Rifapentine, Moxifloxacin, or DNA Vaccine Improves Treatment of Latent Tuberculosis in a Mouse Model

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Rationale: **Priorities for developing improved regimens for treatment of latent tuberculosis (TB) infection include (*1*) developing shorter and/or more intermittently administered regimens that are easier to supervise and (*2*) developing and evaluating regimens that are active against multidrug-resistant organisms.**

Objectives and Methods: **By using a previously validated murine model that involves immunizing mice with *Mycobacterium bovis* bacillus Calmette-Guérin to augment host immunity before infection with virulent *Mycobacterium tuberculosis*, we evaluated new treatment regimens including rifapentine and moxifloxacin, and assessed the potential of the *Mycobacterium leprae* heat shock protein-65 DNA vaccine to augment the activity of moxifloxacin.**

Measurements: **Quantitative spleen colony-forming unit counts, and the proportion of mice with culture-positive relapse after treatment, were determined.**

Main Results: **Three-month, once-weekly regimens of rifapentine combined with either isoniazid or moxifloxacin were as active as daily isoniazid for 6–9 mo. Six-month daily combinations of moxifloxacin with pyrazinamide, ethionamide, or ethambutol were more active than pyrazinamide plus ethambutol, a regimen recommended for latent TB infection after exposure to multidrug-resistant TB. The combination of moxifloxacin with the experimental nitroimidazopyran PA-824 was especially active. Finally, the heat shock protein-65 DNA vaccine had no effect on colony-forming unit counts when given alone, but augmented the bactericidal activity of moxifloxacin.**

Conclusions: **Together, these findings suggest that rifapentine, moxifloxacin, and, perhaps, therapeutic DNA vaccination have the potential to improve on the current treatment of latent TB infection.**

Keywords: DNA vaccine; latency; moxifloxacin; rifapentine; tuberculosis

The objective of treatment for latent tuberculosis infection (LTBI) is to prevent the development of overt tuberculosis (TB) disease in infected, but asymptomatic, individuals. At present, a 9-mo course of daily isoniazid (INH) is recommended as first-line therapy for LTBI (1). Alternative regimens include a shorter 6-mo course of INH, which is likely inferior to a 9-mo course (2), or a 4-mo course of daily rifampin (RIF), which is largely untested (1, 3). Although it is comparable in efficacy to 6 or 12 mo of INH (4–6), a 2-mo course of RIF plus pyrazinamide (PZA) is no longer recommended for use because of concerns over excessive hepatotoxicity (7). Priorities for developing improved regimens for treatment of LTBI include (*1*) developing shorter and/or more intermittently administered regimens that are easier to supervise and (*2*) developing and evaluating regimens that are active against multidrug-resistant organisms (8).

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Regarding the first objective, the long-acting rifamycin, rifapentine (RPT), has appeal. Its half-life of 10 to 15 h is substantially longer than that of RIF (2–3 h) (9, 10), permitting weekly dosing. Moreover, it has been well tolerated at doses of 15 (and up to 20) mg/kg administered once weekly (11), resulting in the potential for substantially greater activity than that obtained with the dose of 10 mg/kg currently recommended for treatment of active TB (12, 13). A clinical trial comparing a 3-mo, once-weekly regimen of INH plus RPT (15 mg/kg) with 9 mo of daily INH is currently underway.

When LTBI is identified in an individual exposed to an infectious source with multidrug-resistant TB (MDR-TB), defined as TB caused by organisms resistant to at least INH and RIF, none of the preceding regimens are expected to be efficacious.

Current recommendations for empiric treatment of LTBI after MDR-TB exposure (MDR-LTBI) are for PZA combined with either ethambutol (EMB) or a fluoroquinolone for 6 to 12 mo, but there are few data to support them (1). In addition, in some outbreak settings, combinations of PZA with ofloxacin or levofloxacin have been associated with treatment-limiting hepatotoxicity (14–17). The paucity of data regarding the efficacy of these or other chemoprophylactic regimens for treatment of MDR-LTBI is of great concern given the increasing prevalence of MDR-TB in many parts of the world and the potential use of MDR-TB strains as agents of bioterrorism.

