Meropenem-vaborbactam restoration of first-line drug efficacy and comparison of meropenem-vaborbactam-moxifloxacin versus BPaL MDR-TB regimen

Sanjay Singh[a], Tawanda Gumbo[b,c], Jan-Willem Alffenaar[d,e,f], Gunavanthi D. Boorgula[a], Prem Shankar[a], Tania A. Thomas[g], Keertan Dheda[h], Lesibana Malinga[i], Prithvi Raj[j], Santosh Aryal[k], Shashikant Srivastava[a,l,*]

[a] *Department of Medicine, School of Medicine, University of Texas at Tyler, Tyler, TX, USA*
[b] *Quantitative Preclinical & Clinical Sciences Department, Praedicare Inc., Dallas, TX, USA*
[c] *Hollow Fiber System & Experimental Therapeutics Laboratories, Praedicare Inc, Dallas, TX, USA*
[d] *Sydney Institute for Infectious Diseases, The University of Sydney, Sydney, New South Wales, Australia*
[e] *School of Pharmacy, The University of Sydney Faculty of Medicine and Health, Sydney, New South Wales, Australia*
[f] *Westmead Hospital, Sydney, New South Wales, Australia*
[g] *Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA*
[h] *The Center for Lung Infection and Immunity Unit, Division of Pulmonology, Department of Medicine, University of Cape Town, Cape Town, South Africa*
[i] *Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa*
[j] *Department of Immunology, UT Southwestern Medical Center, Dallas, TX, USA*
[k] *Department of Pharmaceutical Sciences and Health Outcomes, The Ben and Maytee Fisch College of Pharmacy, University of Texas at Tyler, Tyler, TX, USA*
[l] *Department of Cellular and Molecular Biology, UT Health Science Centre at Tyler, Tyler, TX, US*

Keywords:
Beta-lactams
Pharmacokinetics/pharmacodynamics
Multidrug-resistant tuberculosis
BPaL
Hollow fiber model system

A B S T R A C T

Background: Meropenem in combination with β-lactamase inhibitors (BLIs) and other drugs was tested to identify alternative treatment regimens for multidrug-resistant tuberculosis (MDR-TB).

Methods: The following were performed: (1) MIC experiments; (2) static time-kill studies (STKs) with different BLIs; and (3) a hollow fibre model system of TB (HFS-TB) studies with meropenem-vaborbactam combined with human equivalent daily doses of 20 mg/kg or 35 mg/kg rifampin, or moxifloxacin 400 mg, or linezolid 600 mg vs. bedaquiline-pretonamid-linezolid (BPaL) for MDR-TB. The studies were performed using *Mycobacterium tuberculosis* (*M. tuberculosis*) H37Rv and an MDR-TB clinical strain (named *M. tuberculosis* 16D) that underwent whole genome sequencing. Exponential decline models were used to calculate the kill rate constant (K) of different HFS-TB regimens.

Results: Whole genome sequencing revealed mutations associated with resistance to rifampin, isoniazid, and cephalosporins. The meropenem-vaborbactam MIC of *M. tuberculosis* was H37Rv 2 mg/L and > 128 mg/L for *M. tuberculosis* 16D. Relebactam and vaborbactam improved both the potency and efficacy of meropenem in STKs. Meropenem-vaborbactam alone failed to kill *M. tuberculosis* 16D but killed below day 0 burden when combined with isoniazid and rifampin, with the moxifloxacin combination being the most effective and outranking bedaquiline and pretomanid. In the HFS-TB, meropenem-vaborbactam-moxifloxacin and BPaL had the highest K ($\log_{10}$ cfu/mL/day) of 0.31 (95% CI 0.17–0.58) and 0.34 (95% CI 0.21–0.56), while meropenem-vaborbactam-rifampin (35 mg/kg) had a K of 0.18 (95% CI 0.12–0.25). The K for meropenem-vaborbactam-moxifloxacin-linezolid demonstrated antagonism.

Conclusion: Adding meropenem-vaborbactam could potentially restore the efficacy of isoniazid and rifampin against MDR-TB. The meropenem-vaborbactam-moxifloxacin backbone regimen has implications for creating a new effective MDR-TB regimen.

* Corresponding author: Department of Medicine, School of Medicine, UT Health Science Center at Tyler, 11937 US Highway 271, Tyler, TX 75708, USA.
E-mail address: Shashi.kant@uthct.edu (S. Srivastava).

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), remains a significant cause of mortality globally. The COVID-19 pandemic adversely affected TB detection and prevention efforts worldwide [1]. The World Health Organization estimated that nearly 10 million people developed TB in 2020; however, 5.8 million cases were detected [2,3]. This reduced TB care due to the pandemic also resulted in an increased death rate, as evidenced by ca. 1.5 million TB-related deaths worldwide in 2020; this is the highest year-on-year increase in TB deaths since 2005 [3].

Significant progress has been made in recent years to develop combination regimens to treat multidrug-resistant TB (MDR-TB) infections. Preclinical models such as the hollow fibre model of TB (HFS-TB) have played an increasing role in pharmacokinetics/pharmacodynamics (PK/PD)-based design of treatment regimens for drug-susceptible and MDR-TB (i.e. pan-TB), including treatment shortening [4–11]. As an example, the linezolid PK/PD optimised dose for treatment of MDR-TB in the HFS-TB model was identified as 600 mg/day based on achieving PK/PD exposure targets in 99% of patients, with < 20% of the patients predicted to encounter serious adverse events [12]. To reiterate the translational applicability of preclinical model findings, results of the Nix-TB clinical trial (ClinicalTrials.gov Identifier: NCT02333799) comparing the safety and efficacy of linezolid 600 mg/day versus 1200 mg/day have now been published [12–15]. This clinical trial tested the efficacy of a bedaquiline-pretonamid-linezolid (BPaL) combination regimen to provide information on linezolid dose balancing between efficacy and adverse events, especially given the long therapy duration for MDR-TB. Based on microbial outcomes, efficacy was 96% for 600 mg/day administered for 26 weeks, and severe adverse events were encountered in 20% of patients on 600 mg/day [15]. Thus, the HFS-TB could accurately predict the clinical outcomes (efficacy and toxicity) of a drug or regimen, and such output could reduce the proportion of patients treated with toxic or suboptimal doses in clinical trials [12,15–18]. The results also mean that the BPaL regimen with linezolid 600 mg/day dose, while safer than 1200 mg/day, still has toxicity; moreover, acquired resistance to component drugs has recently been noted in the clinic [15].

