.65 ± 0.37		1.40 ± 0.27	0.08 ± 0.18	0.00 ± 0.00	
RIF-MXF-PZA-EMB		3.12 ± 0.24	0.99 ± 0.42		1.86 ± 0.56	0.50 ± 0.48	0.22 ± 0.49	
C3HeB/FeJ	8.83 ± 0.55			6.70 ± 1.21				
RIF-INH-PZA-EMB		3.43 ± 0.17	1.09 ± 0.38		2.04 ± 1.03	0.48 ± 0.07	0.12 ± 0.29	0.50 ± 0.58
RIF-INH-PZA-MXF		2.92 ± 0.20	0.63 ± 0.66		3.86 ± 0.86	0.08 ± 0.18	0.00 ± 0.00	
RIF-MXF-PZA-EMB		2.90 ± 0.38	0.48 ± 0.92		4.60 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	

[a] D0, day 0; M1, month 1, etc.

Relapse after treatment completion. Relapse results are presented in Table 3. Among BALB/c mice at JHU, treatment with the first-line regimen RIF-INH-PZA-EMB for 2, 3, and 4 months resulted in relapse in 100, 86, and 7%, respectively. Substitution of MXF for EMB did not significantly reduce the rate of relapse after 3 months of treatment, and 3 months of the RIF-INH-PZA-MXF regimen was inferior to 4 months of the first-line regimen ($P = 0.0005$). On the other hand, substitution of MXF for INH reduced the proportion relapsing after 3 months of treatment to 3 (20%) of 15 mice ($P = 0.0007$), a rate comparable to that observed after 4 months of the first-line regimen. Among BALB/c mice at CSU, treatment with the first-line regimen produced relapse rates of 100, 43, and 0% after 2, 3, and 4 months of treatment, respectively. Substitution of MXF for EMB and INH reduced the proportion of mice relapsing after 3 months of treatment to 1 (7%) of 15 ($P = 0.04$) and 0 (0%) of 15 ($P = 0.006$), respectively, values similar to that observed after 4 months of treatment with the first-line regimen. When adjusted for multiple comparisons, only the substitution of MXF for INH produced a statistically significant difference at CSU after 3 months of treatment ($P < 0.05$).

Among C3HeB/FeJ mice at JHU, treatment with RIF-INH-PZA-EMB resulted in relapse in 100, 60, and 13% of mice after 2, 3, and 4 months of treatment, respectively. Substitution of MXF for EMB and INH reduced the proportion of mice relapsing after 3 months of treatment to 20%. These differences narrowly missed the criterion for statistical significance ($P = 0.06$), and the proportion of mice relapsing after 3 months of each MXF-containing regimen was similar to the proportion relapsing after 4 months of treatment with the first-line regimen. Among C3HeB/FeJ mice at CSU, treatment with RIF-INH-PZA-EMB resulted in relapse in 100, 100, and 27% of mice after 2, 3, and 4 months of treatment, respectively. Although substitution of MXF for EMB did not alter the number of relapses, substitution of MXF for INH reduced the proportion of mice relapsing after 3 months of treatment to 60% ($P = 0.02$). Nevertheless, the relapse rates observed after 3 months of each MXF-containing regimen were higher than the relapse rate observed with 4 months of the first-line regimen, especially in the case of RIF-INH-PZA-MXF ($P < 0.0001$).

DISCUSSION

This study was designed to compare the efficacy of two MXF-containing regimens to the standard first-line regimen in two pathologically distinct murine models of TB against two different strains of *M. tuberculosis* in two different laboratories. Typical of most mouse strains used for nonclinical efficacy studies, BALB/c mice develop predominantly cellular foci of chronic inflammation with occasional small foci of necrosis without caseation at the site of *M. tuberculosis* infection in the lungs. In contrast, C3HeB/FeJ mice develop caseating, and even liquefying and cavitating, necrotic granulomas which together may better represent the pathology of progressive human TB (5–8, 11, 23). One way of confirming the relevance of such pathological differences for transla-

TABLE 3 Relapse rates in BALB/c and C3HeB/FeJ mice after treatment with the indicated regimens and durations

