Short-Course Chemotherapy with TMC207 and Rifapentine in a Murine Model of Latent Tuberculosis Infection

Tianyu Zhang^{1,2}, Si-Yang Li¹, Kathy N. Williams¹, Koen Andries³, and Eric L. Nuermberger^{1,4}

¹Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; ²State Key Laboratory of Respiratory Disease, Center for Infection and Immunity, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong, China; ³Tibotec BVBA, Johnson & Johnson, Beerse, Belgium; and ⁴Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Rationale: Multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) is an emerging global health threat. Proper management of close contacts of infectious patients is increasingly important. However, no evidence-based recommendations for treating latent TB infection (LTBI) after MDR/XDR-TB exposure (DR-LTBI) exist. An ultrashort regimen for LTBI caused by drug-susceptible strains (DS-LTBI) is also desirable. TMC207 has bactericidal and sterilizing activity in animal models of TB and improves the activity of current MDR-TB therapy in patients.

Objectives: The objective of this study was to determine whether TMC207 might enable short-course treatment of DR-LTBI and ultrashort treatment of DS-LTBI.

Methods: Using an established experimental model of LTBI chemotherapy in which mice are aerosol-immunized with a recombinant bacillus Calmette-Guérin vaccine before low-dose aerosol infection with *Mycobacterium tuberculosis*, the efficacy of TMC207 alone and in combination with rifapentine was compared with currently recommended control regimens as well as once-weekly rifapentine + isoniazid and daily rifapentine ± isoniazid.

Measurements: Outcomes included monthly lung colony-forming unit counts and relapse rates.

Main Results: Lung colony-forming unit counts were stable at about $3.75 \log_{10}$ for up to 7.5 months postinfection in untreated mice. Rifamycin-containing regimens were superior to isoniazid monotherapy. TMC207 exhibited sterilizing activity at least as strong as that of rifampin alone and similar to that of rifampin + isoniazid, but daily rifapentine +/- isoniazid was superior to TMC207. Addition of TMC207 to rifapentine did not improve the sterilizing activity of rifapentine in this model.

Conclusions: TMC207 has substantial sterilizing activity and may enable treatment of DR-LTBI in 3–4 months.

Keywords: bacillus Calmette-Guérin; mouse; isoniazid; rifampin; pyrazinamide

Multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) is an emerging crisis that threatens to undermine efforts to control TB (1). For every case of MDR/XDR-TB, there are close contacts who carry a substantial risk of developing active MDR/XDR-TB (2). Current guidelines recommend that close contacts of patients with MDR/XDR-TB should be carefully monitored for at least 2 years to facilitate prompt and appropriate

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Close contacts of patients with multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) have a significant risk of developing MDR/XDR-TB themselves. Current recommendations for treating latent TB infection among close contacts call for 6–12 months of treatment with pyrazinamide plus a fluoroquinolone or ethambutol. A shorter regimen active against all strains of MDR/XDR-TB is highly desirable. TMC207 has strong bactericidal activity in animal models of TB and improves existing therapies for MDR-TB, but the sterilizing activity of TMC207 alone has not been examined.

What This Study Adds to the Field

This study provides further validation of a paucibacillary murine model of latent TB infection (LTBI) treatment and demonstrates that TMC207 has sterilizing activity as great as that of currently recommended rifamycin-containing short-course regimens for drug-susceptible LTBI and, therefore, may effectively treat LTBI after MDR/XDR-TB exposure in 3–4 months.

treatment if they develop active TB (3). Treatment of latent TB infection (LTBI) among close contacts of patients with MDR/XDR-TB may prevent the development of active disease, with its attendant morbidity and cost, and help to control the spread of drug-resistant strains. However, clear guidance on the treatment of LTBI among contacts of patients with MDR/XDR-TB (DR-LTBI) is lacking. Current recommendations for empirical treatment of DR-LTBI call for pyrazinamide (PZA) combined with either ethambutol or a fluoroquinolone for 6 to 12 months (3). However, there are few data to support these recommendations and, in some outbreak settings, combinations of PZA with ofloxacin or levofloxacin have been associated with treatment-limiting hepatotoxicity (4–7). A simple and safe short-course regimen of proven efficacy to treat DR-LTBI would be an important advance.

Ultrashort regimens to treat LTBI due to drug-susceptible strains of *Mycobacterium tuberculosis* are also desirable. Using an improved paucibacillary mouse model of LTBI treatment, we showed that daily treatment with rifapentine (RPT) was more effective than rifampin (RIF) plus isoniazid (INH), and as effective as RIF + PZA (8), suggesting that daily RPT-based regimens may be capable of treating LTBI in 2 months or less.