β-lactam antibiotics, including carbapenems, are efficacious against MDR-TB in the HFS-TB model [14,19–23]. *Mycobacterium tuberculosis* has an Ambler class A β-lactamase, BlaC, which are serine hydrolase susceptible to several clinically available β-lactamase inhibitors (BLIs) such as clavulanate [24]. In a systematic literature search, van Rijn et al. found that in vitro the activity of three carbapenems (imipenem, meropenem, and ertapenem) against *M. tuberculosis* improved when used in combination with clavulanate [25]. However, clavulanate is not commercially available alone and, therefore, impractical to prescribe for TB unless to use in combination with amoxicillin. In a 14-day early bactericidal activity (EBA) study of patients randomised to one of four intravenous meropenem-based arms (all in combination with amoxicillin/clavulanate), the EBA ($\log_{10}$ cfu/mL/day) was 0.22 for 2 g every 8 hours (TID), 0.12 for meropenem 2 g TID plus 20 mg/kg/day rifampin (indicating obvious rifampin vs. meropenem-clavulanate antagonism), and 0.059 for meropenem 1 g TID [26]. However, tolerability was poor at all doses due to amoxicillin/clavulanate gastrointestinal toxicity [26]. Fortunately, the *M. tuberculosis* BlaC has a wide binding pocket that accommodates many other BLIs beyond clavulanate, including the synthetic diazabicyclooctane avibactam, bridged bicyclic urea molecule relebactam, and boronic acid-based vaborbactam [27]. These BLIs are potentially less toxic than amoxicillin/clavulanate and have a higher binding affinity than clavulanate.

This study tested the hypothesis that meropenem could restore the efficacy of isoniazid and rifampin against MDR-TB [25]. The following were performed: (1) MIC experiments; (2) static time-kill studies (STKs) with meropenem alone or in combination with either avibactam or relebactam or vaborbactam; and (3) HFS-MDR-TB study with several meropenem-vaborbactam combination experimental regimens compared with the BPaL regimen (positive control) recommended to treat MDR-TB in patients. Meropenem-vaborbactam was chosen for HFS-TB work since it is commercially available as a fixed-dose 1:1 combination [28].

2. Methods

2.1. Bacteria, drugs, and other supplies

Drug-susceptible *M. tuberculosis* H37Rv and one MDR-TB clinical strain (SAMRC-16D), herein designated *M. tuberculosis* 16D, were used in the experiments. Materials and culture methods are discussed in detail in the Supplementary Methods.

2.2. Whole genome sequencing of multidrug-resistant tuberculosis Mycobacterium tuberculosis *16D strain*

DNA was extracted and subjected to whole genome sequencing (WGS) to confirm the mutations in the drug resistance genes in the MDR-TB clinical strain using the methods published elsewhere and described in the Supplementary Methods [12,29]. The sequencing reads were aligned to the reference *M. tuberculosis* genome (NC_000962) and single nucleotide variants (SNVs) made compared with the wild type.

2.3. MIC experiments

The MICs were determined using the broth microdilution method and Mycobacterial Growth Indicator Tube (MGIT) system [12,30]. Supplementary Methods include the details for the inoculum preparation and other experimental procedures.

2.4. Evaluation of different β-lactamase inhibitors on the efficacy of meropenem against M. tuberculosis

Several β-lactam antibiotics have previously been tested for efficacy against *M. tuberculosis* [14,19–23,31]. Here, *M. tuberculosis* H37Rv was co-cultured with meropenem alone or in combination with avibactam (15 mg/L) [20] or relebactam (6 mg/L) or vaborbactam. The meropenem concentrations ranged 0.125–16 mg/L in a two-fold dilution. A commercially available combination of meropenem-vaborbactam (herein abbreviated V) was used for vaborbactam [28]. The inoculum preparation was the same as described for the MIC experiments. After 7 days of co-incubation with drugs at 37 °C under shaking conditions, the cultures were washed twice with normal saline to remove the carry-over drug, serially diluted, and spread on Middlebrook 7H10 agar supplemented with 10% OADC. Cultures were incubated for 28 days before the cfu/mL with each drug concentration was recorded.

2.5. Meropenem-vaborbactam combination static time-kill studies

Since the treatment of TB requires combination therapy, the next set of experiments was performed to determine which other drugs could be combined with V with drug-susceptible *M. tuberculosis* H37Rv. Supplementary Table 1 lists all the drugs and the concentration achieved with the human equivalent standard clinical dose of each drug used in the experiments. The inoculum was prepared as described above. The cultures were incubated for 7 days at 37 °C, after which the cultures were washed twice with normal

Table 1
Mutation profile of the multidrug-resistant tuberculosis clinical strain *Mycobacterium tuberculosis* 16D using whole genome sequencing.

Gene	Description	Coding region change	Amino acid change	Non-synonymous
rpoB	DNA-directed RNA polymerase subunit beta	NP_215181.1:c.1349C>T	NP_215181.1:p.Ser450Leu	Yes
rpoB	DNA-directed RNA polymerase subunit beta	NP_215181.1:c.1690T>C	NP_215181.1:p.Tyr564His	Yes
katG	catalase-peroxidase-peroxynitritase T	NP_216424.1:c.944G>C	NP_216424.1:p.Ser315Thr	Yes
embB	indolylacetylinositol arabinosyltransferase	NP_218312.1:c.916A>G	NP_218312.1:p.Met306Val	Yes
gidB	16S rRNA methyltransferase	NP_218436.1:c.299C>T	NP_218436.1:p.Ser100Phe	Yes
gidB	16S rRNA methyltransferase	NP_218436.1:c.47T>G	NP_218436.1:p.Leu16Arg	Yes
pncA	pyrazinamidase	NP_216559.1:c.416T>G	NP_216559.1:p.Val139Gly	Yes
gyrA	DNA gyrase subunit A	NP_214520.1:c.61G>C	NP_214520.1:p.Glu21Gln	Yes
gyrA	DNA gyrase subunit A	NP_214520.1:c.284G>C	NP_214520.1:p.Ser95Thr	Yes
gyrA	DNA gyrase subunit A	NP_214520.1:c.739G>A	NP_214520.1:p.Gly247Ser	Yes
gyrA	DNA gyrase subunit A	NP_214520.1:c.2003G>A	NP_214520.1:p.Gly668Asp	Yes
ponA1	bifunctional penicillin-binding protein 1A/1B	YP_177687.1:c.1891C>T	YP_177687.1:p.Pro631Ser	Yes

saline to remove the carry-over drug, serially 10-fold diluted, and spread on agar to enumerate the bacterial burden with each drug alone or in combination. The colonies were counted after 28 days of incubation at 37 °C. Next, the experiment was repeated with *M. tuberculosis* 16D. This strain was randomly selected from a library of 30 clinical isolates subjected to WGS to confirm the phenotypic susceptibility [21].