Mouse strain and treatment	% (proportion) of mice relapsing after treatment up to the indicated time points[a]					
	JHU			CSU		
	M2	M3	M4	M2	M3	M4
BALB/c						
RIF-INH-PZA-EMB	100 (15/15)	86 (12/14)	7 (1/15)	100 (15/15)	43 (6/14)	0 (0/15)
RIF-INH-PZA-MXF	100 (10/10)	73 (11/15)		87 (13/15)	7 (1/15)	
RIF-MXF-PZA-EMB	100 (15/15)	20 (3/15)		87 (13/15)	0 (0/15)	
C3HeB/FeJ						
RIF-INH-PZA-EMB	100 (15/15)	60 (9/15)	13 (2/15)	100 (15/15)	100 (13/13)	27 (4/15)
RIF-INH-PZA-MXF	100 (15/15)	20 (3/15)		100 (15/15)	100 (14/14)	
RIF-MXF-PZA-EMB	80 (12/15)	20 (3/15)		100 (14/14)	60 (9/15)	

[a] M2, month 2; M3, month 3, etc.

tion of nonclinical efficacy results into human trials is to compare the ability of each mouse strain to forecast the performance of regimens in phase 3 clinical trials. Thus, our experiments were performed in parallel with the phase 3 REMox-TB trial (16), in which the two 4-month MXF-containing regimens ultimately failed to meet criteria for noninferiority relative to the 6-month first-line regimen. The authors of the editorial accompanying publication of the disappointing trial results echoed prevalent concerns that standard mouse models may not adequately inform regimen design and that use of C3HeB/FeJ mice might improve the translational value of murine models (24).

The efficacy of one or both of these MXF-containing regimens has been previously evaluated in multiple experiments conducted by 3 different groups of investigators using two different strains of mice, three different infection models, and two different strains of *M. tuberculosis* (12-14, 25). However, to our knowledge, the present study is the first to compare the regimens head to head in C3HeB/FeJ mice and a more traditional mouse strain and the first to use a low-dose aerosol infection followed by a longer 6- to 8-week incubation period necessary for the characteristic pathology of C3HeB/FeJ mice to develop. The substitution of MXF for INH in the RIF-INH-PZA combination was first evaluated in mice a decade ago, when studies in a high-dose aerosol infection model in BALB/c mice with a 2-week incubation period prior to treatment demonstrated a more rapid reduction in lung CFU counts and shorter treatment duration needed to prevent relapse (12, 13). In the first study enabling comparisons on the basis of relapse, the RIF-INH-PZA regimen resulted in relapse in 92, 42, and 6% of mice after 3, 4, and 5 months of treatment, whereas RIF-MXF-PZA resulted in relapse in 28, 0, and 0%, respectively, indicating a reduction of between 1 and 2 months in the duration of treatment needed to achieve the same number of relapses (12). Additional experiments in the same model demonstrated a similar margin of benefit (26, 27). Moreover, studies using *M. tuberculosis* Erdman in both high-dose aerosol and intravenous infection models in BALB/c mice demonstrated comparable relapse rates for 4 months of treatment with the RIF-MXF-PZA regimen compared to 5 to 6 months and 6 months, respectively, for the standard regimen (14). Finally, a study using *M. tuberculosis* H37Rv in an intravenous infection model in outbred Swiss mice demonstrated 42% relapse after a 4-month RIF-MXF-PZA-based regimen compared to 17% relapse after a 6-month RIF-INH-PZA-based regimen, a difference that was marginally statistically significant and consistent with a treatment-shortening effect of less than 2 months (25). The present study using a low-dose infection model and a 6- to 8-week incubation period in BALB/c mice yielded results consistent with those observed with the aforementioned high-dose, shorter incubation models, although the margin of benefit of $\leq$1 month for incorporating MXF was somewhat smaller than that observed in previous studies. The substitution of MXF for INH in the first-line regimen was associated with a statistically significant reduction in the proportion of mice relapsing after 3 months of treatment in each mouse strain and each laboratory, with the exception of C3HeB/FeJ mice at JHU, where the difference did not quite reach statistical significance. Still, the magnitude of the treatment-shortening effect of this substitution in this latest study was no more than 1 month.

