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Delamanid or pretomanid? A Solomonic judgement!

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Given the low treatment success rates of drug-resistant tuberculosis (TB), novel TB drugs are urgently needed. The landscape of TB treatment has changed considerably over the last decade with the approval of three new compounds: bedaquiline, delamanid and pretomanid. Of these, delamanid and pretomanid belong to the same class of drugs, the nitroimidazoles. In order to close the knowledge gap on how delamanid and pretomanid compare with each other, we summarize the main findings from preclinical research on these two compounds. We discuss the compound identification, mechanism of action, drug resistance, *in vitro* activity, *in vivo* pharmacokinetic profiles, and preclinical *in vivo* activity and efficacy. Although delamanid and pretomanid share many similarities, several differences could be identified. One finding of particular interest is that certain *Mycobacterium tuberculosis* isolates have been described that are resistant to either delamanid or pretomanid, but with preserved susceptibility to the other compound. This might imply that delamanid and pretomanid could replace one another in certain regimens. Regarding bactericidal activity, based on *in vitro* and preclinical *in vivo* activity, delamanid has lower MICs and higher mycobacterial load reductions at lower drug concentrations and doses compared with pretomanid. However, when comparing *in vivo* preclinical bactericidal activity at dose levels equivalent to currently approved clinical doses based on drug exposure, this difference in activity between the two compounds fades. However, it is important to interpret these comparative results with caution knowing the variability inherent in preclinical *in vitro* and *in vivo* models.

Introduction

The approval of bedaquiline for the treatment of drug-resistant tuberculosis (TB) by the FDA in 2012 led to a revival of anti-TB drug development, as it was the first drug with a new mechanism of action to be registered for the treatment of TB in 40 years. In the years that followed, the landscape of drug-resistant TB treatment changed considerably. In 2014, another new compound, delamanid, was approved by the EMA for the treatment of MDR-TB in adults. Currently, the WHO states that delamanid is indicated for the treatment of rifampicin-resistant (RR) TB or MDR-TB in adults and children.[1] More recently, in 2019, pretomanid was the third new drug introduced to the anti-TB drug arsenal. Pretomanid was granted FDA approval, with an indication specified for treating adults with XDR-TB or drug-intolerant or non-responsive MDR-TB. It is to be combined with bedaquiline and linezolid, known as the BPaL-regimen.

The process of drug development is being accelerated by a novel approach developed by the Critical Path to TB Drug Regimens.[2] Within this approach, novel drugs are tested as a part of new multidrug regimens already in early stages of the preclinical developmental pipeline. Within such regimens, new compounds are combined with established TB compounds (e.g. pyrazinamide), other new compounds (e.g. bedaquiline and pretomanid in the BPaL regimen), or drugs that are approved for treating diseases other than TB (as was the case for linezolid). The efficacy of these new regimens is subsequently tested in clinical trials as a unit, rather than as a single drug. This is different from the traditional approach that studies the addition of a new compound to an existing regimen or the replacement of single drugs by new ones. Although the new approach enables quicker clinical implementation of novel TB drugs (illustrated by the approval of pretomanid only within the BPaL regimen), it may leave us with the question how new compounds from the same class of drugs compare with each other. In this context, it would be interesting to rank new compounds based on their efficacy, and to assess whether new drugs could be interchangeable in case of drug resistance or drug intolerance. Such questions are of particular interest for delamanid and pretomanid, since they belong to the same class of drugs. In addition, although their clinical indications differ, it is possible that future expansions of approvals would allow for treatment of individual patients with either drug, within the same regimen.

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In this review, we summarize and discuss preclinical data on delamanid and pretomanid that have contributed to the implementation of these drugs in the clinic, including compound identification, mechanism of action, drug resistance, *in vitro* activity, *in vivo* pharmacokinetic profiles, and *in vivo* activity and efficacy. Their similarities and differences are discussed and remaining knowledge gaps are identified. Evaluation of clinical studies on either compound are not within the scope of this review.