TMC207 (TMC, J) is a novel diarylquinoline ATP synthase inhibitor with potent *in vitro* activity against *M. tuberculosis*, including strains resistant to commonly used first- and second-

(Received in original form March 6, 2011; accepted in final form May 25, 2011)

Supported by National Institutes of Health contract N01-AI-40007.

Author contributions: Conception and design: T.Z., K.A., E.N.; acquisition and analysis of data: T.Z., S.L., K.W., E.N.; interpretation of data and drafting of manuscript: T.Z., E.N.; reviewing the manuscript for important intellectual content: T.Z., K.A., E.N.

Correspondence and requests for reprints should be addressed to Eric L. Nuermberger, M.D., 1550 Orleans St., Baltimore, MD 21231-1002. E-mail: enuerm@jhmi.edu

Am J Respir Crit Care Med Vol 184, pp 732–737, 2011

Originally Published in Press as DOI: 10.1164/rccm.201103-0397OC on June 9, 2011

Internet address: www.atsjournals.org

line TB drugs (9). It retains bactericidal activity against slowly replicating or nonreplicating organisms *in vitro* (10–12). In animal models of active TB disease, TMC exhibits strong bactericidal activity (9, 13–15). Combinations containing TMC and PZA have displayed synergistic effects and a capacity for sterilizing activity that exceeds that of the current first-line regimen (14, 16). The addition of rifapentine (RPT, P) to TMC and PZA increases this sterilizing activity further (17). Last, in patients with MDR-TB, the addition of TMC to a background regimen was well tolerated and led to more rapid sputum culture conversion when compared with the background regimen plus placebo (18).

The current study was conducted in the previously described experimental model of the chemotherapy of LTBI (8, 19, 20) with two objectives in mind. To determine whether TMC might enable a simple, short-course regimen for treatment of DR-LTBI, we compared TMC with various single-agent and combination drug regimens of proven efficacy in LTBI. To build on observations of the strong activity of daily RPT treatment in this model (8), we evaluated whether the addition of TMC, with or without PZA, to RPT could shorten the duration of treatment further.

METHODS

Mycobacterial Strains

M. tuberculosis H37Rv and a recombinant bacillus Calmette-Guérin (BCG) strain overexpressing the 30-kD major secretory protein (rBCG30) were stored and cultivated as previously described (8). rBCG30 was used as an immunizing agent because it is more immunogenic than the parent BCG Tice strain and has a hygromycin resistance selection marker to differentiate it from *M. tuberculosis* (8).

Antimicrobials

INH, RIF, PZA, and RPT were obtained and formulated for oral administration as previously described (21). TMC was kindly provided by Tibotec (Beerse, Belgium) and formulated for oral administration in an acidified 20% hydroxypropyl- β -cyclodextrin solution (pH $\sim$ 2.2), as previously described (15). The minimal inhibitory concentration of TMC207 against *M. tuberculosis* H37Rv on 7H11 agar was 0.03 μ g/ml. Minimal inhibitory concentrations (in μ g/ml) of RIF (0.25), RPT (0.06), and INH (0.1) on 7H11 agar and PZA (11) on Löwenstein-Jensen medium (pH 5.5) were previously reported (8).

Aerosol BCG Immunization and Infection with *M. tuberculosis*

All animal procedures were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University. Aerosol infections were performed as previously described (8). In brief, 5-week-old female BALB/c mice (Charles River, Wilmington, MA) were infected with rBCG30, using an inhalation exposure system (Glas-Col, Terre Haute, IN) and a log-phase broth culture (optical density at 600 nm, $\sim$ 0.5). Six weeks later, mice were infected with *M. tuberculosis* H37Rv, using a 500-fold dilution of a similar broth culture. Two mice from each of four aerosol infection runs were humanely killed 1 day after infection to determine the number of bacteria implanted in the lungs.

Chemotherapy

Beginning 6 weeks after *M. tuberculosis* infection (Day 0), mice were block-randomized by run to each experimental arm (Table 1) and initiated on treatment. Mice in the following arms were treated 5 days/week (5/7): INH alone, RIF alone, RIF + INH, RPT alone, RPT + INH, TMC alone, TMC_{12.5} + RPT, TMC + RPT, and TMC + RPT + PZA. In the TMC_{12.5} + RPT regimen, the TMC dose was halved to compensate for an approximately 50% reduction in TMC exposure in humans due to induction of metabolism by rifamycins, which does not occur in mice (data not shown). Additional mice received RPT + INH

once weekly (1/7) at doses commensurate with its clinical use (22). Drugs were administered by gavage. Drug doses (in mg/kg) for 5/7 regimens were as follows: INH (10), RIF (10), PZA (150), RPT (10), and TMC (25 or 12.5, as indicated). Doses for the 1/7 regimen were as follows: INH (50) and RPT (15) (9, 23).