Results demonstrate that the new methoxyfluoroquinolone, moxifloxacin (MXF), has significantly greater bactericidal activity than other fluoroquinolones against actively multiplying and nonactively multiplying *Mycobacterium tuberculosis* both *in vitro* and *in vivo* in the mouse model (18–25). These results raise hopes that MXF, either alone or in combination with other drugs active against MDR-TB isolates, might constitute regimens with greater activity and better tolerability than existing regimens for the treatment of MDR-LTBI or even drug-susceptible LTBI.

The investigational nitroimidazopyran PA-824 is active against MDR-TB isolates and has demonstrated promising activity against hypoxia- and drug-induced persisters *in vitro* and in murine models of TB (26–28). Thus, it constitutes another promising companion drug for MXF in the treatment of MDR-LTBI.

Finally, there is increasing interest in the potential role of plasmid DNA vaccines as therapeutic, as opposed to preventive, agents (29). When administered to mice already infected with *M. tuberculosis*, a DNA vaccine encoding *Mycobacterium leprae* heat shock protein (Hsp)-65 resulted in significant and lasting reductions in lung and spleen colony-forming unit counts (30), although other investigators have not observed such therapeutic activity (31). The same vaccine has also been shown to augment the activity of INH plus PZA and to be effective in preventing reactivation of tubercle bacilli from an antibiotic-induced noncultivable state (32), implying that the vaccine may be effective as an adjunctive measure, either during or after chemotherapy, to prevent development of active disease. To date, however, the vaccine has not been evaluated in a model of LTBI in which human bioequipotent drug doses were used to better represent the treatment of LTBI.

The current study had three major objectives. The first was to determine whether once-weekly combinations of RPT with INH or MXF given for 3 mo were as active as the recommended first-line regimen of INH monotherapy given for 6 to 9 mo. The second was to determine whether various MXF-containing drug regimens given for 6 mo were as active as INH monotherapy or the combination of PZA plus EMB currently recommended for the treatment of MDR-LTBI. Finally, the third was to assess the therapeutic efficacy of the *M. leprae* Hsp65 plasmid DNA vaccine used alone or in combination with MXF.

These objectives were pursued in a murine model of LTBI that involves immunizing mice with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) 6 wk before infection with virulent *M. tuberculosis.* In this setting, BCG-immunized mice are better able to restrict the growth of *M. tuberculosis* infection, leading to smaller bacterial populations that are more representative of LTBI in humans (33–35). This model proved its utility when it was used to demonstrate the superior activity of short-course RIF plus PZA over that of INH (33), a finding that prompted the clinical development of the former highly efficacious combination regimen (4–6).

Some of the results of these studies have been previously reported in the form of an abstract (36).

METHODS

Mycobacterial Strains

BCG Pasteur and *M. tuberculosis* H37Rv were mouse-passaged, frozen, and then subcultured in Middlebrook 7H9 broth (Fisher, Pittsburgh, PA) with 10% oleic acid–albumin–dextrose–catalase (OADC; Difco, Detroit, MI)–0.05% Tween 80 (Sigma, St. Louis, MO). Drug-resistant strains were not used.

Antimicrobials

MXF was provided by Bayer (Rolling Meadows, IL), and RPT was provided by Sanofi-Aventis (Bridgewater, NJ). Other drugs were purchased from Sigma or Fisher (PZA). Solutions were prepared weekly in distilled water. PA-824 was prepared as previously described (26).

Aerosol BCG Immunization

All procedures involving animals were approved by the institutional animal care and use committee. Ten-week-old female BALB/c mice (Charles River, Wilmington, MA) were infected with BCG, using the Glas-Col inhalation exposure system (Glas-Col, Terre Haute, IN) and a log-phase culture of BCG ($OD_{600\,nm}$, 1.0).

Aerosol Challenge with *M. tuberculosis*

Six weeks after BCG immunization, mice, including nonimmunized control animals, were infected with a 10-fold dilution of a log-phase culture of *M. tuberculosis* H37Rv ($OD_{600\,nm}$, 0.55). Five immunized and nonimmunized mice were killed the next day to determine colony-forming unit counts for BCG and *M. tuberculosis*, respectively. Five untreated mice from both groups were killed 3, 6, 22, and 30 wk (and 42 wk for immunized mice) after challenge to assess the effect of BCG vaccination on *M. tuberculosis* multiplication.