2.6. Efficacy of meropenem-vaborbactam combination against multidrug-resistant tuberculosis in the hollow fibre model system of TB

Next, an HFS-TB model study was performed to determine whether the same V efficacy could be achieved with fluctuating drug concentrations; meropenem and vaborbactam have an identical half-life ($t_{1/2}$), and in the fixed-dose combination are mixed 1:1 [9]. The peripheral compartment of each HFS-TB unit was inoculated with 20 mL logarithmic phase growth cultures of *M. tuberculosis* 16D. The systems were treated with each different two-drug or three-drug combination of meropenem-vaborbactam once daily for 28 days, one HFS-TB unit per regimen except two units for the nontreated controls; the PKs used for the design are shown in the Supplementary Methods. Given the high MIC against the MDR-TB clinical strain, V alone was not tested in the HFS-TB. The experimental drug combination regimen and human equivalent doses were consistent with clinically acceptable dosing strategies as detailed: **Regimen 1:** combination of V 4 g plus moxifloxacin 400 mg; **Regimen 2:** combination of V 4 g plus rifampin 20 mg/kg; **Regimen 3:** combination of V 4 g plus rifampin 35 mg/kg; **Regimen 4:** combination of V 4 g plus linezolid 600 mg; **Regimen 5:** combination of V 4 g plus moxifloxacin 400 mg plus linezolid 600 mg; **Regimen 6**: WHO-recommended BPaL regimen (bedaquiline 400 mg, pretonamid 200 mg, and linezolid 600 mg) for MDR-TB [32,33] as a comparator (positive control); and **Regimen 7**: nontreated control (negative control). Sampling times and processing of samples for drug PKs and *M. tuberculosis* burden are further described in the Supplementary Methods.

2.7. Drug concentration measurements and data analysis

With the exception of meropenem and vaborbactam, previously published methods were used to measure isoniazid, rifampin, moxifloxacin, linezolid, bedaquiline, and pretomanid in the HFS-TB samples [11,34–36]. Supplementary Methods give details of the drug concentration assays.

2.8. PK/PD analyses and modelling

The PK analysis is described in the Supplementary Methods and Supplementary Tables 2 and 3 [37]. Using the measured drug concentration, noncompartmental analysis using Phoenix WinNonlin (Certara, v8.1) was performed as described in the Supplementary Methods [37]. The PD analyses were performed using the four-parameter inhibitory sigmoid E_{max} model:

$$\text{Effect}\left(\log_{10}\text{cfu/mL}\right) = E_{con} - E_{max} \times IC^{H}/\left(IC^{H} + C_{50}^{H}\right) \quad (1)$$

Where E_{con} is the *M. tuberculosis* burden in non-treated controls, E_{max} (cfu/mL) is E_{con} minus the bacterial burden in the concentration mediating maximal effect (E_{max} defines efficacy), and IC_{50} is the concentration associated with 50% of E_{max}; it defines potency. H is the Hill slope, which gives information on the antibiotic's (meropenem) binding sites (and thus mechanism) [38,39].

3. Results

3.1. Whole genome sequencing

Whole genome sequencing of the clinical strain *M. tuberculosis* 16D, isolated from a South African patient, was performed to confirm the phenotypic drug susceptibly results. The clinical isolate demonstrated mutations shown in Table 1. The isolate had *rpoB* Ser450Leu (rifampin), *katG* Ser315Thr (isoniazid), *gidA* Ser100Phe (streptomycin), *embB* Met306Val (ethambutol), *pncA* Val139Gly (pyrazinamide), and ponA1 Pro631Ser (cephalosporins), indicating high-level resistance to all first-line drugs, aminoglycosides, and the β-lactam class of third-generation cephalosporins. Specifically, there were no mutations in the following genes: *blaC* (BLI resistance), or *rrs* and *rplC* (associated with linezolid resistance) [40], or *atpE*, Rv0678, *pepQ* (associated with bedaquiline resistance) [40,41], or *fbiD* (associated with pretonamid resistance) [42].

3.2. MICs of drugs against M. tuberculosis *H37Rv and clinical isolate*

The meropenem MIC for *M. tuberculosis* H37Rv alone was 16 mg/L, which changed to 4 mg/L in combination with avibactam and 2 mg/L in combination with either relebactam or vaborbactam. The MICs of other drugs against *M. tuberculosis* H37Rv were as follows: isoniazid 0.064 mg/L, rifampin 0.016 mg/L, moxifloxacin 0.016 mg/L, linezolid 0.5 mg/L, tedizolid 0.25 mg/L, bedaquiline 0.03 mg/L, and pretomanid 0.06 mg/L. The MIC of meropenem-vaborbactam against the MDR-TB clinical strain *M. tuberculosis* 16D was > 128 mg/L. Other drug MICs were as follows: isoniazid > 1mg/L, rifampin 32 mg/L, moxifloxacin 0.125 mg/L, linezolid 0.5 mg/L, tedizolid 0.25 mg/L, bedaquiline 0.25 mg/L, and pretomanid 0.125 mg/L.

3.3. Meropenem concentration versus effect studies

Supplementary Figure 1 shows the bacterial kill with different meropenem concentrations alone or in combination with the three

Table 2
Effect of β-lactamase inhibitors on efficacy and potency of meropenem against *Mycobacterium tuberculosis*.

Meropenem plus	No BLI	Avibactam	Relebactam	Vaborbactam
E_{con} (log_{10} cfu/mL)	6.75 (6.60–7.00)	6.80 (6.57–7.04)	6.68 (6.60–7.13)	5.26 (5.03–5.50).
E_{max} (log_{10} cfu/mL)	2.48 (1.81–4.07)	6.09 (5.28– 7.42)	6.65 (5.82–7.34)	4.00 (3.49–4.52)
p-value meropenem alone vs BLI: E_{max}	–	**0.029**	**0.039**	0.052
IC_{50} (mg/L)	14.25 (6.13–40.14)	9.44 (6.20–13.79)	1.52 (0.96–2.0)	3.75 (2.37–6.59)
p-value meropenem alone vs BLI: IC_{50}	–	0.486	**0.018**	**0.008**
r^2	0.85	0.95	0.91	0.97

BLI, β-lactamase inhibitor

BLIs (avibactam, relebactam, and vaborbactam), based on Eq. 1. In the first or initial analyses, the 95% CI for H all crossed 1.0, which meant [1] that they were similar for meropenem alone and meropenem plus BLI, and thus the relevant binding sites for microbial kill were those of meropenem and not BLI [2]. Since the Hill coefficient was 1.0, it is proof of meropenem (ligand) binding to a single target ("receptor"); therefore, for comparisons of E_{max} and IC_{50} and estimates of EC_{50}, E_{max}, and E_{con}, H was fixed at 1.0 and the null hypothesis that E_{max} and IC_{50} were the same for all tested datasets. Results are shown in Table 2. First, each of the three BLIs improved the efficacy of meropenem. However, only relebactam (9.38-fold) and vaborbactam (3.80-fold) improved the potency (IC_{50}) of meropenem against the drug-susceptible *M. tuberculosis* H37Rv. Thus, relebactam and vaborbactam can potentially improve microbial kill while reducing the amount of meropenem required several-fold because of improvement in IC_{50}. Since meropenem-vaborbactam are commercially available, this drug combination was chosen in subsequent studies.