Assessment of Treatment Efficacy

Efficacy was assessed on the basis of the lung colony-forming unit counts during treatment and the proportion of mice with culture-positive relapse after treatment completion. Colony-forming unit counts were determined after 1 month of treatment in all groups. Untreated mice were also assessed 2, 4, and 6 months after the initiation of treatment for other groups. To determine colony-forming unit counts, quantitative cultures of lung homogenates were performed in parallel on (1) selective 7H11 agar enriched with 10% oleic acid-albumin-dextrose-catalase (basic agar), (2) basic agar supplemented with 2-thiophenecarboxylic acid hydrazide (TCH, 4 μ g/ml; Sigma, St. Louis, MO) to select for *M. tuberculosis* (8), (3) basic agar supplemented with hygromycin (40 μ g/ml; Roche Diagnostics, Indianapolis, IN) to select for rBCG30, which has a hygromycin resistance marker, and (4) basic agar supplemented with 0.4% activated charcoal (Sigma) to adsorb residual TMC in lung homogenates and limit the effect of drug carryover. Plates were incubated for 28 days at 37°C before final colony-forming unit counts were determined. Unless otherwise indicated, colony-forming unit counts reported for rBCG30 and *M. tuberculosis* are the counts determined on hygromycin-containing and TCH-containing plates, respectively.

The proportion of mice with culture-positive relapse was determined by holding cohorts of 15 mice for an additional 3 months after treatment completion, as previously described (8), including at least one culture plate supplemented with 0.4% charcoal to monitor for drug carryover effects. Use of 15 mice per group for relapse assessment provides greater than 80% power to detect 40 percentage point differences in the relapse rate, after setting α at 0.01 to adjust for up to 5 simultaneous comparisons. Smaller differences are unlikely to be meaningful in terms of shortening the duration of treatment.

Lung Pathology

To assess the pathological changes arising during long-term paucibacillary infection, five untreated mice were killed at the end of the treatment period (7.5 mo after infection with *M. tuberculosis*). One lung was homogenized for colony-forming unit counts and the other was fixed in 10% neutral buffered formalin for histopathological assessment. After 1 week of fixation, lungs were sectioned and embedded in paraffin. Sections were cut to a thickness of 5 μ m and placed on negatively charged glass slides. The sections were then stained with hematoxylin and eosin and an acid-fast stain.

Statistical Analysis

Colony-forming unit counts (x) were log-transformed as ($x + 1$) before analysis. Group means were compared by one-way analysis of variance with Bonferroni's or Dunnett's post-test, as appropriate. Group relapse proportions were compared by Fisher's exact test, adjusting for multiple comparisons. GraphPad Prism version 5 (GraphPad, San Diego, CA) was used for all analyses.

RESULTS

Stability of Paucibacillary Infection after BCG Immunization

Aerosol infection with rBCG30 implanted $2.63 \pm 0.11 \log_{10}$ cfu in the lungs. Six weeks later, aerosol infection with *M. tuberculosis* implanted $1.18 \pm 0.19 \log_{10}$ cfu. Another 6 weeks later, on Day 0, the mean *M. tuberculosis* colony-forming unit count reached $4.31 \pm 0.24 \log_{10}$. Among untreated mice, mean colony-forming unit counts fell to approximately $3.75 \log_{10}$ by 2.5 mo after infection and remained stable at that level until 7.5 mo after infection, when higher values for two of five mice increased the mean colony-forming unit count to 4.08 ± 0.68 (Figure 1). A review of lung histopathology revealed

TABLE 1. SCHEME OF EXPERIMENT

Drug Regimen [†]	Time Point and Number of Mice Allocated per Group*								
	W-12	W-6	D0	W2 (+3M)	M1 (+3M)	M2 (+3M)	M3 (+3M)	M4 (+3M)	M6 (+3M)
Untreated	8	8	4		4	4		4	4
INH					4			(15)	(15)
RIF					4	(15)	(15)	(15)	
RIF + INH					4	(15)	(15)		
RPT ₁₅ + INH ₅₀ (1/7)					4	(15)	(15)		
RPT					4 (15)	(15)			
RPT + INH					4 (15)	(15)			
TMC					4	(15)	(15)	(15)	
TMC _{12.5} + RPT					4 (15)				
TMC + RPT				(15)	4 (15)				
TMC + RPT + PZA				(15)	4 (15)				

Definition of abbreviations: INH = isoniazid; PZA = pyrazinamide; RIF = rifampin; RPT = rifapentine; TMC = TMC207.