Drug Treatment

After *M. tuberculosis* infection, BCG-immunized mice were randomized to one of the following treatment groups (15 mice/group): INH (6 or 9 mo), PZA plus EMB, INH plus RPT, MXF plus RPT, MXF alone, MXF plus PZA, MXF plus EMB, MXF plus ethionamide (ETH), MXF plus PA-824, *M. leprae* Hsp65 plasmid DNA vaccine (DNA) alone, or MXF plus DNA. Treatment began 6 wk later, with all drugs administered by gavage 5 d/wk, except for once-weekly regimens. Except for the 3-mo, once-weekly regimens and the 9-mo INH control regimen, all regimens were administered for 6 mo. The drug dosages (in mg/kg) were as follows: INH (25 daily or 75 weekly), RPT (15), PZA (150), EMB (100), MXF (100), ETH (50), and PA-824 (100), as previously published (24, 26, 37). For RPT, 15 mg/kg in mice is equipotent to 15 mg/kg (900 mg) in humans (38).

DNA Vaccination

The *M. leprae* Hsp65 plasmid DNA vaccine was obtained from S. Rowland (Aeras Global TB Vaccine Foundation, Bethesda, MD). Fifty micrograms of vaccine was administered intramuscularly in each thigh, as previously described (30), at 0, 3, 6, and 9 wk of treatment.

Assessment of Treatment Efficacy

Five mice were killed at the initiation and completion of treatment. Ten additional mice went untreated for an additional 3 mo before being killed to determine the proportion with culture-positive relapse. At death, mice were weighed before spleens were removed, weighed, and homogenized. Quantitative spleen cultures were performed with OADC-enriched 7H10 agar medium (Difco) and differential media to distinguish BCG and *M. tuberculosis* (34).

Statistical Analysis

Colony-forming unit counts were log-transformed before analysis. Group means were compared by unpaired *t* tests (untreated BCG-immunized vs. nonimmunized mice) or one-way analysis of variance with Dunnett's *post hoc* test (experimental groups vs. each control group). Group proportions were compared using Fisher's exact test, adjusting α for multiple comparisons. All analyses were performed with GraphPad Prism version 4.01 (GraphPad, San Diego, CA).

RESULTS

Aerosol BCG Immunization

The lung $\log_{10}$ colony-forming unit count on the day after aerosol BCG immunization was 3.41 ± 0.17 (mean ± SD). Six weeks later, at the time of *M. tuberculosis* challenge, the mean BCG colony-forming unit count in the spleen was 3.19 ± 0.21 $\log_{10}$.

Impact of BCG Immunization on *M. tuberculosis* Multiplication

The mean lung $\log_{10}$ colony-forming unit count for *M. tuberculosis* on the day after aerosol challenge was 3.04 ± 0.34. By the initiation of treatment 6 wk later, the mean *M. tuberculosis* colony-forming unit count in the spleen was 4.55 ± 0.36. Comparison of mean spleen $\log_{10}$ colony-forming unit counts in BCG-immunized versus nonimmunized mice obtained over the 30 wk after challenge revealed a significant effect of immunization in limiting the multiplication of *M. tuberculosis* ($p \leq 0.01$ at each time point). Immunized mice consistently had 1 $\log_{10}$ fewer colony-forming units in the spleen than did nonimmunized mice (Figure 1). The

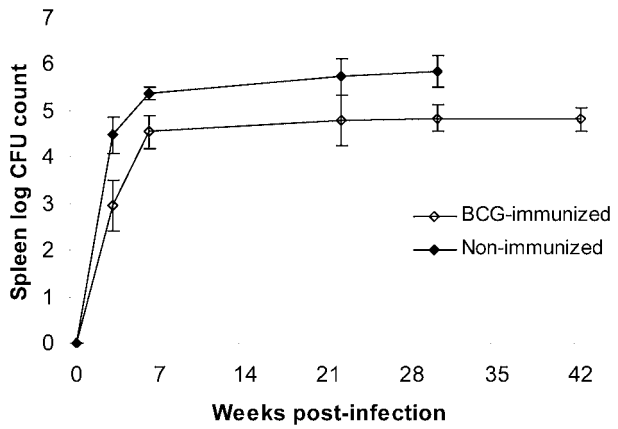

Figure 1. Change in spleen $\log_{10}$ colony-forming unit (CFU) counts over time after challenge with *M. tuberculosis* in BCG-immunized versus nonimmunized mice.

spleens of immunized mice were also significantly smaller than those of nonimmunized mice at each time point, beginning 3 wk after *M. tuberculosis* challenge ($p \leq 0.01$ at each time point; data not shown).

