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

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SUPPLEMENTARY MATERIALS & METHODS

Bacteria, drugs, and other supplies

Before each experiment, cultures were grown in Middlebrook 7H9 broth supplemented with 10% oleic acid, albumin, dextrose, and catalase (OADC) at 37ºC under 5% CO_2 and shaking conditions. Meropenem, avibactam, relebactam, isoniazid, rifampin, linezolid, tedizolid, pretonamid, and bedaquiline were synthesized by the BOC Sciences (Shirley, New York). Moxifloxacin and meropenem-vaborbactam combination were purchased from the UT Health Science Center at Tyler campus pharmacy. Cellulosic hollow fiber cartridges were purchased from FiberCell (Fredrick, Maryland). Mycobacterial Growth Indicator Tube (MGIT) system and supplies and EpiCenter software to record the time-to-positive (TTP) were purchased from Becton, Dickinson and Company (NJ, USA). For the drug concentration measurement, analytical and internal standards were purchased from Cayman Chemical (Ann Arbor, MI, USA) and Toronto Research Chemicals (Toronto, Canada), respectively.

Whole genome sequencing of MDR-TB strain

DNA was extracted using the CTAB/NaCl method described previously[1] followed by sequencing library preparation using the KAPA Biosystem Hyper kit (KK8504). After size selection, adapter-ligated genomic libraries were amplified using four PCR cycles, cleaned using XP beads, and nine pM of each library was used for sequencing on Hiseq 2500 PE100 (paired-end 100bp) lane. SAMtools (http://samtools.sourceforge.net/) was used to sort the sequencing reads, followed by steps to remove adapter artifacts CLC Genomics workbench (v9.5.2) was used to determine the read quality, nucleotide content, and sequence redundancy. Finally, reads were aligned to the reference *Mtb* genome (NC_000962), and single nucleotide variants (SNVs) were compared to the wild type.[2]

MICs

First, the meropenem MIC of the standard laboratory strain *M. tuberculosis* H37Rv was determined using the broth micro-dilution method.[3] Briefly, the turbidity of the log-phase growth cultures was adjusted to McFarland (McF) 0.5 standard followed by 100-fold dilution to get ~10^5 CFU/mL bacterial burden in the inoculum. Next, 180 μL of the inoculum was added to each of the wells containing meropenem (final concentration ranging from 0.125mg/L to 256mg/L in a two-fold serial dilution). The plates were then sealed in a zip-lock bag and incubated at 37^0C. Visual inspections were performed starting day 14, and the drug concentration with no visible bacterial pellet when the nontreated control wells showed growth was recorded as the MIC.

Second, the MIC of meropenem-vaborbactam combination as well as isoniazid, rifampin, moxifloxacin, linezolid, tedizolid, bedaquiline, and pretonamid for *Mtb* H37Rv and clinical strain, SAMRC-16D, was determined using the Mycobacterial Growth Indicator Tube (MGIT) system.[2] Inoculum was prepared by adjusting the turbidity of the log-phase growth cultures to McF 0.5 standard followed by 100-fold dilution to get ~10^5 CFU/mL bacterial burden in the inoculum. Next, 500μL of the inoculum was added to each MGIT tube prefilled with Middlebrook 7H9 media supplemented with 10% OADC and different concentrations of each drug. The MGIT tubes were incubated at 37^0C, and growth units (GU) were recorded hourly using EpiCenter software. Any MGIT tube with GU>70 was flagged culture positive. The MGIT tubes with the lowest drug concentration that remained negative 48hr after the nontreated control (A) flagged positive was determined as the MIC. One additional growth control (B) was used, where the inoculum was further 100-fold diluted to ~10^3 CFU/mL bacterial burden. The drug-containing MGIT tube that was still negative on the day the control B flagged positive was recorded as the MIC. Each experiment was performed twice, with two replicates for each drug concentration.

Drug concentrations used in the static time-kill studies

The drugs and the concentrations achieved with the human equivalent standard clinical dose of each drug in the blood, except rifampin, where the concentration in the lung lesion, are listed below in **Supplementary Table 1**.

Supplementary Table 1. Meropenem-vaborbactam efficacy alone or in combination against drug-susceptible and MDR-TB.

Drugs and human equivalent dose	Drug concentration (mg/L)
Meropenem+Vaborbactam	46
Meropenem+Vaborbactam plus Isoniazid (300mg)	46,6
Meropenem+Vaborbactam plus Rifampin (600mg)	46, 2 (ELF)
Meropenem+Vaborbactam plus Isoniazid plus Rifampin	46, 6, 2 (ELF)
Meropenem+Vaborbactam plus Moxifloxacin (400mg)	46, 4.2
Meropenem+Vaborbactam plus Tedizolid (200mg)	46, 1.77
Meropenem+Vaborbactam plus Bedaquiline (100mg)	46, 1.2
Meropenem+Vaborbactam plus Pretonamid (200mg)	46, 1.1

EFL, epithelial lining fluid

HFS sampling and processing of samples measurements of bacterial burden.