[†] Unless otherwise specified, drug doses (in mg/kg) are as follows: INH, 10; RIF, 10; RPT, 10; TMC, 25; PZA, 150.

* Time points: W-12, day after immunization with rBCG30; W-6, day after infection with *M. tuberculosis*; D0, day of treatment initiation; W2, 2 weeks after treatment initiation; M1, 1 month after treatment initiation, and so on. (+3) indicates that the number of mice in parentheses was held for 3 months after treatment completion before being killed for the relapse assessment.

a spectrum of pathological changes, dependent on the lung colony-forming unit count. In the two mice with lung colony-forming unit counts less than $3.5 \log_{10}$ cfu, the pathology was largely limited to discrete, organized foci of chronic inflammation, typically with dense lymphocytic aggregation around a central clearing, resembling a granuloma (Figures 2A and 2B). Rare acid-fast bacilli (AFB) were visible within the central clearing (Figure 2C). Individual foamy macrophages or small nests of such cells were observed in association with some lesions. In the two mice with lung colony-forming unit counts equal to or greater than $4.5 \log_{10}$ cfu, the pathology was characterized by larger, disorganized zones of chronic inflammation with numerous foamy macrophages (Figures 2D and 2E) and often with clefts suggestive of cholesterol crystallization. Rare Langhans giant cells were observed. AFB were more numerous and visible inside foamy macrophages in these zones (Figure 2F). Obstruction of small airways with cellular debris (Figure 2G) and foci of developing necrosis (Figure 2H) were occasionally evident within these zones. The latter harbored extracellular AFB (Figure 2I).

Treatment Efficacy, as Assessed by Lung Colony-forming Unit Counts

Mean lung colony-forming unit counts after 1 month of treatment are displayed in Figure 3. INH monotherapy was not significantly better than no treatment. The RIF, RIF + INH, and RPT + INH (1/7) control regimens were each more effective than INH ($P < 0.001$), producing colony-forming unit counts that were 1.44, 1.65, and 1.63 $\log_{10}$ lower, respectively, than those observed in untreated mice. Each of the remaining regimens was more effective than RIF ($P < 0.05$ for RPT and TMC alone, $P < 0.01$ for RPT + INH, $P < 0.001$ for others). When compared with the RIF + INH and RPT + INH (1/7) control regimens, however, only the TMC + RPT-containing regimens were significantly more active ($P < 0.001$). TMC alone was not statistically superior to RPT alone. Full-dose TMC + RPT, with or without PZA, was more active than RPT or TMC alone ($P < 0.05$), but not RPT + INH. Only TMC + RPT + PZA was more active than RPT + INH ($P < 0.001$).

When limiting the comparisons to TMC alone versus each of the control regimens [INH alone, RIF alone, RIF + INH, and RPT + INH (1/7)] with one-way analysis of variance followed

by Dunnett's post-test, TMC was statistically superior to each control regimen ($P < 0.001$ vs. INH, $P < 0.05$ vs. others).

Treatment Efficacy, as Assessed by Culture-positive Relapse after Treatment

The relapse results are displayed in Table 2. Two weeks of treatment with TMC + RPT with or without PZA was unable to prevent relapse in any mouse. However, 1 month of daily treatment with RPT was sufficient to prevent relapse in 33%. The addition of INH or TMC to RPT did not significantly affect the relapse rate, but TMC + RPT + PZA was superior to TMC + RPT and the other regimens, preventing relapse in all but one mouse. This result is consistent with previous observations of additive or synergistic activity when rifamycins and TMC are combined with PZA (14, 24).

After 2 months of treatment, 100, 93, and 87% of mice receiving RIF, RIF + INH, and RPT + INH (1/7), respectively, relapsed. TMC alone resulted in a similar 87% relapse rate. On the other hand, 2 months of daily RPT, with or without INH, was sufficient to prevent relapse in all mice.

After 3 months of treatment, 87, 54, and 47% of mice receiving RIF, RIF + INH, and RPT + INH (1/7), respectively, relapsed. These differences did not reach statistical significance. Only 14% of mice treated with TMC alone relapsed, a relapse rate significantly lower than in RIF-treated mice and lower than

Figure 1. Mean *Mycobacterium tuberculosis* colony-forming unit counts ($\pm$ SD) in the lungs of untreated rBCG30-immunized mice.