3.4. Meropenem-vaborbactam plus different drug combinations in static time-kill studies

The results of bacterial kill below day 0 or stasis (i.e. log_{10} cfu/mL on day 0 minus log_{10} cfu/mL on day 7) for V in combination with several anti-TB drugs against the drug-susceptible *M. tuberculosis* H37Rv are shown in Figure 1A. The results are arranged by *M. tuberculosis* log_{10} cfu/mL, with the least effective on the left and the most effective on the right. V killed 1.37 log_{10} cfu/mL below stasis, consistent with the good MIC. The most effective combinations were V-rifampin, V-rifampin-isoniazid, and V-moxifloxacin, which reduced the *M. tuberculosis* below limits of quantitation (> 5.77 ± 0.13 log_{10} cfu/mL below stasis).

Figure 1B shows the results of the same combinations in the MDR-TB strain, *M. tuberculosis* 16D. The results are arranged by *M. tuberculosis* log_{10} cfu/mL below stasis, with the least effective on the left and the most effective on the right. V on its own had no effect, and microbial kill equalled the non-treated controls; thus, bacteria grew above day 0 (negative kill). V-pretomanid was similar to the non-treated controls, despite the good MIC of pretomanid. The addition of rifampin to V resulted in the combination holding *M. tuberculosis* at stasis, thus reversing both rifampin and V resistance. V-bedaquiline resulted in < 1 log_{10} cfu/mL kill, which was statistically similar to V-rifampin. V-INH demonstrated remarkable microbial kill in this isoniazid-resistant strain. In common with the *M. tuberculosis* H37Rv (Figure 1A), the most effective combination for the MDR-TB strain was V-moxifloxacin, which killed 3.45 ± 0.88 log_{10} cfu/mL, suggesting that this would be a good regimen to test vs. the BPaL regimen used in MDR-TB, as was performed in the HFS-TB.

3.5. Hollow fibre model system of TB experiments

Figure 2 represents the concentration-time profile of the drugs achieved in the HFS-TB. PK modelling revealed the elimination rate

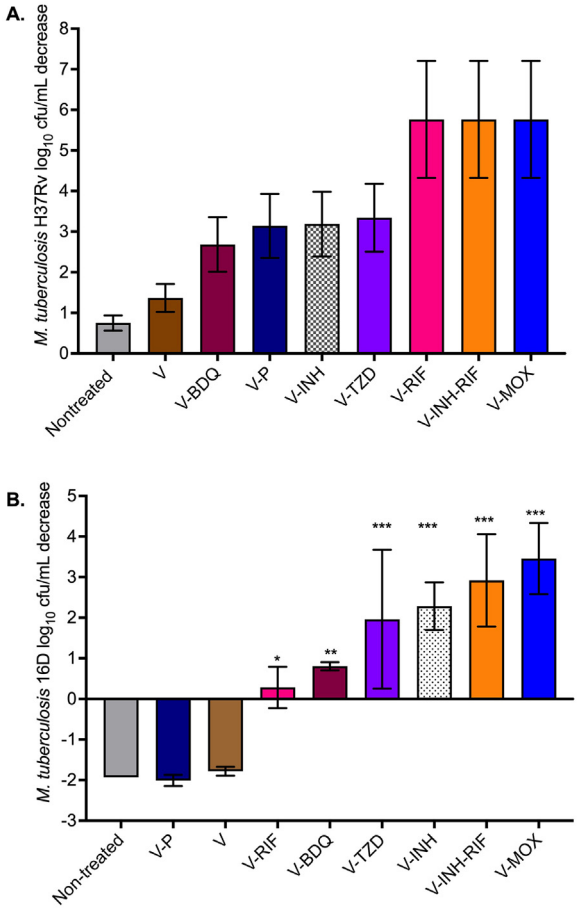

Figure 1. *Mycobacterium tuberculosis* kill below stasis with meropenem-vaborbactam alone or in combination with other anti-tuberculosis drugs in static concentration time-kill studies. **(A)** *Mycobacterium tuberculosis* H37Rv, V-RIF, V-INH-RIF, and V-MOX, mean and standard deviation estimates look the same because they reduced *M. tuberculosis* burden below the assay limits. The extent of killing with INH, TZD, BDQ, and P was not different from each other. **(B)** For the MDR-TB *Mycobacterium tuberculosis* 16D strain, V alone had no effect, but the best combinations were for V-MOX. V-INH-RIF effect was statistically equal to V-MOX. Abbreviations: V, meropenem-vaborbactam; INH, isoniazid; RIF, rifampin; MOX, moxifloxacin; TZD, tedizolid; BDQ, bedaquiline; P, pretonamid.
$^{*}P < 0.05$, $^{**}P < 0.01$, $P < 0.001$, and $^{***}P < 0.0001$ compared with non-treated controls. Error bars are standard deviations.

constant (Ke), half-life ($t_{1/2}$), and 0-24 hour AUC (AUC_{0-24}) for the drugs used in different combination regimens to treat MDR-TB in the HFS-TB model are shown in Table 3. Since the circulating medium in the HFS-TB did not contain any protein, the drug concentrations reported here represent the free fraction of the drugs.

Figure 3A shows the results of the HFS-TB study, with MDR-TB clinical strain, using the TTP readouts (the higher the TTP, the lower the bacterial burden). As shown in Figure 3A, both high-dose rifampin combinations with V were able to kill the

Table 3
Pharmacokinetic parameters, as calculated by noncompartmental analysis, in the hollow fibre model system of TB.

Drug	T_{max} (hr)	C_{max} (mg/L)	AUC_{0-24} ($mg^{*}hrL^{-1}$)	Ke (L/hr)	V (L)	$t_{1/2}$ (hr)
Meropenem	3.8	10.983 ± 2.683	111.083 ± 30.221	0.337 ± 0.083	1.263 ± 0.307	3.180 ± 0.153
Vaborbactam	3.4	68.80 ± 10.91	715.22 ± 197.22	0.05 ± 0.01	0.29 ± 0.02	4.36 ± 1.31
Rifampin	1	1.652 and 5.162	12.536 and 39.176	0.068 ± 0.034	0.696 ± 0.024	4.749 ± 0.345
Moxifloxacin	1	3.188 ± 0.089	51.652 ± 3.866	0.012 ± 0.002	0.408 ± 0.010	19.657 ± 2.606
Linezolid	1	9.832 ± 1.829	115.083 ± 30.221	0.041 ± 0.015	0.0541 ± 0.100	9.893 ± 0.153

Abbreviations: Ke, elimination rate constant; $t_{1/2}$, half-life; AUC_{0-24}, 0-24 hour AUC; V, meropenem-vaborbactam