The substitution of MXF for EMB has not been studied as extensively as the substitution of MXF for INH in mice. In a high-dose aerosol infection model in BALB/c mice in which the addition of EMB does not increase the bactericidal activity of RIF-INH-PZA, the addition of MXF to RIF-INH-PZA was associated with an additional 0.6-$\log_{10}$ reduction in lung CFU counts after 2 and 3 months of treatment (13). In a similar model using the Erdman strain rather than the H37Rv strain of *M. tuberculosis*, the same addition of MXF was associated with an $\sim$1-$\log_{10}$ reduction after 2 months of treatment. This result was statistically significant when a RIF formulation and dosing schedule similar to the earlier study was used but not when the RIF formulation and dosing schedule were different (14). Relapse was not examined at an informative time point in either study. The present study confirms similar modest reductions of $<$1 $\log_{10}$ in lung CFU counts at 2 months in both mouse strains when MXF is substituted for EMB. It also assessed, for the first time, the treatment-shortening effects of this substitution. Although there were trends toward lower relapse rates after 3 months of treatment with the RIF-INH-PZA-MXF regimen compared to the same duration of the first-line regimen in C3HeB/FeJ mice at JHU and in BALB/c mice at CSU, the size of the treatment-shortening effect was no greater than 1 month. Moreover, for BALB/c mice at JHU and C3HeB/FeJ mice at CSU, 3 months of the RIF-INH-PZA-MXF regimen was inferior to 4 months of the first-line regimen, indicating a treatment-shortening effect of $<$1 month. Thus, synthesizing past and present mouse model studies evaluating these MXF-containing regimens reveals a treatment-shortening benefit of at least 1 month, but less than 2 months, obtained by substituting MXF for INH and a benefit of $\leq$1 month obtained by substituting MXF for EMB.

The lack of well-validated rapid surrogate markers for sterilizing activity and limited resources available to conduct clinical trials evaluating new TB regimens put a premium on nonclinical efficacy results for selecting regimens, drug doses, dosing schedules, and treatment duration. Mouse models of TB are commonly maligned for not adequately representing the distinctive pathology of human TB. Nevertheless, they have been used successfully in the past to demonstrate the treatment-shortening potential of RIF and PZA that was subsequently confirmed in clinical trials (1, 3). Until the advent of the potent fluoroquinolone-containing regimens studied here, no new regimens had been shown to shorten treatment in mice that advanced to phase 3 clinical trials and enabled a full assessment of their treatment-shortening potential. By comparing the efficacy of regimens in two pathologically distinct mouse models in parallel with phase 3 clinical trials, we evaluated the predictive ability of these models. Analysis of the present results, both in isolation and in the context of prior studies, demonstrates that the treatment-shortening effects observed in mice are entirely consistent with the results of the REMox-TB trial and other phase 3 clinical trials in which substitution of MXF for INH or EMB modestly improved the rate of sputum culture conversion but did not enable shortening of TB treatment by 2 months (15–21). Although no clear advantage of using C3HeB/FeJ mice compared to BALB/c mice was evident from this study, recent evidence that some drugs do perform differently in these two mouse strains (7, 8, 28) supports further studies of this kind to better define the role of C3HeB/FeJ mice in TB drug development.