Figure 2. (A and B) Histopathology of mouse lungs 7.5 months after low-dose challenge with *Mycobacterium tuberculosis*, demonstrating circumscribed, well-organized granuloma-like lesions (original magnification: A, $\times 40$; B, $\times 200$) in a mouse with $3.35 \log_{10}$ cfu in the lungs. (C) The few evident bacilli (thin arrows) were localized to central areas in such lesions (original magnification, $\times 1,000$). (D and E) Larger zones of chronic inflammation (original magnification: D, $\times 20$) with many foamy macrophages (original magnification: E, $\times 200$) were noted in the mice with greater than $4.5 \log_{10}$ cfu in the lungs. (F) Acid-fast bacilli (AFB) were more numerous and localized to foamy macrophages (original magnification, $\times 1,000$). (G–I) Airway obstruction with cell debris (original magnification: G, $\times 200$; thick arrow) and evolving necrosis (original magnification: H, $\times 200$) with extracellular AFB (original magnification: I, $\times 1,000$) were visible in the same lesion.

in RIF + INH-treated mice before, but not after, controlling for multiple comparisons. However, after 4 months of treatment, similar proportions of mice receiving RIF alone and TMC alone, 46 and 29%, respectively, relapsed. All mice treated with INH for 4 and 6 months relapsed, consistent with the poor sterilizing activity of this drug.

DISCUSSION

In this study, we further demonstrated the suitability of this paucibacillary murine TB model for evaluating new drugs and drug regimens to treat LTBI (8). By extending the observation period for untreated mice to 7.5 months postinfection, we demonstrated the stability of the paucibacillary state in rBCG30-immunized mice. However, it is noteworthy that, by 7.5 months postinfection, 2 of the 5 remaining mice had lung colony-forming unit counts higher than any of the 12 mice killed in the preceding 5 months. As these mice were approaching 11 months of age, it is tempting to speculate that they had begun to experience immune senescence leading to failure to contain the infection, as has been described previously after low-dose infection with *M. tuberculosis* (25). This situation may be akin to reactivation of LTBI. The pathological findings provide additional support for this speculation. Whereas the pathology in the lungs of mice with low colony-forming unit counts at 7.5 months postinfection was dominated by well-circumscribed and organized cellular lesions resembling granulomas with rare intracellular AFB, the lungs of the two mice with high colony-forming unit counts demonstrated larger, disorganized zones of chronic inflammation with numerous foamy macrophages, occasional multinucleated giant cells, and more numerous AFB inside the foamy macrophages. Developing necrosis, with disintegration of foamy macrophages and release of bacilli into the extracellular space, was also evident in one lesion in the mouse with the highest colony-forming unit count. These findings are consistent with prior reports of lipid pneumonia as the primary pathological event in the Cornell model of TB reactivation in mice and in human postprimary infection before evolution of caseous necrosis (26). They also lend further support to assertions that persistent *M. tuberculosis* resides in foamy macrophages in murine as well as human hosts (27). Of course, whether these two

individual mice with higher colony-forming unit counts at 7.5 months originally had stable, well-contained infection with colony-forming unit counts of approximately $3.75 \log$ that then “reactivated” or whether they had a more poorly contained higher burden infection throughout their course is open to speculation. Only more detailed histopathological analyses conducted at multiple time points and for longer periods of time will answer that question and define the full spectrum of lesion evolution in this model.

Extending the treatment period for clinically relevant control regimens beyond that of our previous study (8) produced valuable information on their sterilizing activity in this paucibacillary model. The reduced activity of INH relative to rifamycin-based regimens suggests there is only limited bacterial replication during the treatment phase, consistent with the current understanding of LTBI. Four months of RIF and 3 months of RIF + INH each cured approximately 50% of mice. Together with previous results (8), these results are consistent with current recommendations for the use of these rifamycin-containing regimens to treat LTBI. Similar efficacy was observed with 3 months of

Figure 3. Mean *Mycobacterium tuberculosis* lung colony-forming unit counts ($\pm$ SD) after 1 month of treatment. Abbreviations: 1/7, drugs administered once weekly; H, isoniazid; J, TMC207; P, rifapentine; R, rifampin; Z, pyrazinamide.

