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An overview of drugs for the treatment of *Mycobacterium kansasii* pulmonary disease

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ABSTRACT

Objectives: The aim of this study was to determine and compare the efficacy of drugs to treat *Mycobacterium kansasii* (*Mkn*) pulmonary disease by performing minimum inhibitory concentration (MIC) determination and time–kill studies.

Methods: We determined the MICs to 13 drugs against the *Mkn* standard laboratory strain ATCC 12478 and 20 clinical isolates and performed time–kill studies with 18 drugs from different classes using the standard laboratory strain of *Mkn*. The β-lactam antibiotics were tested with or without the combination of the β-lactamase inhibitor avibactam. An inhibitory sigmoid E_{max} model was used to describe the relationship between drug concentrations and bacterial burden.

Results: Among the 13 tested drugs in the MIC experiments, the lowest MIC was recorded for bedaquiline. Among the 18 drugs used in the time–kill studies, maximum kill with cefdinir, tebipenem, clarithromycin, azithromycin, moxifloxacin, levofloxacin, tedizolid, bedaquiline, pretomanid and telacebac was greater than that for some of the drugs (isoniazid, rifampicin and ethambutol) used in standard combination therapy.

Conclusion: We report preclinical data on the efficacy and potency of drugs that can potentially be repurposed to create a safe, effective and likely shorter-duration regimen for the treatment of *Mkn* pulmonary disease.

1. Introduction

The incidence of pulmonary disease caused by nontuberculous mycobacteria (NTM) is on the rise [1]. *Mycobacterium kansasii* (*Mkn*) is a NTM with a clinical presentation very similar to the disease caused by *Mycobacterium tuberculosis* (*Mtb*) [2,3]. The current chemotherapeutic regimen [2] to treat *Mkn* pulmonary disease consists of isoniazid or a macrolide in combination with rifampicin and ethambutol; however, it requires a duration of therapy of ≥12 months. Thus, there is an unmet need to develop more effective, safe and shorter-course treatment regimens for *Mkn* pulmonary disease. Due to the lack of randomised controlled trials as well as pharmacokinetic/pharmacodynamic (PK/PD)-informed studies to determine the optimal drug dose, regimen composition and even the susceptibility breakpoint for critical drugs within a multidrug regimen, the therapeutic approach has been largely empirical and remains extrapolated from *Mtb*. Recently, a four-drug regimen of current and repurposed drugs demonstrated the ability to shorten *Mtb* treatment to 4 months from the conventional 6 months of therapy [4]. However, the quest for such a shorter-duration oral regimen for *Mkn* pulmonary diseases continues.

In contrast to most bacterial infections, there is a general belief that the drug minimum inhibitory concentration (MIC) and clinical response do not correlate for most drugs used to treat NTM diseases [5]. However, in our opinion, the MIC is a good starting point

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to make an informed decision on antibiotics to treat a given bacterial infection. Studies on the role of MIC in NTM disease have been complicated by inconsistent methodologies, including grouping of species and subspecies into a single analysis, the considerable challenge of predicting intracellular concentrations of a drug for mycobacterial infections that exist in various local anatomic and immunological environments, as well as a lack of consideration of individual host PK variability. These challenges notwithstanding, the MIC is still an important factor to determine the PK/PD-optimised drug exposure, the clinical dose to achieve the optimal drug exposure target, and the susceptibility breakpoint above which the drug at optimal dose will fail to kill the bacteria, in this case *Mkn*. The present study summarises the MIC and dose–response (time–kill) studies with several drugs from different classes to determine their efficacy and potency against *Mkn*. The efficacy of a drug can be described as the maximum effect (E_{max}) that a drug can produce regardless of the dose. Once E_{max} is achieved, increasing the drug dose will not produce an increased effect. Whereas potency can be described as the amount of drug required to produce a given effect. For example, EC_{50} is the concentration of the drug that can produce 50% of the maximum effect (bacterial kill). The findings presented here can help repurpose drugs for the treatment of *Mkn* pulmonary disease.