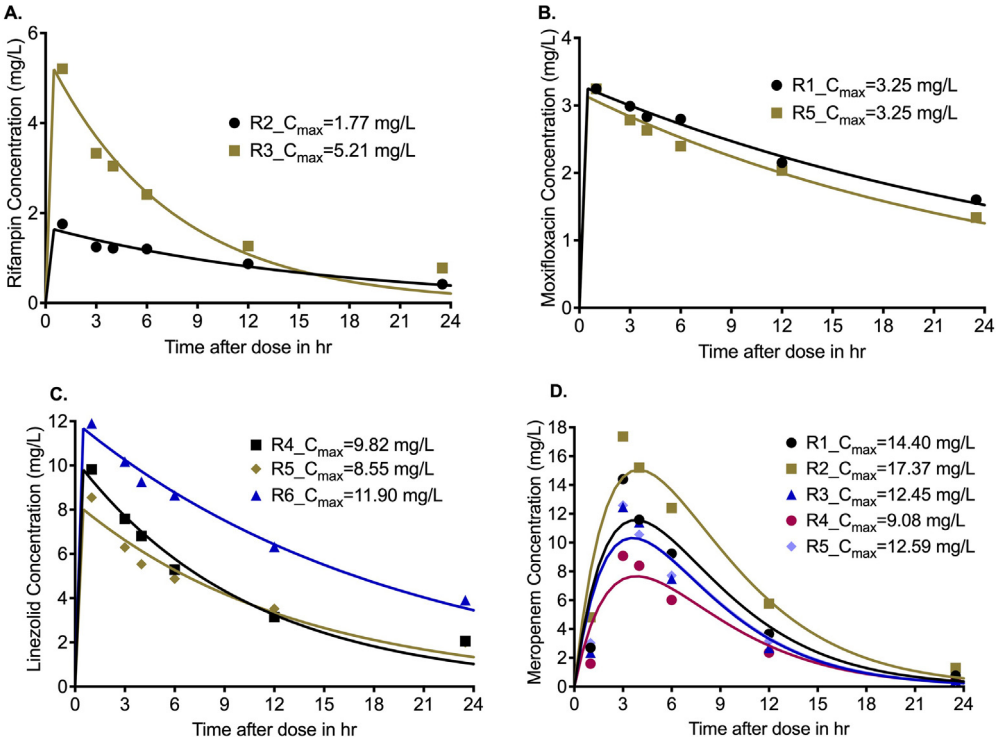

Figure 2. Concentration-time profiles of drugs achieved in the hollow fibre model system of TB. (A) Rifampin, (B) Moxifloxacin, (C) Linezolid, (D) Meropenem. R1–R6 represents different drug combination regimens.

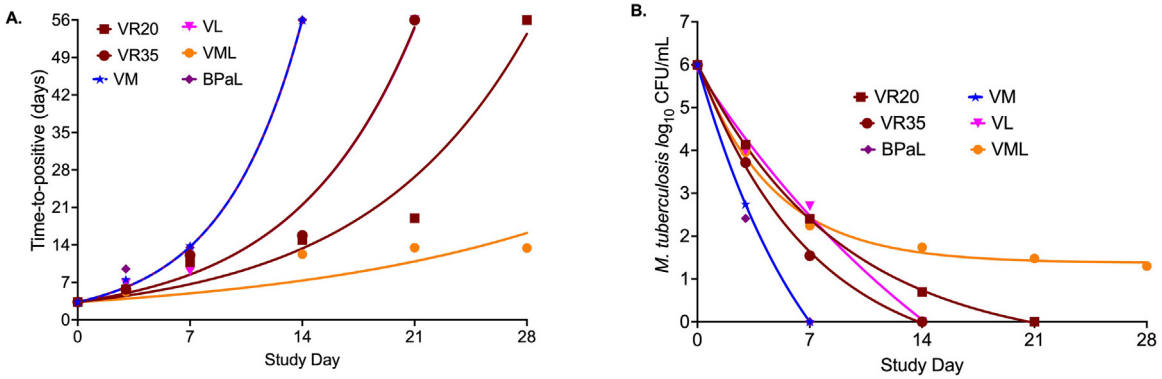

Figure 3. Meropenem-vaborbactam alone or in combination with other anti-tuberculosis drugs against multidrug-resistant tuberculosis clinical strain in the hollow fibre model system of TB. **(A)** Changes in the TTP with different treatment regimens. The higher the TTP, the lower the bacterial burden. Both VR combinations killed multidrug-resistant tuberculosis, the VM combination showed similar efficacy as with the BPaL regimen, and the VM combination was as effective as the high-dose rifampin combination. However, when linezolid and moxifloxacin were used together, VML failed to sterilise the systems. **(B)** Time-kill curves with each V combination and the BPaL regimen using the cfu/mL readouts. Similar to the TTP results, except VML combination, all other regimens sterilised the HFS-TB units. However, the time to sterilisation differed between the regimens.
Abbreviations: V, meropenem-vaborbactam; R, rifampin; M, moxifloxacin; L, linezolid; B, bedaquiline; P, pretonamid.

MDR-TB. However, the TTP was 14 days with the 35 mg/kg rifampin combination compared with 21 days with the 20 mg/kg combination. The VM combination was as effective as the high-dose rifampin combination. However, when linezolid and moxifloxacin were combined, VML failed to sterilise the systems, indicating antagonism at the tested dose combination. The TTP with the VM combination and BPaL regimen was virtually the same. Figure 3B is the same data but using log_{10} cfu/mL readout. First, non-treated controls in this *M. tuberculosis* MDR-TB strain initially demonstrated a decline in bacterial burden, likely because it took time for this strain (not used in HFS-TB till now) to adapt to the HFS-TB environment. Second, V combinations were active

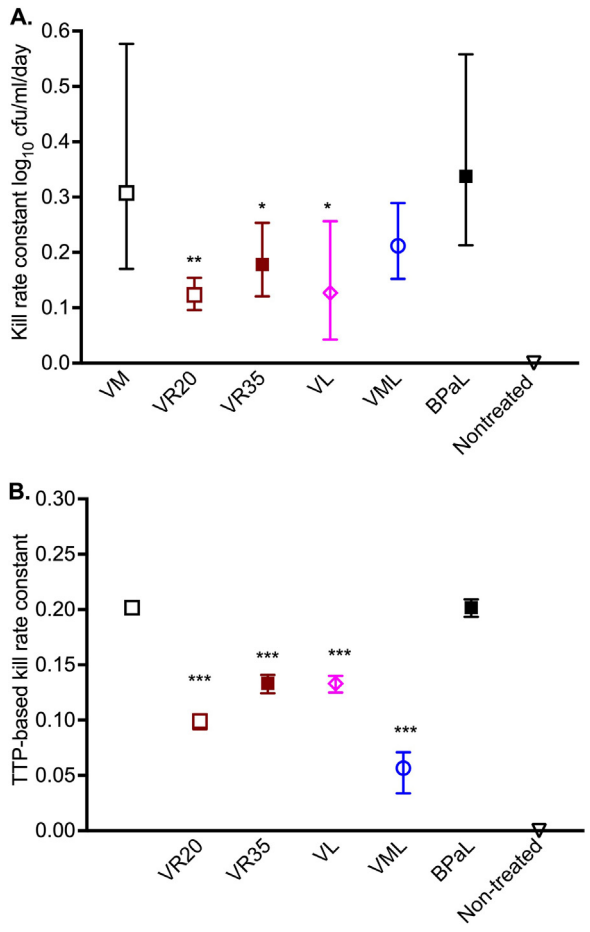

Figure 4. Exponential decline models for combination regimens in hollow fibre model system of TB. **(A)** Based on $\log_{10}$ CFU/mL/day, VM had similar kill rates to BPaL. **(B)** Based on TTP, VML had a lower kill rate constant than either VM or VL, demonstrating antagonism.
Abbreviations: V, meropenem-vaborbactam; R, rifampin; M, moxifloxacin; L, linezolid; B, bedaquiline; P, pretonamid
Error bars are 95% confidence intervals of the kill slopes.

despite the 128 mg/L V MIC. Third, all treatment regimens (except V-moxifloxacin-linezolid) eventually sterilised the HFS-TB by day 28.