TABLE 2. RESULTS OF RELAPSE ASSESSMENTS

Treatment Group*	Percentage (Proportion) with Positive <i>M. tuberculosis</i> Cultures 3 mo after Completing Treatment for:					
	2 wk	1 mo	2 mo	3 mo	4 mo	6 mo
INH					100 (15/15)	100 (15/15)
RIF			100 (15/15) [†]	87 (13/15)	46 (6/13)	
RIF + INH			93 (14/15)	54 (7/13)		
RPT ₁₅ + INH ₅₀ (1/7)			87 (13/15)	47 (7/15) [†]		
RPT		67 (10/15)	0 (0/15)			
RPT + INH		60 (9/15) [†]	0 (0/15)			
TMC			87 (13/15)	14 (2/14)	29 (4/14)	
TMC _{12.5} + RPT		80 (12/15)				
TMC + RPT	100 (15/15)	53 (8/15)				
TMC + RPT + PZA	100 (15/15)	7 (1/15)				

Definition of abbreviations: (1/7), drugs were administered once weekly; INH = isoniazid; *M. tuberculosis* = *Mycobacterium tuberculosis*; PZA = pyrazinamide; RIF = rifampin; RPT = rifapentine; TMC = TMC207.

* Unless otherwise specified, drug doses (in mg/kg) are as follows: INH, 10; RIF, 10; RPT, 10; TMC, 25; PZA, 150.

[†] Relapse in one mouse was associated with just 1 cfu.

RPT + INH (1/7) and is supported by the results of a large randomized trial showing this regimen to be at least as effective as 9 months of INH for LTBI (28). The reason for which 6 months of INH was unable to cure any mice, in spite of its observed efficacy in human LTBI, is not clear. Nor is it clear whether extending INH treatment to 9 months would have produced cures. Although this discrepancy will require further study to resolve, the close correspondence between our results and the clinical evidence of efficacy of rifamycin-containing regimens and their superior potency over INH monotherapy provides further validation of this model for evaluating new treatment regimens for LTBI.

The principal objective of the current study was to evaluate the potential of TMC as a treatment for LTBI, including DR-LTBI. TMC exhibits significant sterilizing activity in drug combinations that exploit the synergistic activity of TMC and PZA, with or without addition of a rifamycin (16, 17). However, because MDR/XDR-TB isolates commonly display phenotypic and/or genotypic evidence of PZA resistance and because use of PZA in LTBI regimens has been associated with high rates of hepatotoxicity, PZA is not likely to be part of a routinely recommended regimen for treating DR-LTBI. Therefore, it is important to understand the sterilizing activity of TMC in the absence of PZA. To our knowledge, the present study is the first to apply relapse rate assessments after TMC monotherapy in an effort to address this issue. Indeed, TMC alone does exhibit substantial sterilizing activity. The finding that 3 months of TMC was as effective as 3 months of RIF + INH or RPT + INH (1/7) and possibly more effective than RIF suggests that it may effectively treat LTBI, including that associated with MDR/XDR-TB strains, in as little as 3 or 4 months, provided the safety profile is sufficient to recommend its use. Despite producing similar colony-forming unit counts after 1 month of treatment, TMC did not have the sterilizing activity of RPT. This finding is in agreement with the observation in a murine model of active TB that although TMC + PZA + moxifloxacin (MXF) produced a more rapid fall in colony-forming unit counts, RPT + PZA + MXF appeared to have greater sterilizing activity (17). Thus, comparing the rate of reduction of colony-forming unit counts during treatment does not always predict the sterilizing activity of the regimens in question. Still, the finding from the present study that TMC may have sterilizing activity at least as great as that of RIF suggests TMC has great potential to shorten the duration of treatment for active or latent TB infection caused by MDR/XDR-TB strains.

We observed that daily RPT was more effective than RIF + INH and as effective as RIF + PZA (8), suggesting that daily

RPT-based regimens may be capable of treating LTBI in 2 months or less. In the current experiment, combinations of TMC and RPT, with or without PZA, were evaluated in hopes of identifying regimens capable of shortening the duration of drug-susceptible LTBI treatment even further. Despite producing a further reduction in the M1 colony-forming unit count, the addition of TMC to RPT did not significantly improve the sterilizing activity of the regimen unless PZA was also added. Because we did not have an RPT + PZA regimen in the present experiment, we cannot discern the contribution of TMC to this three-drug combination. However, prior evidence that (1) the addition of PZA alone to RPT did not increase the sterilizing activity of the regimen in the paucibacillary model (8), and (2) the addition of TMC to RPT + PZA + MXF did increase the activity of the regimen in an active disease model (17) suggests that TMC did contribute to the activity of the TMC + RPT + PZA combination. That 1 month of this regimen cured virtually all mice whereas 3 to 4 months of RIF-based treatments still permitted relapse in roughly half the animals gives hope that ultrashort regimens comprising 2 to 3 weeks of TMC, an optimized rifamycin, and PZA may be highly efficacious for LTBI and limit the hepatotoxic potential of PZA.