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REFERENCES

1. **Grosset J.** 1978. The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. Bull Int Union Tuberc **53:**5–12.
2. **Grumbach F, Canetti G, Grosset J, Le LM.** 1967. Late results of long-term intermittent chemotherapy of advanced, murine tuberculosis: limits of the murine model. Tubercle **48:**11–26. http://dx.doi.org/10.1016/S0041-3879(67)80047-3.
3. **Mitchison DA, Chang KC.** 2009. Experimental models of tuberculosis: can we trust the mouse? Am J Respir Crit Care Med **180:**201–202. http://dx.doi.org/10.1164/rccm.200905-0708ED.
4. **Nuermberger E.** 2008. Using animal models to develop new treatments for tuberculosis. Semin Respir Crit Care Med **29:**542–551. http://dx.doi.org/10.1055/s-0028-1085705.
5. **Harper J, Skerry C, Davis SL, Tasneen R, Weir M, Kramnik I, Bishai WR, Pomper MG, Nuermberger EL, Jain SK.** 2012. Mouse model of necrotic tuberculosis granulomas develops hypoxic lesions. J Infect Dis **205:**595–602. http://dx.doi.org/10.1093/infdis/jir786.
6. **Rosenthal IM, Tasneen R, Peloquin CA, Zhang M, Almeida D, Mdluli KE, Karakousis PC, Grosset JH, Nuermberger EL.** 2012. Dose-ranging comparison of rifampin and rifapentine in two pathologically distinct murine models of tuberculosis. Antimicrob Agents Chemother **56:**4331–4340. http://dx.doi.org/10.1128/AAC.00912-12.
7. **Driver ER, Ryan GJ, Hoff DR, Irwin SM, Basaraba RJ, Kramnik I, Lenaerts AJ.** 2012. Evaluation of a mouse model of necrotic granuloma formation using C3HeB/FeJ mice for testing of drugs against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **56:**3181–3195. http://dx.doi.org/10.1128/AAC.00217-12.
8. **Irwin SM, Gruppo V, Brooks E, Gilliland J, Scherman M, Reichlen MJ, Leistikow R, Kramnik I, Nuermberger EL, Voskuil MI, Lenaerts AJ.** 2014. Limited activity of clofazimine as a single drug in a mouse model of tuberculosis exhibiting caseous necrotic granulomas. Antimicrob Agents Chemother **58:**4026–4034. http://dx.doi.org/10.1128/AAC.02565-14.
9. **Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, Higgins DE, Daly MJ, Bloom BR, Kramnik I.** 2005. Ipr1 gene mediates innate immunity to tuberculosis. Nature **434:**767–772. http://dx.doi.org/10.1038/nature03419.
10. **Ordonez AA, Pokkali S, DeMarco VP, Klunk M, Mease RC, Foss CA, Pomper MG, Jain SK.** 2014. Radioiodinated DPA-713 imaging correlates with bactericidal activity of tuberculosis treatments in mice. Antimicrob Agents Chemother **59:**642–649. http://dx.doi.org/10.1128/AAC.04180-14.
11. **Marzo E, Vilaplana C, Tapia G, Diaz J, Garcia V, Cardona PJ.** 2014. Damaging role of neutrophilic infiltration in a mouse model of progressive tuberculosis. Tuberculosis **94:**55–64. http://dx.doi.org/10.1016/j.tube.2013.09.004.
12. **Nuermberger EL, Yoshimatsu T, Tyagi S, Williams K, Rosenthal I, O'Brien RJ, Vernon AA, Chaisson RE, Bishai WR, Grosset JH.** 2004. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. Am J Respir Crit Care Med **170:**1131–1134. http://dx.doi.org/10.1164/rccm.200407-885OC.
13. **Nuermberger EL, Yoshimatsu T, Tyagi S, O'Brien RJ, Vernon AN, Chaisson RE, Bishai WR, Grosset JH.** 2004. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. Am J Respir Crit Care Med **169:**421–426. http://dx.doi.org/10.1164/rccm.200310-1380OC.
14. **De Groote MA, Gilliland JC, Wells CL, Brooks EJ, Woolhiser LK, Gruppo V, Peloquin CA, Orme IM, Lenaerts AJ.** 2011. Comparative studies evaluating mouse models used for efficacy testing of experimental drugs against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **55:**1237–1247. http://dx.doi.org/10.1128/AAC.00595-10.