2. Materials and methods

2.1. Drugs, bacteria and supplies

We used the following 18 drugs: isoniazid; rifampicin; ethambutol; cefpodoxime; cefdinir; tebipenem; linezolid; tedizolid; clarithromycin; azithromycin; moxifloxacin; levofloxacin; minocycline; omadacycline; bedaquiline; pretomanid; sulfamethoxazole; and telacebac. The β-lactam antibiotics were tested with or without the combination of the β-lactamase inhibitor avibactam [6,7] at a concentration of 15 mg/L. Drugs were purchased either from the University of Texas Health Science Center at Tyler pharmacy or were synthesised by BOC Sciences (Sheryl, NY, USA). We used the standard *Mkn* laboratory strain ATCC 12478 and a collection of 20 clinical isolates (Table 1) to determine the MICs of the drugs. As the intent of the current study was to screen antibiotics for efficacy against *Mkn*, no patient demographic or clinical data (including the drugs in the combination regimen used to treat patients from which these clinical isolates were collected) was recorded. The concentration–response studies were performed only with the standard laboratory strain (ATCC 12478).

2.2. Minimum inhibitory concentration (MIC) and concentration–response studies

MICs were determined by the broth microdilution method [8]. Before each experiment, bacteria were first grown to log-phase growth followed by preparation of a 0.5 McFarland turbidity-adjusted inoculum preparation using Middlebrook 7H9 broth supplemented with 10% oleic acid–albumin–dextrose–catalase (OADC) [8–12]. Second, the turbidity-adjusted inoculum was 100-fold diluted to achieve an initial bacterial burden of $\sim 10^5$ $\log_{10}$ CFU/mL. In the third step, 180 μL of the inoculum was added to each of the 96-wells of a tissue culture plate prefilled with 20 μL of each drug concentration (10 $\times$). The plates were sealed in a Ziplock bag to prevent evaporation and cultures were incubated at 37°C. After 7 days of incubation, plates were read using an inverted mirror and the drug concentration completely inhibiting bacterial growth (absence of bacterial pellet) was recorded as the MIC. Experiments were performed twice with three replicates per drug concentration.

For the static concentration–response studies, the drug concentration range was comparable with those used in the MIC experiments. The experiment was performed in 15 mL screw-capped tubes with a total volume of 5 mL. The inoculum was prepared as described above and bacteria were co-incubated with drugs for 7 days at 37°C. On study Day 7, cultures were washed twice with normal saline to remove the carry-over drug, 10-fold serially diluted in normal saline, and spread on Middlebrook 7H10 agar supplemented with 10% OADC. Agar cultures were incubated at 37°C, sealed in a Ziplock bag, and CFU were recorded after 10 days of incubation. In addition to the monotherapy experiments, we also performed experiments with isoniazid, rifampicin and ethambutol as two- and three-drug combinations to benchmark the efficacy of drugs in the standard regimen [13] used to treat *Mkn* pulmonary disease. We used the EC_{80} (concentration of the drug that can produce 80% of the maximum effect) of isoniazid, rifampicin and ethambutol in the combination studies.

2.3. Data analysis

The individual MIC against 20 clinical isolates was used to calculate the MIC_{50} and MIC_{90} of each drug. We used the four-parameter inhibitory sigmoid E_{max} model to describe the relationship between drug concentrations and bacterial burden. The four parameters in the model were E_{con} (growth in the non-treated controls), E_{max} (maximum bacterial kill compared with the non-treated controls on study Day 7), EC_{50} (effective concentration mediating 50% of E_{max}) and the steep portion of the slope (H) as Hill coefficient. GraphPad Prism v.9 (GraphPad Software Inc., La Jolla, CA, USA) was used for plotting the data.

3. Results

Table 1 lists the concentration range of each drug used in the MIC experiments as well as showing the MIC, MIC_{50} and MIC_{90} of the drugs against the 20 individual clinical isolates. The results of the experiments with isoniazid, rifampicin and ethambutol are shown in Fig. 1A–C and Table 2. In the monotherapy concentration–response studies, the *Mkn* kill below stasis (or bacterial burden in the inoculum on Day 0) was 1.88 $\log_{10}$ CFU/mL with isoniazid (Fig. 1A), 2.28 $\log_{10}$ CFU/mL with rifampicin (Fig. 1B) and 1.66 $\log_{10}$ CFU/mL with ethambutol (Fig. 1C). The E_{max} (compared with non-treated control on study Day 7) and EC_{50}, based on the inhibitory sigmoid model, were 1.88 $\log_{10}$ CFU/mL and 0.88 mg/L for isoniazid, 2.28 $\log_{10}$ CFU/mL and 0.09 mg/L for rifampicin and 1.66 $\log_{10}$ CFU/mL and 1.39 mg/L for ethambutol. Table 2 summarises the results of the combination studies where each drug was used at EC_{80} concentration (isoniazid, 2.5 mg/L; rifampicin, 0.2 mg/L; and ethambutol, 4 mg/L). The bacterial burden in the inoculum (stasis) was 5.75 $\log_{10}$ CFU/mL, which grew to 8.12 $\log_{10}$ CFU/mL in 7 days. As shown in Table 2, the *Mkn* kill with the two- and three-drug combinations was higher than each drug alone. Moreover, the *Mkn* kill with the isoniazid + rifampicin two-drug combination was not significantly different from the three-drug combination, suggesting that ethambutol could be replaced with another potent drug in the standard regimen.