In our previous study (8), mice receiving 1 month of RPT + INH appeared more likely to relapse than mice receiving the same duration of RPT alone, although the difference was not statistically significant and the former group of mice had lower lung colony-forming unit counts after 2 weeks of treatment. In the current study, mice receiving RPT and RPT + INH had equivalent outcomes, providing further evidence that the difference observed between RPT and RPT + INH in our previous experiment was not significant. The present finding that 1 month of RPT ± INH resulted in relapse rates similar to 4 months of RIF and 3 months of RIF + INH or RPT + INH (1/7) supports the future clinical evaluation of daily RPT + INH regimens of 1 to 2 months' duration for treatment of drug-susceptible LTBI.

Interpretation of the results of the current study is subject to several limitations. As we have discussed previously (8), mice do not develop latent infection with *M. tuberculosis* as we know it, so there can be no assurances that activity in this model will predict activity in LTBI. Moreover, LTBI likely represents a spectrum of conditions ranging from cleared infection to incipient disease. Models with inbred animal strains, uniform infections, and similar drug exposures cannot reproduce the heterogeneity in presentation or treatment response. That said, the model used in this study accurately ranks the potency of existing LTBI regimens in a manner that is consistent with their

clinical use and, at least for rifamycin-based regimens, cures mice that receive the recommended treatment durations. No other animal model of LTBI treatment has been validated in this way. A second limitation is that we did not use an MDR/XDR strain of *M. tuberculosis* for these experiments. However, because TMC is a new drug with a novel mechanisms and no known cross-resistance with first- and second-line drugs already in use, we believed a drug-susceptible strain would be an acceptable and safer surrogate strain for this experiment (20). A final issue is that of statistical power. Despite using 15 mice per time point for relapse assessments, only differences of 40% or more in relapse proportions are likely to be identified. However, as observed in Table 2, the rate of reduction in the proportion of inbred mice with relapse is typically at least 40 percentage points per month, indicating that smaller differences in relapse proportions are not clinically significant.

Author Disclosure: K.A. is employed by, and holds stock in, Johnson & Johnson. E.N.'s institution has received grants from Otsuka Pharmaceuticals, Pfizer, and Sanofi-aventis. None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank Dr. Paul Converse for assistance in reviewing the lung histopathology and obtaining the photomicrographs, and Dr. Jacques Grosset for critical review of the manuscript.

