Efflux inhibition with verapamil potentiates bedaquiline in *Mycobacterium tuberculosis*

**Running title:** Verapamil enhances killing by bedaquiline of TB

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Abstract:

Drug efflux is an important resistance mechanism in *Mycobacterium tuberculosis* (*M. tb*.). We found that verapamil, an efflux inhibitor, profoundly decreases the minimum inhibitory concentration of bedaquiline and clofazimine to *M. tb* by 8 to 16-fold. This exquisite susceptibility was noted among drug-susceptible and drug-resistant clinical isolates. Thus, efflux inhibition is an important sensitizer of bedaquiline and clofazimine, and efflux may emerge as a resistance mechanism to these drugs.

Body:

The emergence of drug-resistant tuberculosis (TB) has complicated global TB control efforts. The World Health Organization (WHO) estimates that up to half a million multidrug-resistant TB (MDR-TB) cases occur annually (1). Current treatment regimens for drug-resistant TB are lengthy, costly, toxic and less effective than regimens for drug-susceptible TB. There is an urgent need to develop novel therapeutic regimens that are efficacious against drug-resistant TB and well tolerated.

Drug efflux has recently been highlighted as an important resistance mechanism in *Mycobacterium tuberculosis* (*M. tb*.). (2) In contrast, efflux inhibition may augment the bactericidal and sterilizing efficacy of existing drugs in a regimen by either increasing the intracellular drug concentration or by decreasing the immune cell-induced tolerance to these drugs. The addition of efflux pump inhibitors to TB regimens has the potential to enhance antimycobacterial killing and prevent the emergence of drug-resistance (3–5).
Verapamil is an FDA approved efflux pump inhibitor that appears promising as adjunctive chemotherapy for TB. We have recently shown that the addition of verapamil accelerates both the bactericidal and sterilizing activities of standard TB treatment in the mouse (6). In an in vitro system, we have shown that after two hours of incubation with verapamil, rifampin levels inside bacterial cells were increased by 2-fold (6). Inhibition of efflux pumps of *M. tb.* by verapamil reduces the macrophage-induced bacterial drug tolerance in lung granulomas (4). Lastly, there is evidence that verapamil may reverse some forms of drug-resistance, as it is able to restore rifampin efficacy in mice infected with rifampin-resistant *M. tb.* strain (7). While the impact of efflux pump inhibition has been demonstrated for rifampin and other first-line medications, it is unclear whether this benefit extends to additional and newer classes of TB drugs.

Bedaquiline (also known as Sirturo™, TMC-207, R207910 or the ‘J’ compound) is the first anti-TB drug of a novel class to be approved by the US Food and Drug Administration (FDA) in 40 years (8). A diarylquinoline, bedaquiline inhibits the mycobacterial proton pump, ATP synthase (9). Clinical trials have demonstrated its safety and efficacy, leading to its recent approval for the treatment of MDR-TB (10-12). While the therapeutic potential of bedaquiline is encouraging, WHO guidelines warn that improper use could promote the emergence of bedaquiline resistance and possible loss of the first new TB chemotherapeutic drug (13). Thus, there is an urgent need to protect bedaquiline from the emergence of resistance.
To determine the effect of efflux inhibition on the antimycobacterial activity of bedaquiline, clofazimine, meropenem and moxifloxacin, we determined the MICs of these drugs in the presence of verapamil using a microplate alamar blue assay (MABA), as previously described (14). Briefly, $10^4$ CFU of clinical isolates of *M. tb.* and the lab strain H37Rv were plated on a 96-well plate in the presence of serial drug-dilutions with or without 50 $\mu$g/mL of verapamil. The lowest concentration of drug leading to at least a 90% reduction of bacterial growth signal by MABA was recorded as the MIC. Each assay was done three times, and the results of one representative experiment are shown in Table 1.

Eight clinical isolates of *M. tb.* with various first-and second line drug-susceptibility patterns (see Table 2) were obtained from Project SEREFO-NIAID/University of Bamako Research Collaboration on HIV/TB, in Bamako, Mali for evaluation in this study (15, 16).