The β-lactams are the largest class of antibiotics and are used to treat several bacterial infections. However, their potential to treat *Mkn* pulmonary disease has not been systematically explored. The results of the experiments with cefpodoxime, cefdinir and tebipenem are shown in Fig. 1D–F. Experiments with tebipenem were performed with and without avibactam, whereas cefpodoxime and cefdinir were tested only in combination with avibactam at a concentration of 15 mg/L. As shown in Fig. 1D, cefpodoxime in combination with avibactam killed 1.84 $\log_{10}$ CFU/mL

Table 1
Minimum inhibitory concentrations (MICs) of 13 drugs against *Mycobacterium kansasii* standard laboratory strain ATCC 12478 and 20 clinical isolates [a]

Strain	MIC (mg/L)												
	INH [0.25–32]	RIF [0.03–4]	EMB [1–64]	SMX [2–128]	CLA [0.06–8]	AZI [0.25–32]	TZD [0.06–8]	LZD [0.12–8]	PTM [1–64]	MNO [0.12–16]	BDQ [0.03–2]	CFD [0.25–32]	TBP+AVI [0.12–128]
ATCC	1	1	8	128	1	32	1	4	64	8	0.03	32	0.5
UVA_1	2	1	16	128	0.5	32	0.5	2	64	16	0.03	32	ND
UVA_2	32	2	32	128	0.5	32	1	4	64	16	0.03	32	ND
TY_1	2	0.5	8	128	8	32	2	4	64	2	2	32	0.25
TY_2	1	1	4	128	0.5	8	1	2	8	4	0.03	16	0.25
TY_3	0.5	0.25	2	128	0.12	8	0.5	2	1	2	0.03	32	0.25
TY_4	0.5	0.5	2	128	0.5	16	0.5	2	4	2	0.03	16	0.25
TY_5	1	0.5	2	128	0.5	16	0.5	2	4	1	0.03	8	0.25
TY_6	0.5	0.5	4	128	0.5	8	0.5	2	4	2	0.03	32	0.25
TY_7	0.5	0.5	4	128	0.5	16	0.5	2	4	2	0.03	16	0.25
TY_8	1	0.5	4	128	0.5	16	0.5	2	2	2	0.03	16	1
TY_9	32	4	64	128	8	32	8	8	64	16	0.03	32	0.25
TY_10	1	2	4	128	2	32	1	4	8	4	0.03	32	0.5
TY_11	32	4	64	128	8	32	8	8	64	16	2	32	0.25
TY_12	0.5	0.5	4	128	0.5	32	1	4	16	4	0.03	0.5	0.25
TY_13	0.5	0.5	2	128	0.25	8	0.5	2	8	2	0.03	16	1
TY_14	1	0.5	4	128	1	32	1	4	8	4	0.03	32	0.5
TY_15	8	4	64	128	2	32	1	8	64	16	0.03	32	0.5
TY_16	1	0.5	4	128	1	16	0.5	2	8	4	0.03	16	0.5
TY_17	0.5	1	4	128	1	32	0.5	2	8	4	0.03	32	1
TY_18	32	4	64	128	2	10	8	8	64	16	0.03	32	2
MIC_{50}	1	0.5	4	128	0.5	32	1	2	8	4	0.03	32	0.25
MIC_{90}	32	4	64	128	8	32	8	8	64	16	1.6	32	1

INH, isoniazid; RIF, rifampicin; EMB, ethambutol; SMX, sulfamethoxazole; CLA, clarithromycin; AZI, azithromycin; TZD, tedizolid; LZD, linezolid; PTM, pretomanid; MNO, minocycline; BDQ, bedaquiline; CFD, cefdinir; TBP, tebipenem; AVI, avibactam; ND, not done.
[a] The concentration range tested is given in brackets.