Activity of 3-Mo, Once-Weekly Rifapentine-containing Regimens

The mean spleen $\log_{10}$ colony-forming unit counts at the completion of treatment were no different between the two 3-mo, once-weekly regimens and the 6-mo daily INH control regimen (Table 1). No more than one mouse of five in each group had negative spleen cultures at the completion of therapy. Although only 6 of 10 mice treated with 3 mo of once-weekly INH plus RPT had positive cultures when assessed for relapse as compared with 9 of 10 mice in the 9-mo daily INH group or the 3-mo, once-weekly MXF plus RPT group, this difference was not statistically significant. Thus, the activity of the 3-mo, once-weekly regimens could not be distinguished from that of 6 to 9 mo of daily INH.

Activity of 6-Mo, MXF-containing Regimens

The mean spleen $\log_{10}$ colony-forming unit counts after 6 mo of treatment are presented in Figure 2. Each MXF-containing regimen was compared with each control regimen (INH or PZA + EMB). MXF alone was less active than INH ($p < 0.05$) and no more active than PZA plus EMB, but combinations of MXF with PZA, ETH, or EMB were more active than PZA plus EMB ($p < 0.01$ for the two former combinations; $p < 0.05$ for the last) and at least as active as INH. Most striking, however, was the activity of the MXF plus PA-824 combination. All five spleens from this group were culture negative. The difference in colony-forming unit counts after treatment with INH versus MXF plus PA-824 was statistically significant before, but not after, adjustment for multiple comparisons.

Comparisons of the proportion of culture-positive spleens on completion of therapy and at the point of assessment for relapse are presented in Table 2. MXF plus PA-824 was clearly the most effective regimen, resulting in a greater proportion of mice with culture-negative spleens at completion of therapy and at relapse, compared with 9 mo of INH and 6 mo of PZA plus EMB ($p < 0.05$ after adjustment for multiple comparisons). MXF plus PA-824 provided a durable cure for all mice whereas the next most effective regimen, MXF plus PZA, produced a durable cure in only two of nine mice. The number of mice with culture-negative spleens at the end of therapy was greater in the MXF plus PZA group (four of five) compared with the PZA plus EMB group (zero of five) before, but not after, adjusting for multiple comparisons. None of the other regimens could be differentiated from either control regimen.

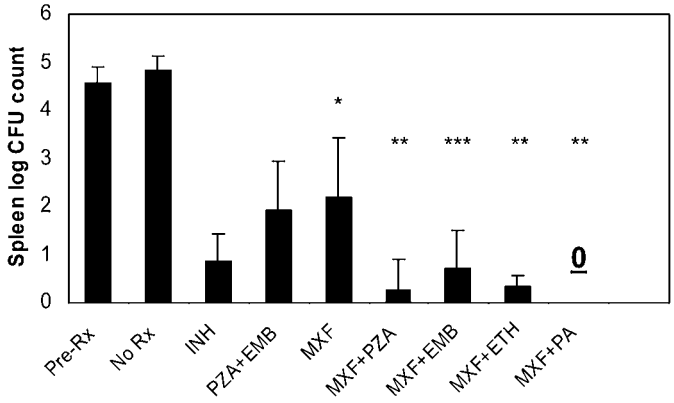

Figure 2. Spleen $\log_{10}$ CFU counts on completion of 6 mo of treatment. *0,* all spleens were culture negative. *$p < 0.05$ versus INH; **$p < 0.01$ versus PZA plus EMB; ***$p < 0.05$ versus PZA plus EMB.

Therapeutic Efficacy of Plasmid DNA Vaccination

Postexposure vaccination with the *M. leprae* Hsp65 DNA vaccine alone had no effect on spleen colony-forming unit counts 6 mo after the initiation of therapy compared with untreated control animals (Table 3). Interestingly, however, combined therapy with MXF and the DNA vaccine was significantly more active than therapy with MXF alone ($p < 0.05$). Addition of the DNA vaccine resulted in an additional 1.8 $\log_{10}$ fall in spleen colony-forming unit counts compared with MXF alone. In addition, the combination of MXF plus DNA resulted in three of five spleens being culture negative on completion of therapy, compared with zero of five spleens in the MXF-alone group, but this difference did not reach statistical significance. In addition, eight of nine mice treated with MXF plus DNA were culture positive 3 mo after completion of treatment, implying that the DNA vaccine had limited ability to prevent regrowth after MXF was discontinued.