When microbial kill was analysed using exponential decline models of bacterial burden vs. time, the kill rate constants were identified as shown in Figure 4. Figure 4A shows the kill rate constants based on cfu/mL readout; the r^2 for all regressions was $>$ 0.98 in all regimens. The null hypothesis that K was the same for V-moxifloxacin vs. the rest of the regimens was rejected for V-rifampin (both) and V-linezolid but not for V-moxifloxacin-linezolid ($P = 0.171$) and BPaL ($P = 0.699$), based on the $\log_{10}$ cfu/mL readout. Figure 4B is based on time-to-positive based kill rates; r^2 for all regressions ranged from 0.95 to $>$ 0.99 in all regimens. Figure 4B shows that the null hypothesis that K was the same for V-moxifloxacin vs. the rest of the regimens was rejected ($P < 0.0001$), except with BPaL ($P = 0.9947$). In addition, a comparison of K for V-linezolid vs. V-moxifloxacin-linezolid revealed that K was lower with V-moxifloxacin-linezolid ($P < 0.0001$). Overall, the best kill rates were encountered with the V-moxifloxacin combination, which was statistically equal to the BPaL regimen by both readouts. The V-rifampin combinations (both 20 mg/kg and 35 mg/kg) had better kill rates than non-treated controls ($P < 0.0001$) for both readouts, although this was lower than V-moxifloxacin and BPaL combinations. However, this is still consistent with reversing rifampin-resistance in this MDR-TB strain. Linezolid demonstrated antagonism to the V-moxifloxacin-linezolid regimen, in the HFS-TB.

4. Discussion

This study showed that similar to the prior observations of carbapenem efficacy against *M. tuberculosis* in the combination of the BLI clavulanic acid, meropenem alone killed drug-susceptible *M. tuberculosis*, but potency (IC_{50}) was enhanced by the BLIs relebactam and vaborbactam, at the same time improving E_{max} (potency), which resulted in better efficacy [25]. The finding that the Hill slope was 1.0 gave partial insight into how BLIs work, in this case, reducing hydrolysis of the active compound meropenem, and the efficacy likely reflects a single binding site/protein that is inhibited by meropenem. It is unclear why E_{max} improved, since the number of these binding sites was not increased and saturable [25,43]. Conversely, the V MIC against the MDR-TB strain compared with the drug-susceptible laboratory strain was multiple-fold higher; however, there was no BlaC mutation on WGS. In any case, V microbial efficacy was restored by other drugs such as rifampin (in the presence of *rpoB* Ser450Leu mutation) and isoniazid (in the presence of a *katG* Ser315Thr mutation) in static time-kill studies, suggesting that the high V MIC was not due to mutations in the meropenem target. Thus, if MIC is the sole determinant of the drug's efficacy and a decision for clinical use, the meropenem-vaborbactam combination may not be included in the MDR-TB treatment regimens.

In the STK test tube experiments, V restored rifampin and isoniazid activity in a highly resistant strain (with multiple mutations in *katG* and *rpoB* genes) in the MDR-TB strain; this is exciting but the mechanistic basis is still unclear. In the HFS-TB, the rifampin effect was enhanced in a dose-dependent fashion (hold the dose of V constant); this observation will require further testing in the HFS-TB and animal models to determine the potential clinical utility. Given the limited options of effective drugs against MDR-TB, reversing rifampin resistance and using this time-honoured drug could be helpful. The findings of reversing resistance are also important for rifampin dosing. While clinical studies support the efficacy of higher rifampin doses and being safe, there is still some reservation on increased doses [44–46]. On the other hand, the addition of pretomanid to V in the susceptible drug strain was moderate, but in MDR-TB resulted in an effect indistinguishable from non-treated controls. In contrast, the addition of bedaquiline to V had a moderate effect in both the drug-susceptible *M. tuberculosis* strain and MDR-TB strain. Thus, these drugs are not ideal companions for V in combination.

The STK studies overestimate the efficacy of a drug because the concentration does not change over time. In people, the drug concentrations decline with a half-life of ca. 2.5 hours for meropenem and vaborbactam, and around the same for isoniazid and rifampin. That means that over 24 hours, the total exposures (e.g. measured as AUC) are multiple folds in patients, and drugs stay 100% above the MIC, which is why a combination of preclinical models and methodologies are used [12,14,19–23,31,34,35,47–54]. One strength of the HFS-TB model is to multiplex different drug PKs in a combination to simulate in vivo kinetics on lung lesions. In its qualification opinion, the EMA recommended that the HFS-TB be used for five purposes, including: "to provide preliminary proof of concept for developing a specific drug or combination to treat tuberculosis" and "to provide data to support PK/PD analyses leading to initial dose selection for non-clinical and clinical studies" [9,10]. The combination of V with several other drugs—such as moxifloxacin, pretomanid, bedaquiline, and linezolid—were compared with BPaL, using PKs of these drugs encountered in human lung lesions, and in the case of rifampin using two different

doses. Consistent with STK experiments, the V-moxifloxacin regimen was the most effective and equalled the standard of care for MDR-TB of BPaL. Linezolid antagonised V and V-moxifloxacin when combined at 600 mg dose. The V-rifampin data suggest that the meropenem-rifampin antagonism noted in EBA studies could be overcome using higher doses. However, the BLI used in that study was clavulanate, not vaborbactam, and thus not directly comparable.

This study had several limitations. First, all models were *in vitro*, and some *in vivo* support of these observations will be required. Second, more *M. tuberculosis* strains than tested here will be needed to better generalise these findings. Third, while meropenem-vaborbactam is reported as a promising agent to potentially restore the efficacy of isoniazid and rifampin against MDR-TB, the drug in its current formulation can only be administered intravenously; therefore, utility outside ambulatory care may be limited. On the other hand, the meropenem-vaborbactam was administered once a day, which may make administration more amenable compared with the three times a day schedule. The fourth limitation was a lower than intended C_{max} of meropenem in the HFS-TB. One possible explanation could be that the drug syringes were changed every 72 hours. An earlier study by Watt et al. [55], while developing a meropenem dosing schedule for the treatment of TB, described that meropenem was unstable at 37 °C, where a loss of 50% potency was reported after 24 hours followed by 25% every additional day for the drug in solution. Similarly, loss of potency for ertapenem, another carbapenem, was also reported elsewhere [31]. Therefore, in the HFS-TB study where the drug syringes were changed every 72 hours with fresh drug, the instability may result in lower than expected V efficacy than if the drug was prepared each day.