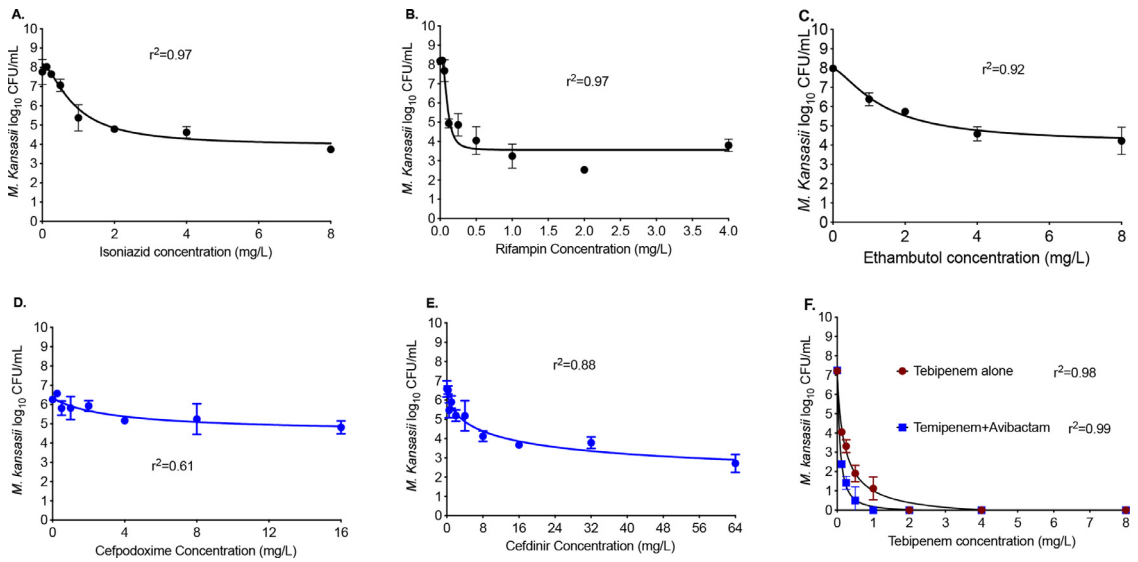

Fig. 1. Efficacy of drugs in the standard regimen and three β-lactam antibiotics against *Mycobacterium kansasii* (*Mkn*). Relationship between bacterial burden and (A) isoniazid, (B) rifampicin and (C) ethambutol concentration on Day 7 and r^2 to show good model fit. *Mkn* kill below stasis was 1.88 $\log_{10}$ CFU/mL with isoniazid, 2.28 $\log_{10}$ CFU/mL with rifampicin and 1.66 $\log_{10}$ CFU/mL with ethambutol. (D–F) Among the three β-lactam antibiotics, (D) and (E) show the results for cefpodoxime and cefdinir in combination with avibactam. There was no kill below stasis with cefpodoxime, whereas cefdinir showed a 1.50 $\log_{10}$ CFU/mL *Mkn* kill below stasis. In comparison, *Mkn* kill with tebipenem (F) was independent of the β-lactamase inhibitor avibactam. Kill below stasis with tebipenem was 4.48 $\log_{10}$ CFU/mL.

Table 2
Extent of *Mycobacterium kansasii* kill with isoniazid (H), rifampicin (R) and ethambutol (E) alone or in combination at static concentration

	H	R	E	HR	HE	RE	HRE
Kill below stasis ($\log_{10}$ CFU/mL)	1.69	1.39	1.44	3.66	2.41	2.51	3.46
E_{max} (on Day 7) ($\log_{10}$ CFU/mL)	3.76	3.76	3.82	6.03	4.79	4.88	5.83