The systems were set to mimic 2hr half-life of meropenem, 3hr for isoniazid and rifampin, and 12hr, 30hr, 18hr, and 8hr half-life for moxifloxacin, bedaquiline, pretonamid, and linezolid, respectively.[4-9] The circulating medium was Middlebrook 7H9 broth supplemented with 2% dextrose at pH 6.8. The drugs were infused using a programable syringe pump with the time to reach the maximum concentration of 1hr for rifampin, moxifloxacin, and linezolid, 3hr for meropenem-vaborbactam, and 4hr for bedaquiline and pretonamid. Since the drugs in the

combination regimens had different half-lives, the dilution rate was set to achieve the short half-life, and the drug with the longer half-life was supplemented using the syringe pumps to achieve the optimal drug exposure with the given clinical dose.

The central compartment of each HFS-TB unit was sampled pre-dose, then 1, 3, 4, 6, 12, and 23.5hr postdosing to measure the drug concentrations and validation of the concentration-time profile of each drug in the combination regimen. The peripheral compartment of each HFS-TB unit was sampled on study days 0, 3, 7, 14, 21, and 28. Samples were washed twice by centrifuging at 12,000 rpm for five minutes. The bacterial pellet was resuspended in one mL of normal saline, followed by inoculation into the MGIT tubes. The MGIT-derived time-to-positive (TTP) for each sample on different study days was recorded using the EpiCenter software. The time-in-protocol for the MGIT tubes was set to 56 days. Any MGIT tube showing no growth unit after 56 days of incubation was recorded as negative, and the results were defined as sterilization of that HFS-TB unit with the given drug combination regimen. As a second pharmacodynamic measure, the samples were also 10-fold serially diluted and cultured on Middlebrook 7H10 agar supplemented with 10% OADC to enumerate the bacterial burden.

Drug concentration measurement

Analytical standards of rifampin, bedaquiline, linezolid, meropenem, moxifloxacin, pretomanid and vaborbactam were purchased from Cayman Chemical (Ann Arbor, MI, USA). Internal standards (IS) linezolid-d3, meropenem-d6, moxifloxacin-d4, tazobactam, bedaquiline-d6, rifampicin-d3 were purchased from Toronto Research Chemicals (Toronto, Canada). LC-MS/MS analysis was performed using Waters Acquity UPLC connected to a Waters Xevo TQ mass spectrometer (Milford, MA). Data was collected using MassLynx version 4.1 SCN810 software. Separation was achieved on a Waters Acquity UPLC HSS T3 column (50x2.1mm; 1.8µm). All standard and internal standard (IS) stock solutions were prepared at 1mg/mL in 80:20

methanol:water and stored at -20ºC except for bedaquiline, and the IS were prepared in DMSO. The calibration curve and low and high-quality control samples (LQC and HQC) were prepared by diluting the stock solution in the blank medium. In a 96-well plate, 20µl of the sample was added to 180µL of IS solution in 0.1% aqueous formic acid (FA) or Methanol with 0.1% FA. The plates were vortexed. For all compounds, except vaborbactam, the following LC conditions were met- the mobile phase was a gradient mixture of 0.1% aqueous FA (solvent A) and 0.1% FA in methanol (solvent B). The flow rate was 0.2mL/min, and the total run time was six minutes. For vaborbactam, the mobile phase was a gradient mixture of 0.1% aqueous FA (solvent A) and 0.1% FA in Acetonitrile (solvent B). The flow rate was 0.4mL/min, and the total run time was six minutes. Compounds were detected using ESI in positive or negative MRM mode. **Supplementary Tables 2 and 3** summarize the MS parameters and percentage coefficient of variation (%CV).

Supplementary Table 2. Transition ions and scan conditions.

Compounds	Transition (m/z)	Cone Voltage (V)	Collison Energy (eV)
Linezolid	338 > 296	35	20
Linezolid-d3	341 > 297	35	20
Moxifloxacin	402 > 384	35	20
Moxifloxacin-d4	406 > 388	35	20
Pretonamid	360 > 175	28	24
Meropenem	384 > 141	20	16
Meropenem-D6	390 > 147	20	16
Rifampin	823 > 791	34	18
Rifampin-d3	826 > 794	34	18
Vaborbactam	296 > 234	30	20
Tazobactam	299 > 138	18	14
Bedaquiline	555 > 58	30	20

Bedaquiline-d6	561 > 64	38	22

Supplementary Table 3. Drug calibration range and quality control parameters.