Compound discovery

Delamanid and pretomanid are nitroimidazoles, a class of drugs active against a broad spectrum of microorganisms, including protozoa and anaerobic bacteria.[3] Another well-known member of the nitroimidazoles is metronidazole, for which antibacterial activity was originally discovered in 1962.[4] In the 1970s, a subclass of nitroimidazoles was identified that harboured antimycobacterial activity.[5] This property was further explored,[6] and preclinical studies demonstrated that the bicyclic 5-nitroimidazooxazole CGI-17341 was active against *Mycobacterium tuberculosis* both *in vitro* and *in vivo*.[7] Although potential mutagenicity hampered further development of this particular compound, it paved the way towards the identification of other antimycobacterial nitroimidazoles.[6,8]

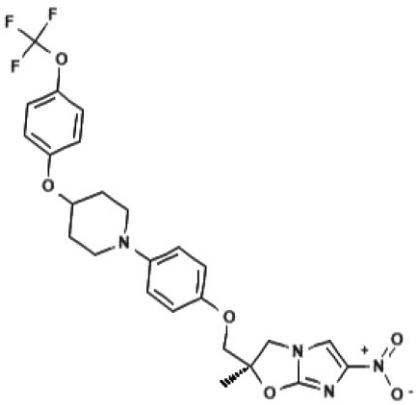
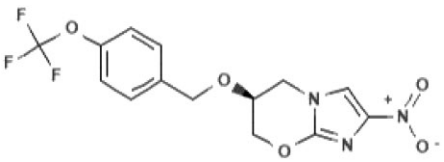
Delamanid

Otsuka Pharmaceutical Co. Ltd aimed to develop an antimycobacterial compound that targets mycolic acid synthesis.[9] By random screening, three structures were identified: dihydrophenazine, urea-type and dihydroimidazooxazole derivatives. Special attention was given to the latter, given the recent positive results on the antimycobacterial activity of CGI-17341. All nitroimidazoles in the Otsuka library were screened for mutagenicity and results showed that mutagenic properties were probably related to the functional groups attached to the core structure.[9–11] In particular, derivatives containing dimethyl residues were associated with higher mutagenicity.[9] Among a series of (*R*)-form 6-nitro-2,3-dihydroimidazo[2,1-b]oxazoles with various phenoxymethylgroups and a methyl group at the 2-position, delamanid was identified (Table 1). Its promising preclinical activity made delamanid the lead compound for further safety and efficacy studies.[9–11]

Pretomanid

In terms of the discovery of the nitroimidazoles for TB, drug discovery efforts leading to identification of pretomanid preceded those leading to delamanid. Researchers at PathoGenesis Corporation noticed the potency of CGI-17341 as well.[12] The company took an interest in nitroimidazooxazines rather than nitroimidazooxazoles, which have a six-membered ring fused to the nitroimidazole instead of a five-membered ring (Table 1). By comparing the antimycobacterial activity of a series of 328 bicyclic nitroimidazooxazines with that of CGI-17341, pretomanid was identified.[12] Pretomanid was found to be active against drug-susceptible as well as drug-resistant *M. tuberculosis* strains,[12] as was also seen for delamanid.[10] More information

Table 1. Chemical name and structure, and mechanism of action of delamanid and pretomanid

Characteristic	Delamanid (OPC-67683)	Pretomanid (PA-824)
Developed by:	Otsuka Pharmaceutical Co., Ltd.	PathoGenesis Corporation
Chemical name[a]:	(2R)-2-methyl-6-nitro-2-[(4-{4-[4-(trifluoromethoxy)phenoxy] piperidin-1-yl}phenoxy)methyl]-2,3-dihydroimidazo[2,1-b][1,3]oxazole	(6*S*)-2-nitro-6-[[4-(trifluoromethoxy)phenyl]methoxy]-6,7-dihydro-5*H*-imidazo[2,1-b][1,3]oxazine
Chemical structure[a]		
Mechanism of action	1. Inhibition of mycolic acid synthesis (methoxymycolates and ketomycolates) 2. Respiratory poisoning (reactive intermediates are yet to be identified)	1. Inhibition of mycolic acid synthesis (ketomycolates) 2. Respiratory poisoning by the release of reactive nitrogen species upon metabolic activation