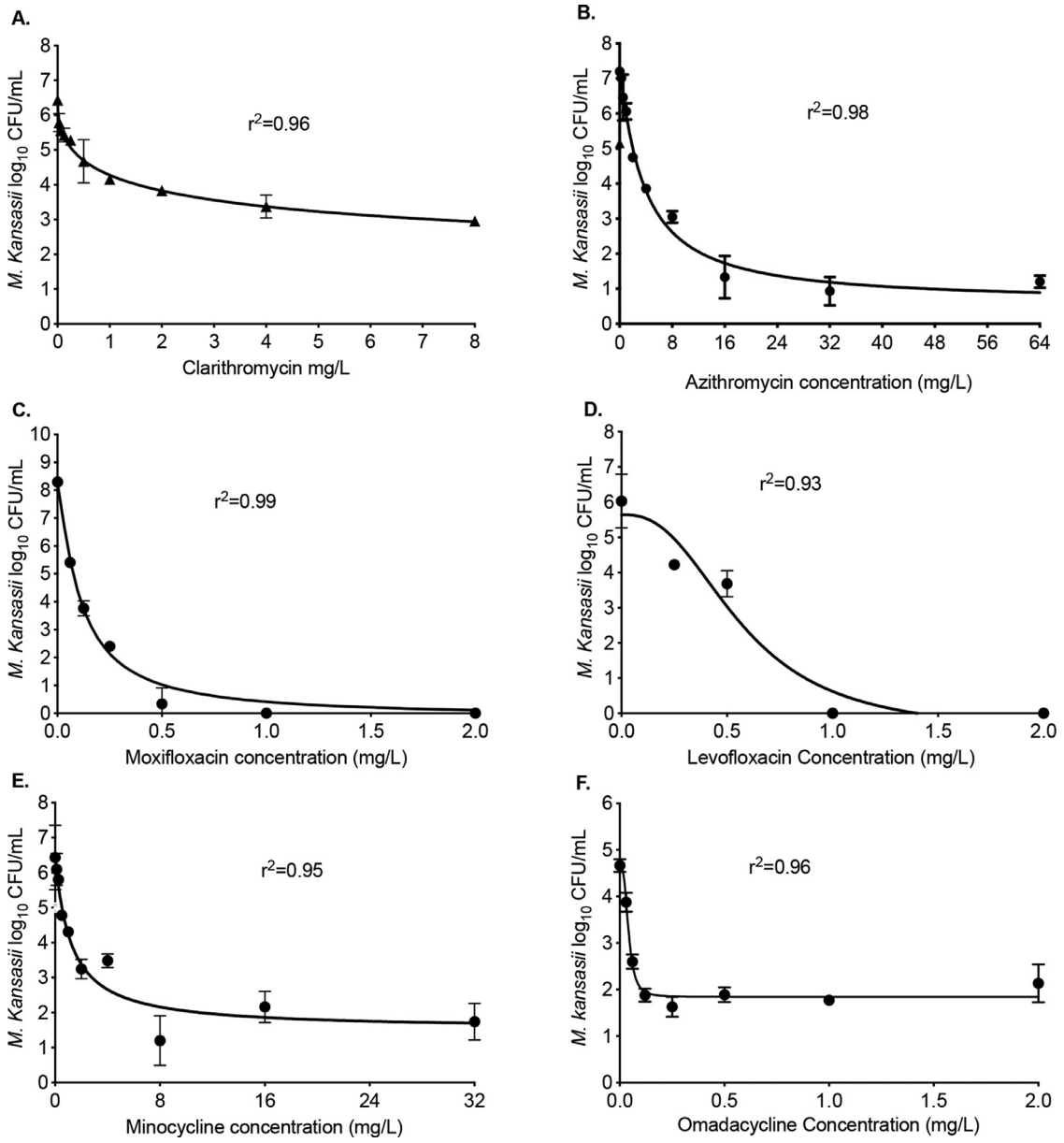

Fig. 2. Efficacy of macrolide, fluoroquinolone and tetracycline classes of antibiotics against *Mycobacterium kansasii* (*Mkn*). As shown in the figure, (A) clarithromycin, (B) azithromycin, (C) moxifloxacin, (D) levofloxacin, (E) minocycline and (F) omadacycline were able to kill *Mkn* in the test tube experiments. The extent of kill varied between antibiotics of the same class. The highest concentrations of moxifloxacin and levofloxacin used in the experiments were 32 mg/L and 64 mg/L. However, for clarity of presentation, the *x*-axis has been truncated. Kill below stasis was 1.64 $\log_{10}$ CFU/mL with clarithromycin, 3.95 $\log_{10}$ CFU/mL with azithromycin, 4.13 $\log_{10}$ CFU/mL with moxifloxacin, 4.53 $\log_{10}$ CFU/mL with levofloxacin, 2.87 $\log_{10}$ CFU/mL with minocycline and 2.64 $\log_{10}$ CFU/mL with omadacycline.

Mkn in 7 days, where the EC_{50} was calculated as 3.53 mg/L. The results of the cefdinir + avibactam combination are shown in Fig. 1E, showing 5.13 $\log_{10}$ CFU/mL *Mkn* kill in 7 days at static concentration with an EC_{50} of 12.09 mg/L. Fig. 1F shows that tebipenem alone or in combination with avibactam killed 7.25 $\log_{10}$ CFU/mL *Mkn* in 7 days. However, the EC_{50} was 3-fold lower in combination with avibactam (0.21 mg/L vs. 0.07 mg/L).