TABLE 1. SPLEEN $\log_{10}$ COLONY-FORMING UNIT COUNTS AND PROPORTION OF MICE WITH POSITIVE SPLEEN CULTURES 3 MO AFTER TREATMENT WITH 3-MO, ONCE-WEEKLY RIFAPENTINE-CONTAINING REGIMENS

Regimen	Spleen log CFU Count at End of Treatment*	Proportion with Culture-positive Spleens at Relapse
Control regimens		
6-mo INH	0.85 ± 0.59	10/10
9-mo INH	ND	9/10
Experimental regimens		
3-mo INH plus RPT	0.76 ± 0.27	6/10
3-mo MXF plus RPT	1.32 ± 0.54	9/10

Definition of abbreviations: CFU = colony-forming unit; INH = isoniazid; MXF = moxifloxacin; ND = not determined; RPT = rifapentine.

* Spleen log colony–forming unit count was 4.55 ± 0.36 at the initiation of treatment.

TABLE 2. PROPORTION OF MICE WITH POSITIVE SPLEEN CULTURES AND RELAPSE AFTER TREATMENT WITH MOXIFLOXACIN-CONTAINING REGIMENS

	Proportion with Culture-positive Spleen at:	
Regimen	End of Treatment	Relapse
Controls		
6-mo INH	4/5	10/10
9-mo INH	ND	9/10
6-mo PZA plus EMB	5/5	ND†
Experimental regimens		
6-mo MXF	5/5	ND†
6-mo MXF plus PZA	1/5	7/9
6-mo MXF plus EMB	3/5	7/8
6-mo MXF plus ETH	4/5	8/8
6-mo MXF plus PA-824	0/5*	0/9‡

Definition of abbreviations: EMB = ethambutol; ETH = ethionamide; INH = isoniazid; MXF = moxifloxacin; ND = not determined; PZA = pyrazinamide; RPT = rifapentine.

* $p < 0.05$ versus PZA plus EMB.

† Groups in which all mice were culture positive at the end of treatment were not considered for relapse determinations.

‡ $p < 0.05$ versus INH.

TABLE 3. SPLEEN $\log_{10}$ COLONY-FORMING UNIT COUNTS AFTER TREATMENT WITH REGIMENS CONTAINING Hsp65 PLASMID DNA VACCINE

	Spleen CFU Count
Control regimen	
6-mo untreated	4.83 ± 0.29
Experimental regimens	
6-mo DNA alone	5.32 ± 0.51
6-mo MXF	2.19 ± 1.26
6-mo MXF plus DNA	0.39 ± 0.73*

Definition of abbreviations: CFU = colony-forming unit; MXF = moxifloxacin.
* $p < 0.05$ versus MXF alone.

DISCUSSION

A previous study in a similar model of BCG-immunized mice yielded the highly efficacious short-course RIF plus PZA regimen (33). In the aftermath of excessive hepatotoxicity observed with this regimen and in the context of expanding hot spots of MDR-TB in certain areas of the world, we have revisited this model to identify alternative regimens that are shorter and easier to administer to patients with drug-susceptible LTBI or are more active against MDR-LTBI. This study bears several findings that might have implications for the current management of LTBI caused by either drug-susceptible or MDR *M. tuberculosis.*

First, 3-mo, once-weekly regimens based on the combination of RPT (15 mg/kg) with either INH or MXF were both highly active and indistinguishable from 6 to 9 mo of daily INH. These results indirectly corroborate the results of a clinical trial comparing a 3-mo regimen of once-weekly INH plus RPT (15 mg/kg) with a 2-mo regimen of daily RIF plus PZA (a regimen with efficacy similar to that of 6–12 mo of daily INH) (4–6). That trial was stopped early when the risk of hepatotoxicity due to RIF plus PZA was recognized. Analysis of 399 patients enrolled up to that point, however, revealed no difference in efficacy (39). Our results also predict that the ongoing clinical trial comparing the same once-weekly INH plus RPT regimen with 9 mo of daily INH will show similar efficacy for the two regimens. If so, the INH plus RPT regimen would provide an important alternative regimen of significantly shorter duration that is particularly suited for directly observed therapy of LTBI. Furthermore, our results suggest that substitution of MXF for INH in this regimen is unlikely to improve its efficacy, likely because the rifamycin is the most active component against latent or persisting bacilli and because the activity of MXF against persisters is no better than that of INH, at least when MXF is given at a daily dose of 100 mg/kg (26).