Compounds	**Calibration range (μg/mL)**	**r^2**	**LQC, HQC (μg/mL)**	**LQC, HQC Inter-day %CV**	**LQC, HQC Intra-day %CV**	**LLOQ (μg/mL)**
Linezolid	0.02-10	>0.99	0.2, 4	5, 6	4, 3	0.01
Moxifloxacin	0.005-5	>0.99	0.2, 4	17, 2	8, 2	0.0025
PA-824	0.025-5	>0.99	0.2, 4	18, 3	16, 2	0.005
Meropenem	0.1-100	>0.99	0.2, 80	6,2	9,4	0.05
Rifampin	0.02-20	>0.99	0.4, 8	7, 13	6, 6	0.1
Vaborbactam	0.1-100	>0.99	0.2,80	6,1	6,3	0.05
Bedaquiline	0.01-0.5	>0.99	0.06, 0.8	9, 3	11, 4	0.01

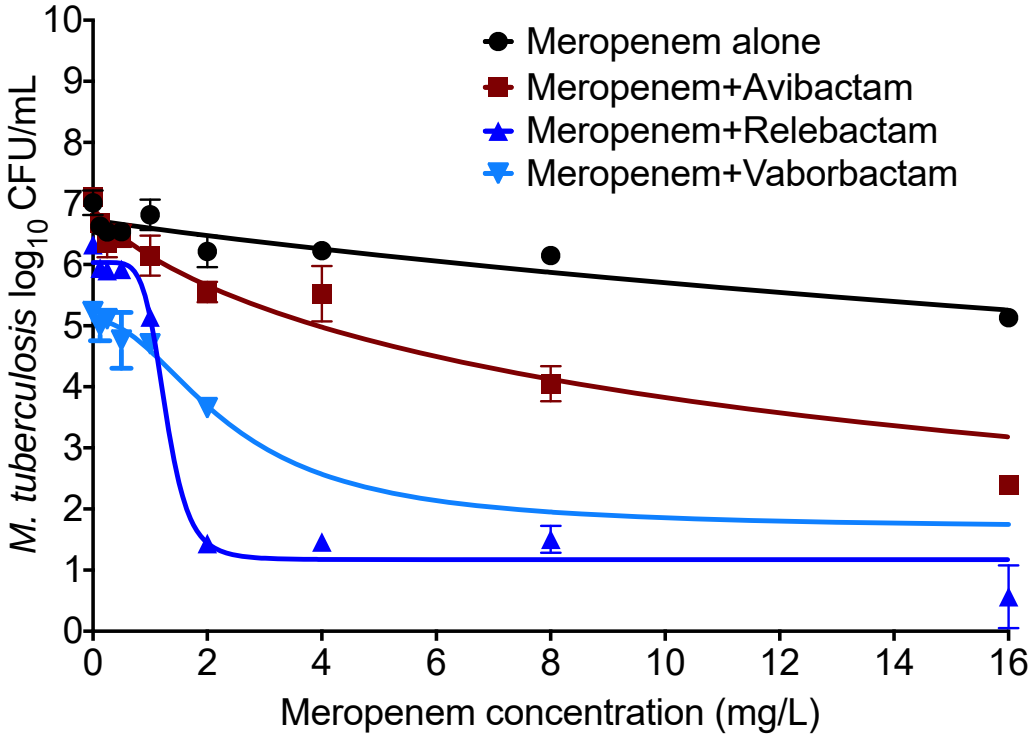
LQC, low-quality control; HQC, high-quality control, LLOQ, lower limit of quantitation; CV, coefficient of variation.

PK and PD analyses

Pharmacokinetics analysis of the measured drug concentration was performed using Phoenix WinNonlin (Certara, v8.1)[10]. Samples below the limit of quantification (BLQ) were assigned a value of “0” for analysis. The relationship between the bacterial burden and drug concentration, for both test-tube and HFS-TB studies, was determined using the four-parameter inhibitory sigmoid maximal effect model. Linear regression was used to calculate the kill slopes, and the exponential growth model was used to calculate the rate constant when TTP was used as the measure of bacterial burden in the HFS-TB. The combination regimens were compared using a two-way analysis of variance.

SUPPLEMENTARY RESULTS

Supplementary Figure 1. Efficacy of meropenem alone or in combination with different β-lactamase inhibitors against drug-susceptible *M. tuberculosis* H37Rv.



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