The British Thoracic Society (BTS) recommends combining macrolides with ethambutol and rifampicin to treat *Mkn* pulmonary diseases [14]. In Fig. 2A,B, we show the results for two macrolides (clarithromycin and azithromycin). Maximal kill (E_{max}) with clarithromycin after 7 days of co-incubation at static concentrations was 3.48 $\log_{10}$ CFU/mL compared with the non-treated controls (Fig. 2A). The clarithromycin EC_{50} was calculated as 2.21 mg/L. In comparison, azithromycin showed an E_{max} of 6.61 $\log_{10}$ CFU/mL in 7 days with an EC_{50} of 3.75 mg/L (Fig. 2B). Thus, both macrolides showed good efficacy against *Mkn* in the test tube experiments.

Fluoroquinolones are another class of antibiotics with the potential to be used for the treatment of *Mkn* pulmonary disease. In Fig. 2C,D, we show the results of the two fluoroquinolones (moxifloxacin and levofloxacin). After 7 days of co-incubation, the E_{max} of moxifloxacin was 8.38 $\log_{10}$ CFU/mL compared with the non-treated controls with an EC_{50} of 0.11 mg/L (Fig. 2C). In comparison, levofloxacin showed an E_{max} of 6.21 $\log_{10}$ CFU/mL on Day 7 of the study with an EC_{50} of 0.57 mg/L (Fig. 2D). Thus, both moxifloxacin and levofloxacin should be further explored for the treatment of *Mkn* pulmonary disease.

Fig. 2E,F shows the results of the two tetracycline antibiotics (minocycline and omadacycline) tested for efficacy against *Mkn*. On Day 7 of the study, compared with the non-treated controls, the E_{max} of minocycline was recorded as 4.96 $\log_{10}$ CFU/mL and the