In summary, meropenem-vaborbactam restores the efficacy of first-line drugs against MDR-TB. These findings suggest that meropenem-vaborbactam in combination with moxifloxacin could be a potential candidate as a backbone to create a pan-TB regimen.

Acknowledgements

We thank Paula Ashcraft, Center of Metabolomics, Institute of Metabolic Disease, Baylor Scott & White Research Institute, Dallas, Texas, USA, for help with LC-MS/MS analysis.

Competing Interests: Tawanda Gumbo founded and is president and CEO of Praedicare Inc., a pre-clinical and translational contract research organisation, and founded Praedicare Africa Pvt. Ltd., a clinical contract research organisation. All other authors have nothing to declare.

Funding source: This work was supported by 1R01HD099756 grant from Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the University of Texas System STARS award (250439/39411), and funding from the Department of Pulmonary Immunology (423500/14000), UT Health Science Center at Tyler, Texas, USA to Shashikant Srivastava.

Ethical approval: Not applicable.

Data availability statement: Upon a reasonable request, the raw data for the results presented in the manuscript are available from the corresponding author.

Author contributions: All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work, and have given their approval for this version to be published. Conceptualisation and design, Shashikant Srivastava and JWA; MIC and HFS-TB experiments, Sanjay Singh, GDB, Shashikant Srivastava; PK/PD modelling and data analysis, SS and TG; Clinical isolate collection, LM; DNA sequencing and genomic data analysis, PR, Shashikant Srivastava; Clinically relevant comments and edits, TT and KD. Shashikant Srivastava wrote the first draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2023.106968.

References

[1] Migliori GB, Thong PM, Alffenaar JW, Denholm J, Tadolini M, Alyaquobi F, et al. Gauging the impact of the COVID-19 pandemic on tuberculosis services: a global study. Eur Respir J 2021;58.

[2] Pai M, Kasaeva T, Swaminathan S. Covid-19's devastating effect on tuberculosis care - a path to recovery. N Engl J Med 2022;386:1490–3.

[3] WHO Global Tuberculosis Report 2021. Geneva: World Health Organization; 2021.

[4] Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. Antimicrob Agents Chemother 2009;53:3720–5.

[5] McLeay SC, Vis P, van Heeswijk RP, Green B. Population pharmacokinetics of bedaquiline (TMC207), a novel antituberculosis drug. Antimicrob Agents Chemother 2014;58:5315–24.

[6] No_Author_Listed. SIVEXTRO (tedizolid phosphate). 2014.

[7] Sotgiu G, Centis R, D'Ambrosio L, Spanevello A, Migliori GB. Linezolid to treat extensively drug-resistant TB: retrospective data are confirmed by experimental evidence. Eur Respir J 2013;42:288–90.

[8] Diacon AH, Dawson R, Hanekom M, Narunsky K, Venter A, Hittel N, et al. Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. Int J Tuberc Lung Dis 2011;15:949–54.

[9] Alffenaar JC, Akkerman OW, Kim HY, Tiberi S, Migliori GB. Precision and Personalized Medicine and anti–TB treatment: Is TDM feasible for programmatic use? Int J Tuberc Lung Dis 2020 S1201-9712(20):30043–6.

[10] European Medicines Agencies. Qualification opinion on *in vitro* hollow fibre system model of tuberculosis (HFS-TB). 2015.

[11] Gumbo T, Chapagain M, Magombedze G, Srivastava S, Deshpande D, Pasipanodya JG, et al. Novel tuberculosis combination regimens of two and three–months therapy duration. bioRxiv 2022 2022.03.13.484155.

[12] Srivastava S, Magombedze G, Koeuth T, Sherman C, Pasipanodya JG, Raj P, et al. Linezolid dose that maximizes sterilizing effect while minimizing toxicity and resistance emergence for tuberculosis. Antimicrob Agents Chemother 2017;61 pii: AAC.00751–17.

[13] Deshpande D, Srivastava S, Pasipanodya JG, Bush SJ, Nuermberger E, Swaminathan S, et al. Linezolid for infants and toddlers with disseminated tuberculosis: first steps. Clin Infect Dis 2016;63:S80–SS7.

[14] Srivastava S, Deshpande D, Pasipanodya J, Nuermberger E, Swaminathan S, Gumbo T. Optimal clinical doses of faropenem, linezolid, and moxifloxacin in children with disseminated tuberculosis: Goldilocks. Clin Infect Dis 2016;63:S102–S1S9.

[15] Conradie F, Bagdasaryan TR, Borisov S, Howell P, Mikiashvili L, Ngubane N, et al. Bedaquiline-pretomanid-linezolid regimens for drug-resistant tuberculosis. N Engl J Med 2022;387:810–23.

[16] Gumbo T, Pasipanodya JG, Nuermberger E, Romero K, Hanna D. Correlations between the hollow fiber model of tuberculosis and therapeutic events in tuberculosis patients: learn and confirm. Clin Infect Dis 2015;61(Suppl 1):S18–24.

[17] Pasipanodya JG, Nuermberger E, Romero K, Hanna D, Gumbo T. Systematic analysis of hollow fiber model of tuberculosis experiments. Clin Infect Dis 2015;61(Suppl 1):S10–17.

[18] Gumbo T, Pasipanodya JG, Romero K, Hanna D, Nuermberger E. Forecasting accuracy of the hollow fiber model of tuberculosis for clinical therapeutic outcomes. Clin Infect Dis 2015;61(Suppl 1):S25–31.

[19] Deshpande D, Srivastava S, Bendet P, Martin KR, Cirrincione KN, Lee PS, et al. Antibacterial and sterilizing effect of benzylpenicillin in tuberculosis. Antimicrob Agents Chemother 2018;62:e02232–e02e46.

[20] Srivastava S, van Zyl J, Cirrincione K, Martin K, Thomas T, Deshpande D, et al. Evaluation of ceftriaxone plus avibactam in an intracellular hollow fiber model of tuberculosis: Implications for the treatment of disseminated and meningeal tuberculosis in children. Pediatr Infect Dis J 2020;39:1092–100.

[21] Srivastava S, Thomas T, Howe D, Malinga L, Raj P, Alffenaar JW, et al. Cefdinir and beta-lactamase Inhibitor Independent efficacy against *Mycobacterium tuberculosis*. Front Pharmacol 2021;12:677005.

[22] Srivastava S, Gumbo T, Thomas T. Repurposing cefazolin-avibactam for the treatment of drug resistant *Mycobacterium tuberculosis*. Front Pharmacol 2021;12:776969.

[23] Deshpande D, Srivastava S, Chapagain M, Magombedze G, Martin KR, Cirrincione KN, et al. Ceftazidime-avibactam has potent sterilizing activity against highly drug-resistant tuberculosis. Sci Adv 2017;3:e1701102.

[24] Wang F, Cassidy C, Sacchettini JC. Crystal structure and activity studies of the *Mycobacterium tuberculosis* beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. Antimicrob Agents Chemother 2006;50:2762–71.