References

- World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response 2010. Geneva: World Health Organization; 2010.WHO/HTM/TB/2010.3.
- Becerra M, Appleton S, Franke M, Chalco K, Arteaga F, Bayona J, Murray M, Atwood S, Mitnick C. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet* 2010;377:147–152.
- American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR Recomm Rep* 2000;49:1–51.
- Horn DL, Hewlett D Jr, Alfalla C, Peterson S, Opal SM. Limited tolerance of ofloxacin and pyrazinamide prophylaxis against tuberculosis. *N Engl J Med* 1994;330:1241.
- Papastavros T, Dolovich LR, Holbrook A, Whitehead L, Loeb M. Adverse events associated with pyrazinamide and levofloxacin in the treatment of latent multidrug-resistant tuberculosis. *CMAJ* 2002;167:131–136.
- Ridzon R, Meador J, Maxwell R, Higgins K, Weismuller P, Onorato IM. Asymptomatic hepatitis in persons who received alternative preventive therapy with pyrazinamide and ofloxacin. *Clin Infect Dis* 1997;24:1264–1265.
- Lou HX, Shullo MA, McKaveney TP. Limited tolerability of levofloxacin and pyrazinamide for multidrug-resistant tuberculosis prophylaxis in a solid organ transplant population. *Pharmacotherapy* 2002;22:701–704.
- Zhang T, Zhang M, Rosenthal IM, Grosset JH, Nuermberger EL. Short-course therapy with daily rifapentine in a murine model of latent tuberculosis infection. *Am J Respir Crit Care Med* 2009;180:1151–1157.
- Andries K, Verhasselt P, Guillemont J, Gohlmann HWH, Neefs J-M, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, *et al.* A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005;307:223–227.
- Koul A, Vranckx L, Dendouga N, Balemans W, Van den Wyngaert I, Vergauwen K, Gohlmann HWH, Willebrords R, Poncelet A, Guillemont J, *et al.* Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. *J Biol Chem* 2008;283:25273–25280.
- Rao SPS, Alonso S, Rand L, Dick T, Pethe K. The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, nonreplicating *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2008;105:11945–11950.
- Coleman D, Waddell SJ, Mitchison DA. Effects of low incubation temperatures on the bactericidal activity of anti-tuberculosis drugs. *J Antimicrob Chemother* 2011;66:146–150.
- Lenaerts AJ, Hoff D, Aly S, Ehlers S, Andries K, Cantarero L, Orme IM, Basaraba RJ. Location of persisting mycobacteria in a guinea pig model of tuberculosis revealed by R207910. *Antimicrob Agents Chemother* 2007;51:3338–3345.
- Ibrahim M, Andries K, Lounis N, Chauffour A, Truffot-Pernot C, Jarlier V, Veziris N. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. *Antimicrob Agents Chemother* 2007;51:1011–1015.
- Lounis N, Veziris N, Chauffour A, Truffot-Pernot C, Andries K, Jarlier V. Combinations of R207910 with drugs used to treat multidrug-resistant tuberculosis have the potential to shorten treatment duration. *Antimicrob Agents Chemother* 2006;50:3543–3547.
- Ibrahim M, Truffot-Pernot C, Andries K, Jarlier V, Veziris N. Sterilizing activity of R207910 (TMC207)-containing regimens in the murine model of tuberculosis. *Am J Respir Crit Care Med* 2009;180:553–557.
- Andries K, Gevers T, Lounis N. Bactericidal potencies of new regimens are not predictive of their sterilizing potencies in a murine model of tuberculosis. *Antimicrob Agents Chemother* 2010;54:4540–4544.
- Diacon A, Pym A, Grobusch M, Patientia R, Rustonjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, *et al.* The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 2009;360:2397–2405.
- Nuermberger EL, Yoshimatsu T, Tyagi S, Bishai WR, Grosset JH. Paucibacillary tuberculosis in mice after prior aerosol immunization with *Mycobacterium bovis* BCG. *Infect Immun* 2004;72:1065–1071.
- Nuermberger E, Tyagi S, Williams KN, Rosenthal I, Bishai WR, Grosset JH. Rifapentine, moxifloxacin, or DNA vaccine improves treatment of latent tuberculosis in a mouse model. *Am J Respir Crit Care Med* 2005;172:1452–1456.
- Rosenthal I, Zhang M, Williams K, Peloquin C, Tyagi S, Vernon A, Bishai W, Chaisson R, Grosset J, Nuermberger E. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. *PLoS Med* 2007;4:e344.
- Schechter M, Zajdenverg R, Falco G, Barnes GL, Faulhaber JC, Coberly JS, Moore RD, Chaisson RE. Weekly rifapentine/isoniazid or daily rifampin/pyrazinamide for latent tuberculosis in household contacts. *Am J Respir Crit Care Med* 2006;173:922–926.
- Rosenthal IM, Williams K, Tyagi S, Vernon AA, Peloquin CA, Bishai WR, Grosset JH, Nuermberger EL. Weekly moxifloxacin and rifapentine is more active than the Denver regimen in murine tuberculosis. *Am J Respir Crit Care Med* 2005;172:1457–1462.
- Almeida D, Nuermberger E, Tasneen R, Rosenthal I, Tyagi S, Williams K, Peloquin C, Grosset J. Paradoxical effect of isoniazid on the activity of rifampin-pyrazinamide combination in a mouse model of tuberculosis. *Antimicrob Agents Chemother* 2009;53:4178–4184.
- Rhoades E, Frank A, Orme I. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent *Mycobacterium tuberculosis*. *Tuber Lung Dis* 1997;78:57–66.
- Hunter RL, Jagannath C, Actor JK. Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. *Tuberculosis (Edinb)* 2007;87:267–278.
- Peyron P, Vaubourgeix J, Poquet Y, Levillain F, Botanch C, Bardou F, Daffe M, Emile JF, Marchou B, Cardona PJ, *et al.* Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. *PLoS Pathog* 2008;4:e1000204.
- Sterling TR, Borisov AS, Shang N, Bliven-Sizemore E, Chaisson RE, Gordin F, Hackman J, Hamilton CD, Horsburgh CR, Kerrigan A, *et al.* Tuberculosis Trials Consortium. The PREVENT TB Study (TB Trials Consortium Study 26) of 3 months of once-weekly rifapentine plus INH vs. 9 months of daily INH for treatment of latent TB infection: first report of results of a multi-center, randomized clinical trial. Presented at the 41st Union Conference on Lung Health, Berlin, November 15, 2010.