Second, regarding treatment of MDR-LTBI, combinations of MXF with PZA, ETH, or EMB were more active than the currently recommended regimen of PZA plus EMB when all regimens were administered daily for 6 mo. The activity of the former two regimens was also at least as good as that of INH given for 6 to 9 mo, as currently recommended for the treatment of LTBI caused by fully drug-susceptible isolates (1). The combination of MXF plus EMB was also not significantly worse than INH. Thus, when used for at least 6 mo, MXF in combination with PZA, ETH, or even EMB may constitute highly efficacious oral regimens for the treatment of MDR-LTBI.

Third, MXF alone was no more active than PZA plus EMB and was less active than INH alone. Still, the use of MXF alone (or in combination with EMB) for 9 to 12 mo may constitute an efficacious oral regimen for persons intolerant of PZA or ETH. Given the apparent frequency with which PZA and regimens containing PZA have been implicated in causing hepatotoxicity (7, 14–17, 40), such a regimen may provide a valuable alternative regimen for patients with MDR-LTBI.

Two additional observations from the current study provide reason for optimism for the future management of LTBI caused by both drug-susceptible and drug-resistant *M. tuberculosis.* First, addition of the investigational nitroimidazopyran PA-824 to MXF resulted in a highly significant improvement in activity and a regimen that performed better than 9 mo of INH. Although the activity of PA-824 alone was not assessed in this study, we have described the potent bactericidal activity of PA-824 against persisting tubercle bacilli in another murine model (26). Together, these promising results suggest that PA-824, which is now in phase I clinical trials, may provide a new building block for regimens that can effectively treat LTBI in less than 6 mo. Second, the *M. leprae* Hsp65 plasmid DNA vaccine demonstrated synergistic therapeutic activity when administered together with MXF, resulting in significantly lower spleen colony-forming unit counts and fewer culture-positive spleens at the completion of treatment compared with treatment with MXF alone. This result is corroborated by, and extends, findings that this and other DNA vaccines, when administered during or after chemotherapy, can augment the antibacterial activity of chemotherapy and/or reduce the potential for relapse (30, 32, 41, 42). In addition to the treatment of LTBI, these findings may also have broader implications for shortening the duration of treatment for active TB, a major goal of drug development research (8).

The findings reported here come with several caveats. First, because mice were infected with a larger dose of *M. tuberculosis* than originally planned, they developed larger bacillary burdens than expected, slightly exceeding 10^4 cfu/spleen, which is a reasonable outer limit for the bacillary burden of human LTBI (34). This also led to substantially greater than expected proportions of mice having positive cultures 3 mo after completion of treatment. Still, it is evident that BCG immunization acted to restrain proliferation of *M. tuberculosis.* Overall, the high frequency of culture positivity 3 mo after completion of treatment in this study should not be equated with the likelihood of failure with use of any of these regimens to treat LTBI in humans. Rather, the use of INH as the positive control provides the ability to make meaningful assessments of the experimental regimens in terms of activity, if not efficacy. That said, the study is likely underpowered to detect differences in parameters other than relapse.

Second, because PA-824 has just entered phase I clinical trials, we can provide no reassurance that the dose of PA-824 used in this study will be equipotent to human doses that will be recommended if the drug is ultimately approved for clinical use. The rationale for using the 100-mg/kg dose is that this dose is the lowest dose that has bactericidal activity in infected mice (26). Nonetheless, the results reported here suggest that PA-824 has great potential for the treatment of LTBI and warrant further studies of PA-824 alone and in combination with INH, the rifamycins, and PZA to develop regimens that are shorter, more intermittent, and/or more active against MDR isolates than current regimens.

Third, regarding the activity of the DNA vaccine in combination with MXF, it must be borne in mind that our study involved BCG immunization followed by DNA vaccination of infected mice. It is possible that this "prime-boost" protocol exaggerated the potential therapeutic activity of the DNA vaccine. Nevertheless, most of the world's population has received BCG vaccination, making it possible that a similar boosting effect might occur in many humans with active TB or LTBI.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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