We found that supplementation with verapamil profoundly decreases the MIC of bedaquiline among drug-susceptible and drug-resistant clinical isolates. Verapamil yielded an 8 to 16-fold decrease in the bedaquiline MIC of all isolates tested, irrespective of first and second-line drug resistance patterns (See Table 2). To confirm these findings, we used a liquid broth dilution assay for MIC determination and obtained similar results.

Similar to bedaquiline, we found that the MIC of clofazimine against *M. tb.* decreases by 8-fold in the presence of 50 $\mu$g/mL verapamil. (See Table 3). Verapamil did
not contribute to MIC-reduction of meropenem and moxifloxacin in these clinical isolates
(data not shown).

This is the first report that efflux pump inhibition by verapamil can potentiate the
killing of *M. tb.* by bedaquiline and clofazimine. In the present study, we found an
impressive 8-fold or more reduction in the MICs of bedaquiline and clofazimine,
suggesting the specificity of verapamil for inhibiting efflux pumps relevant to these
drugs. As with rifampin, verapamil may inhibit the efflux of these drugs from *M. tb.*
resulting in higher intracellular drug levels and enhanced drug activity.

Our finding that verapamil sensitizes *M. tb.* to bedaquiline and clofazimine is
likely to extend to *in vivo* infection. By potentiating the killing of bedaquiline and
clofazimine, verapamil may accelerate clearance of *M. tb.*, thus allowing for shorter
treatment regimens of both drug-susceptible and drug-resistant forms of TB.

Furthermore, given that efflux inhibition renders *M. tb.* exquisitely susceptible to
bedaquiline and clofazamine, these data suggest that efflux may emerge as a common
resistance mechanism to these drugs. As has been noted with first-line drugs, verapamil
might reduce the macrophage-induced drug tolerance to bedaquiline (4). As in other
organisms (17, 18), verapamil may prevent the emergence of further drug resistance, and
thus preserve the activity bedaquiline.
Given the extremely low MIC values achieved with the combination of bedaquiline and verapamil, a drug regimen containing these two agents may be highly effective for the treatment of both drug-resistant and drug-susceptible *M. tb*. While overlapping toxicities must be considered when any two drugs are given in combination, there are no overlapping toxicities that would prevent simultaneous administration of bedaquiline and verapamil. Both bedaquiline and verapamil have effects on the cardiac conduction system, however, their effects are distinct: bedaquiline prolongs the QT interval (11) whereas verapamil prolongs the PR interval (19). Indeed, the MIC lowering of verapamil may enable lower doses of bedaquiline and thereby limit its dose related toxicities.

These findings suggest that efflux inhibition is an important sensitizer of bedaquiline, which has significant microbiologic, pharmacologic and clinical implications for *M. tb*. Further studies are warranted to extend the synergistic effects of verapamil and bedaquiline in an animal model of TB infection.

**Acknowledgments:**
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**References:**


Table 1. Verapamil potentiates the killing of bedaquiline in H37Rv and clinical isolates of *M. tb*. The MICs to bedaquiline with and without verapamil are listed, with the resulting fold change in MIC. MIC values are listed in µg/mL.

Table 2. Eight clinical isolates from Bamako, Mali were utilized for MIC determination. Brief clinical information and drug susceptibility testing (DST) results are listed. First and second-line drug testing includes Isoniazid (H), Rifampin (R), Ethambutol (E), Streptomycin (S), Pyrazinamide (Z), Kanamycin (Kan) and Amikacin (Ami).

Table 3: Broth confirmation of verapamil potentiation of bedaquiline and clofazimine. The MIC of *M. tb* H37Rv to bedaquiline and clofazimine with and without verapamil were determined via a broth MIC-determination method. MIC values are listed in µg/mL.
Table 1. Verapamil potentiates the killing of bedaquiline in H37Rv and clinical isolates of *M. tb*. The MICs to bedaquiline with and without verapamil are listed, with the resulting fold change in MIC. MIC values are listed in µg/mL.

<table>
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<tr>
<th>Bac</th>
<th>MIC TMC only (µg/ml)</th>
<th>MIC TMC + Verapamil (µg/ml)</th>
<th>Fold Change in MIC</th>
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<td>Bac1 MAL010084</td>
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<td>0.00195</td>
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</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>MIC Drug only (µg/ml)</th>
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<td>Clofazimine</td>
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<td>0.03125</td>
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