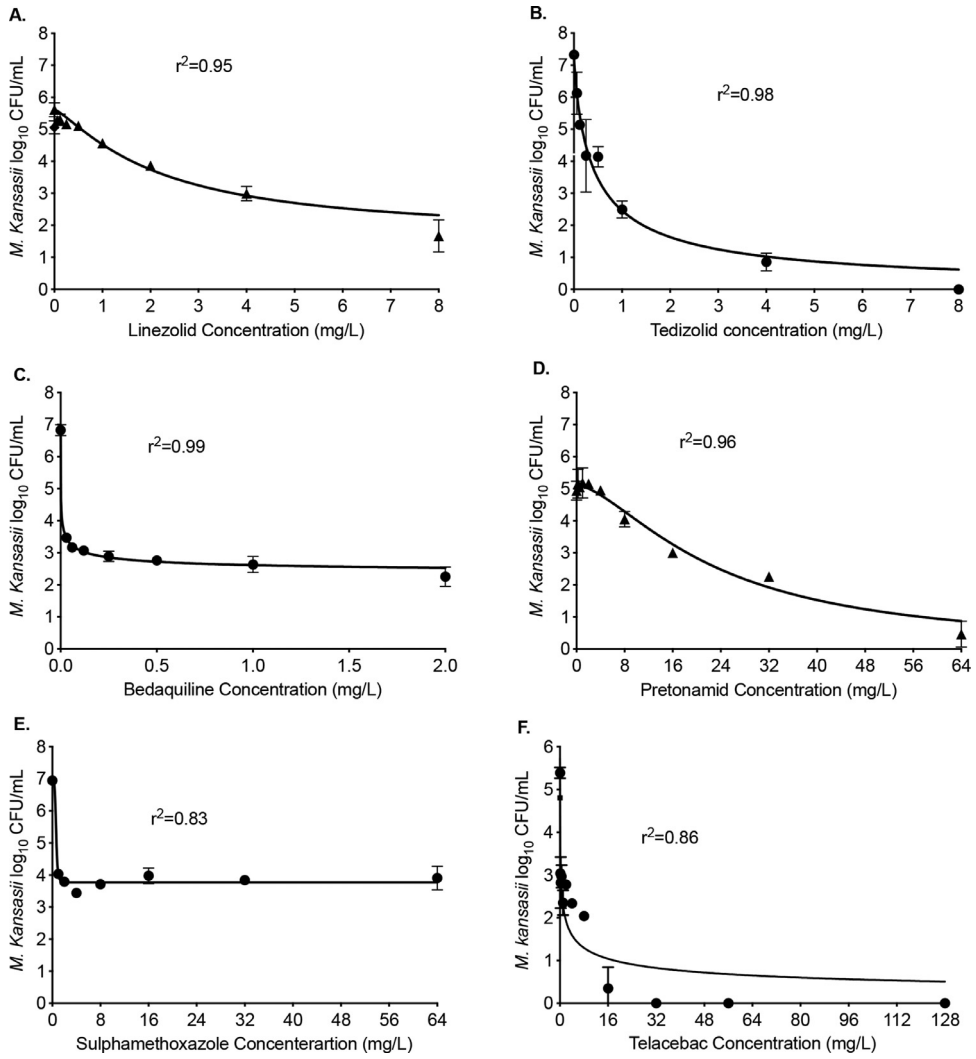

Fig. 3. Efficacy of drugs with potential to repurpose them for the treatment of *Mycobacterium kansasii* (*Mkn*) pulmonary disease. The figure shows excellent bacterial kill compared with the non-treated controls on Day 7 with each of the six drugs, namely (A) linezolid, (B) tedizolid, (C) bedaquiline, (D) pretomanid, (E) sulfamethoxazole and (F) telacebac. Kill below stasis was 3.4 $\log_{10}$ CFU/mL with linezolid, 4.22 $\log_{10}$ CFU/mL with tedizolid, 3.10 $\log_{10}$ CFU/mL with bedaquiline, 4.73 $\log_{10}$ CFU/mL with pretomanid, 1.82 $\log_{10}$ CFU/mL with sulfamethoxazole and 4.80 $\log_{10}$ CFU/mL with telacebac.

EC_{50} was calculated as 1.21 mg/L (Fig. 2E). Omadacycline showed an E_{max} of 2.81 $\log_{10}$ CFU/mL on Day 7 of the study with an EC_{50} of 0.04 mg/L (the lowest EC_{50} of any single drug tested excepting bedaquiline, as shown below) (Fig. 2F). Thus, while the efficacy (E_{max}) of minocycline was higher, omadacycline showed better potency as the EC_{50} was 30-fold lower.

The oxazolidinones is another class of antibiotics that could be potentially repurposed for the treatment of *Mkn* pulmonary disease. Fig. 3A,B shows the results of the two oxazolidinones (linezolid and tedizolid) tested for efficacy against *Mkn*. In the 7-day static concentration experiment, the E_{max} of linezolid was 3.96 $\log_{10}$ CFU/mL compared with the non-treated controls with an EC_{50} 2.18 mg/L (Fig. 3A). In comparison, tedizolid showed a higher E_{max} of 7.32 $\log_{10}$ CFU/mL for the same study duration, with an EC_{50} of 0.43 mg/L (Fig. 3B). Thus, in terms of both efficacy (E_{max}) and potency (EC_{50}), tedizolid could be a better choice for the treatment of *Mkn* pulmonary disease.

Bedaquiline, pretomanid, telacebac and sulfamethoxazole are among the antimicrobials that are either specifically designed to treat *Mtb* or Gram-positive and Gram-negative bacterial infections [15–19]. In Fig. 3C–F, we show the results of these four drugs with the intent to repurpose them for treatment of *Mkn* pulmonary disease. Fig. 3C shows the results of the bedaquiline studies, which killed 4.57 $\log_{10}$ CFU/mL in 7 days with an EC_{50} of 0.002 mg/L. Fig. 3D shows the results for pretomanid concentration–response studies. The E_{max} of pretomanid was 5.12 $\log_{10}$ CFU/mL and the EC_{50} was calculated as 23.05 mg/L. Next, in Fig. 3E we show the results for sulfamethoxazole, which killed 3.18 $\log_{10}$ CFU/mL in the 7-day static concentration experiments and the EC_{50} was calculated as 0.63 mg/L. Finally, Fig. 3F shows the results for telacebac, where maximum *Mkn* kill was recorded as 5.89 $\log_{10}$ CFU/mL with an EC_{50} of 0.39 mg/L. Thus, bedaquiline, pretomanid, sulfamethoxazole and telacebac have the potential to treat *Mkn* pulmonary disease, particularly in combination with other drugs that differentially maximise either efficacy or potency.

In addition to the results described above, Supplementary Table S1 summarises the model parameters for each of the drugs including E_{con}, E_{max}, EC_{50} and Hill constant that could be used to calculate the drug exposure for the combination studies.

4. Discussion

In the USA, *Mkn* is the second most common NTM after *Mycobacterium avium* complex [20]. *Mkn* is considered to be one of the most virulent of the NTM [21,22]. However, data on *Mkn* disease incidence are scarce in part because it has not been

commonly considered a transmissible public-health threat and it is not a reportable pathogen in many municipalities. The 2020, multi-society NTM treatment guidelines recommended daily or intermittent therapy when a macrolide-based regimen is used and daily therapy when an isoniazid-based regimen is used [2]. The 2020 guidelines also recommend that *Mkn* could be treated for a fixed duration of 12 months instead of 12 months beyond culture conversion [2]. The current American Thoracic Society (ATS) guidelines acknowledge the level of evidence for the currently recommended regimen for NTM infections as having the lowest evidence categorisation by GRADE (Grades of Recommendation Assessment, Development and Evaluation) criteria [3,23]. Our findings suggest significant opportunity for the development of new treatment regimens by repurposing drugs.