15. **Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, Bozeman L, Heilig CM, Bernardo J, Choudhri S, Grosset JH, Guy E, Guyadeen P, Leus MC, Maltas G, Menzies D, Nuermberger EL, Villarino M, Vernon A, Chaisson RE.** 2009. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. Am J Respir Crit Care Med **180:**273–280. http://dx.doi.org/10.1164/rccm.200901-0078OC.
16. **Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, Pappas F, Phillips PP, Nunn AJ.** 2014. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med **371:**1577–1587. http://dx.doi.org/10.1056/NEJMoa1407426.
17. **Jindani A, Harrison TS, Nunn AJ, Phillips PP, Churchyard GJ, Charalambous S, Hatherill M, Geldenhuys H, McIlleron HM, Zvada SP, Mungofa S, Shah NA, Zizhou S, Magweta L, Shepherd J, Nyirenda S, van Dijk JH, Clouting HE, Coleman D, Bateson AL, McHugh TD, Butcher PD, Mitchison DA.** 2014. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. N Engl J Med **371:**1599–1608. http://dx.doi.org/10.1056/NEJMoa1314210.
18. **Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, Choudhri S, Daley CL, Munsiff SS, Zhao Z, Vernon A, Chaisson RE.** 2006. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. Am J Respir Crit Care Med **174:**331–338. http://dx.doi.org/10.1164/rccm.200603-360OC.
19. **Conde MB, Efron A, Loredo C, De Souza GR, Graca NP, Cezar MC, Ram M, Chaudhary MA, Bishai WR, Kritski AL, Chaisson RE.** 2009. Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. Lancet **373:**1183–1189. http://dx.doi.org/10.1016/S0140-6736(09)60333-0.
20. **Jawahar MS, Banurekha VV, Paramasivan CN, Rahman F, Ramachandran R, Venkatesan P, Balasubramanian R, Selvakumar N, Ponnuraja C, Iliayas AS, Gangadevi NP, Raman B, Baskaran D, Kumar SR, Kumar MM, Mohan V, Ganapathy S, Kumar V, Shanmugam G, Charles N, Sakthivel MR, Jagannath K, Chandrasekar C, Parthasarathy RT, Narayanan PR.** 2013. Randomized clinical trial of thrice-weekly 4-month moxifloxacin or gatifloxacin containing regimens in the treatment of new sputum positive pulmonary tuberculosis patients. PLoS One **8:**e67030. http://dx.doi.org/10.1371/journal.pone.0067030.
21. **Rustomjee R, Lienhardt C, Kanyok T, Davies GR, Levin J, Mthiyane T, Reddy C, Sturm AW, Sirgel FA, Allen J, Coleman DJ, Fourie B, Mitchison DA.** 2008. A phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. Int J Tuberc Lung Dis **12:**128–138.
22. **Almeida D, Nuermberger E, Tasneen R, Rosenthal I, Tyagi S, Williams K, Peloquin C, Grosset J.** 2009. Paradoxical effect of isoniazid on the activity of rifampin-pyrazinamide combination in a mouse model of tuberculosis. Antimicrob Agents Chemother **53:**4178–4184. http://dx.doi.org/10.1128/AAC.00830-09.
23. **Apt A, Kramnik I.** 2009. Man and mouse TB: contradictions and solutions. Tuberculosis **89:**195–198. http://dx.doi.org/10.1016/j.tube.2009.02.002.
24. **Warner DF, Mizrahi V.** 2014. Shortening treatment for tuberculosis to basics. N Engl J Med **371:**1642–1643. http://dx.doi.org/10.1056/NEJMe1410977.
25. **Ibrahim M, Truffot-Pernot C, Andries K, Jarlier V, Veziris N.** 2009. Sterilizing activity of R207910 (TMC207)-containing regimens in the murine model of tuberculosis. Am J Respir Crit Care Med **180:**553–557. http://dx.doi.org/10.1164/rccm.200807-1152OC.
26. **Rosenthal IM, Zhang M, Williams KN, Peloquin CA, Tyagi S, Vernon AA, Bishai WR, Chaisson RE, Grosset JH, Nuermberger EL.** 2007. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS Med **4:**e344. http://dx.doi.org/10.1371/journal.pmed.0040344.
27. **Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger E.** 2009. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. Antimicrob Agents Chemother **53:**1314–1319. http://dx.doi.org/10.1128/AAC.01182-08.
28. **Lanoix J-P, Lenaerts AJ, Nuermberger EL.** 30 March 2015. Heterogeneous disease progression and treatment response in a C3HeB/FeJ mouse model of tuberculosis. Dis Model Mech. http://dx.doi.org/10.1242/dmm.019513.