[25] van Rijn SP, Zuur MA, Anthony R, Wilffert B, van Altena R, Akkerman OW, et al. Evaluation of carbapenems for treatment of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2019;63.

[26] De Jager V, Gupte N, Nunes S, Barnes GL, van Wijk RC, Mostert J, et al. Early Bactericidal cctivity of meropenem plus clavulanate (with or without rifampin) for tuberculosis: The COMRADE randomized, phase 2A clinical trial. Am J Respir Crit Care Med 2022;205:1228–35.

[27] Kurz SG, Bonomo RA. Reappraising the use of β-lactams to treat tuberculosis. Expert Rev Anti Infect Ther 2012;10:999–1006.

[28] No_Author_Listed. VABOMERE^{TM} (meropenem and vaborbactam) for injection, for intravenous use. U.S.A. Food and Drug Administration; 2017.

[29] Srivastava S, Garg A, Ayyagari A, Nyati KK, Dhole TN, Dwivedi SK. Nucleotide polymorphism associated with ethambutol resistance in clinical isolates of *Mycobacterium tuberculosis*. Curr Microbiol 2006;53:401–5.

[30] CLSI. Susceptibility testing of mycobacteria, nocardia spp., and other aerobic actinomycetesCLSI Standard M24. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

[31] van Rijn SP, Srivastava S, Wessels MA, van Soolingen D, Alffenaar JC, Gumbo T. Sterilizing effect of ertapenem-clavulanate in a hollow-fiber model of tuberculosis and implications on clinical dosing. Antimicrob Agents Chemother 2017;61 e02039–16.

[32] WHO Rapid communication: Key changes to the treatment of drug-resistant tuberculosis (WHO/UCN/TB/2022.2). Geneva: World Health Organization; 2022.

[33] WHO Rapid communication: Key changes to the treatment of drug-resistant tuberculosis. Geneva: World Health Organizaton; 2022.

[34] Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis 2011;204:1951–9.

[35] Srivastava S, Deshpande D, Magombedze G, Gumbo T. Efficacy versus hepatotoxicity of high-dose rifampin, pyrazinamide, and moxifloxacin to shorten tuberculosis therapy duration: there is still fight in the old warriors yet!. Clin Infect Dis 2018;67:S359–SS64.

[36] Gumbo T, Sherman CM, Deshpande D, Alffenaar JW, Srivastava S. *Mycobacterium tuberculosis* sterilizing activity of faropenem, pyrazinamide and linezolid combination and failure to shorten the therapy duration. Int J Infect Dis 2021;104:680–4.

[37] Phoenix® WinNonlin® version 8.1 No_author_listed. Princeton, NJ: Certara USA, Inc; 2020.

[38] Gumbo T, Angulo-Barturen I, Ferrer-Bazaga S. Pharmacokinetic-pharmacodynamic and dose-response relationships of antituberculosis drugs: recommendations and standards for industry and academia. J Infect Dis 2015;211(Suppl 3):S96–S106.

[39] Prinz H. Hill coefficients, dose-response curves and allosteric mechanisms. J Chem Biol 2010;3:37–44.

[40] Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med 2017 S2213-2600:30079-6.

[41] Almeida D, Ioerger T, Tyagi S, Li SY, Mdluli K, Andries K, et al. Mutations in pepQ confer low-level resistance to bedaquiline and clofazimine in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2016;60:4590–9.

[42] Rifat D, Li SY, Ioerger T, Shah K, Lanoix JP, Lee J, et al. Mutations in fbiD (Rv2983) as a novel determinant of resistance to pretomanid and delamanid in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2020;65.

[43] Gumbo T, Alffenaar JC. Pharmacokinetic/pharmacodynamic background and methods and scientific evidence base for dosing of second-line tuberculosis drugs. Clin Infect Dis 2018;67:S267–SS73.

[44] Velasquez GE, Brooks MB, Coit JM, Pertinez H, Vargas Vasquez D, Sanchez Garavito E, et al. Efficacy and safety of high-dose rifampin in pulmonary tuberculosis. A randomized controlled trial. Am J Respir Crit Care Med 2018;198:657–66.

[45] Boeree MJ, Diacon AH, Dawson R, Narunsky K, du Bois J, Venter A, et al. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med 2015;191:1058–65.

[46] van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, et al. Why Do We Use 600 mg of Rifampicin in Tuberculosis Treatment? Clin Infect Dis 2011;52:e194–9.

[47] Srivastava S, Musuka S, Sherman C, Meek C, Leff R, Gumbo T. Efflux-pump-derived multiple drug resistance to ethambutol monotherapy in *Mycobacterium tuberculosis* and the pharmacokinetics and pharmacodynamics of ethambutol. J Infect Dis 2010;201:1225–31.

[48] Srivastava S, Sherman C, Meek C, Leff R, Gumbo T. Pharmacokinetic mismatch does not lead to emergence of isoniazid- or rifampin-resistant *Mycobacterium tuberculosis* but to better antimicrobial effect: a new paradigm for antituberculosis drug scheduling. Antimicrob Agents Chemother 2011;55:5085–9.

[49] Musuka S, Srivastava S, Siyambalapitiyage Dona CW, Meek C, Leff R, Pasipanodya J, et al. Thioridazine pharmacokinetic-pharmacodynamic parameters “wobble” during treatment of tuberculosis: a theoretical basis for shorter-duration curative monotherapy with congeners. Antimicrob Agents Chemother 2013;57:5870–7.

[50] Srivastava S, Deshpande D, Pasipanodya J, Swaminathan S, Gumbo T. Drug concentration thresholds predictive of outcome in children with tuberculosis: not your parents' target concentrations. Clin Infect Dis 2016.

[51] Srivastava S, Deshpande D, Pasipanodya JG, Thomas T, Swaminathan S, Nuermberger E, et al. A combination regimen design program based on pharmacodynamic target setting for childhood tuberculosis: Design rules for the playground. Clin Infect Dis 2016;63:S75–SS9.

[52] Srivastava S, Modongo C, Siyambalapitiyage Dona CW, Pasipanodya JG, Deshpande D, Gumbo T. Amikacin optimal exposure targets in the hollow fiber system model of tuberculosis. Antimicrob Agents Chemother 2016;60:5922–7.

[53] Srivastava S, Pasipanodya JG, Ramachandran G, Deshpande D, Shuford S, Crosswell HE, et al. A long-term co-perfused disseminated tuberculosis-3D liver hollow fiber model for both drug efficacy and hepatotoxicity in babies. EBioMedicine 2016;6:126–38.

[54] Srivastava S, Deshpande D, Nuermberger E, Lee PS, Cirrincione K, Dheda K, et al. The sterilizing effect of intermittent tedizolid for pulmonary tuberculosis. Clin Infect Dis 2018;67:S336–SS41.

[55] Watt B, Edwards JR, Rayner A, Grindey AJ, Harris G. *in vitro* activity of meropenem and imipenem against mycobacteria: development of a daily antibiotic dosing schedule. Tuber Lung Dis 1992;73:134–6.