Towards the goal of developing new effective drug regimens for *Mkn*, we show the MIC distribution of 13 different drugs, including drugs in the standard combination regimen as well as drugs that are specifically designed for *Mtb* (bedaquiline, pretomanid and telacebac) and used for the treatment of drug-resistant tuberculosis, including linezolid. Elsewhere, *Mkn* was reported to be susceptible to sulfamethoxazole [24], however we found that all 20 clinical strains had a sulfamethoxazole MIC $\geq$ 128 mg/L. Among the macrolides, clarithromycin showed lower MICs compared with azithromycin; among the oxazolidinones, the clinical strains had lower MICs for tedizolid compared with linezolid; tebipenem, alone and in combination with avibactam, had MICs comparable with one of the most potent drugs in the standard regimen, namely rifampicin, and bedaquiline showed the lowest MIC among all the 13 drugs used in the MIC experiments.

While drug susceptibility testing (MIC) could help in initial decision-making, it is important to know how well a drug kills the infecting organism. Thus, we compared the kill below stasis with isoniazid, rifampicin and ethambutol (1.88, 2.28 and 1.66 $\log_{10}$ CFU/mL, respectively) to several drugs, namely tebipenem, azithromycin, moxifloxacin, levofloxacin, minocycline, omadacycline, linezolid, tedizolid, bedaquiline, pretomanid and telacebac, and found these drugs to have either a better kill below stasis or were able to kill the entire bacterial burden in the inoculum in the 7-day static concentration experiments. Therefore, the next step should be to examine these drugs at dynamic concentrations, such as in the hollow-fibre model system as proposed by Alffenaar et al. [25] and Rampacci et al. [26], to determine whether the effect persists at fluctuating concentrations and what would be the PK/PD-optimised exposure target for kill and resistance suppression.

It is a common belief that *in vitro* susceptibility testing of NTM, including *Mkn*, is of little help in managing the treatment of these infections. However, there is some evidence that indeed the *in vitro* susceptibility of *Mkn*, even if based on the interpretative criteria used with *Mtb*, could correlate with clinical outcome [27,28]. Therefore, a more expansive understanding of drug susceptibility, as we have developed in this this study, can be used to predict clinical outcome in patients with *Mkn* pulmonary disease and to design drug regimens for clinical studies with the dose to achieve the PK/PD-optimised drug exposure target. For example, we have previously published PK/PD studies with moxifloxacin [12] that determined moxifloxacin 800 mg/day as the PK/PD-optimised dose for the treatment of *Mkn* pulmonary disease. In the next step, moxifloxacin was added to the currently recommended regimen of isoniazid–rifampicin–ethambutol or replaced isoniazid or ethambutol [9]. It was observed that the addition of moxifloxacin resulted in faster *Mkn* kill with the potential to shorten the therapy duration to possibly 6 months or less. In another example, we compared the kill effect of the novel drug combination of rifapentine–tedizolid–minocycline with the ATS-recommended standard regimen and BTS-recommended regimen that included a macrolide [10]. Notably, the experimental regimen performed better than the standard-of-care regimens. Thus, if these drugs are combined at doses determined using formal PK/PD studies, they may lead to safe, tolerable and shorter-duration regimens, a significant leap in the management of an otherwise neglected disease.

Despite these promising results for repurposing drugs for the treatment of *Mkn* pulmonary disease, our study has limitations. One could argue that the experiments were performed at static concentrations that might overestimate the kill due to constant drug exposure. However, the PK/PD studies [10] with rifapentine, tedizolid and minocycline provided the crucial evidence that the effect seen at static concentration will likely persist at dynamic concentration. Additional PK/PD studies are warranted to test and rank various drug combinations that could potentially be advanced in the clinic.

To summarise, development of new drugs specific to *Mkn* has not been a priority for the pharmaceutical industry, and information on potential drugs and regimens to advance in clinical trials is lacking. Therefore, repurposing drugs as we report here is a promising strategy to improve the management of *Mkn* pulmonary disease.

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Competing interests

TG founded and is the president and CEO of Praedicare Inc., a contract research organisation; JGP is an employee of Praedicare Inc. All other authors declare no competing interests.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.12.010.

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