[a]Information extracted from https://pubchem.ncbi.nlm.nih.gov.

on optimization studies of nitroimidazooxazines that resulted in the identification of pretomanid are detailed in published patents.[13,14]

Mechanism of action

Delamanid and pretomanid are thought to have a comparable, dual mode of action: (i) interference with mycolic acid synthesis, and (ii) respiratory poisoning.[15–17] It is noteworthy that most published research on the mechanism of action has been performed with pretomanid.

Inhibition of mycolic acid biosynthesis

Under aerobic conditions, inhibition of mycolic acid synthesis is considered to be the main mode of action of delamanid and pretomanid. Mycolic acids are a major component of the lipids forming the mycobacterial outer membrane, and are restricted to mycobacteria and related genera of the Actinobacteria phylum.[18] Mycolic acids contribute to bacterial virulence by forming a permeability barrier to drugs,[19] contributing to intracellular survival,[20] and modulating the pro-inflammatory response.[20,21] Three classes of mycolic acids are known: α-mycolates (most abundant), methoxymycolates, and ketomycolates.[22] Delamanid inhibits synthesis of ketomycolates and methoxymycolates, but not α-mycolates,[9,10] whereas isoniazid inhibits all three classes.[10] The exact mechanism by which delamanid blocks mycolic acid synthesis is not yet elucidated, as no mutations in delamanid-resistant organisms have been linked to cell wall synthesis.[23] Pretomanid blocks the formation of ketomycolic acid.[12] It is hypothesized that this process involves inhibition of a deazaflavin coenzyme (F_{420})-dependent enzyme that is responsible for oxidation of hydroxymycolate into ketomycolate.[24] Whether pretomanid also inhibits synthesis of the other mycolate classes is unknown.

Respiratory poisoning

Delamanid and pretomanid are prodrugs that need metabolic activation by mycobacteria to exert antimycobacterial activity (Figure 1).[10,12,25] In short, bio-activation of both compounds by mycobacteria depends on redox cycling of deazaflavin cofactor 420, or F_{420}. The enzyme deazaflavin-dependent nitroreductase (Ddn), which participates in the redox cycling of F_{420}, is responsible for bio-activation of both delamanid and pretomanid by the process of des-nitrification,[10,26–28] although the compounds bind differently to Ddn.[29] Human nitroreductases were found to be unable to activate delamanid, potentially due to their use of NAD(P)H as electron donor, which has a higher redox potential compared with F_{420}.[30] Similarly, pretomanid can be metabolized, but not bio-activated, by human liver enzymes, as they do not induce des-nitrification.[31] The activation of delamanid and pretomanid being restricted to mycobacterial Ddn might (in part) explain the selective activity against mycobacteria without being genotoxic to humans.[30,31]

Ddn-mediated metabolic activation of delamanid generates one main metabolite, desnitro-imidazooxazole, which has no antimycobacterial activity.[10] For pretomanid, Ddn reduces the imidazole ring, forming three major metabolites among which is a des-nitro form.[28] The metabolites have been described by Singh *et al.*[28] not to show any activity against *M. tuberculosis*. However, reduction of pretomanid releases reactive nitrogen species, such as nitric oxide (NO) which acts as an active intermediate.[16,28] NO is thought to target cytochrome oxidases in the mycobacterial electron-transport chain, thereby hampering ATP synthesis.[16,32] Since mycobacteria maintain their respiratory function and energy production at low levels under anaerobic conditions, they may be more vulnerable to impairment of ATP homeostasis under such circumstances.[16,33] Transcriptional profiling of *M. tuberculosis* exposed to delamanid revealed that delamanid probably induces respiratory poisoning as well.[17] However, the active intermediate of delamanid is not yet identified. Hayashi *et al.*[34] recently found that mutations in type II

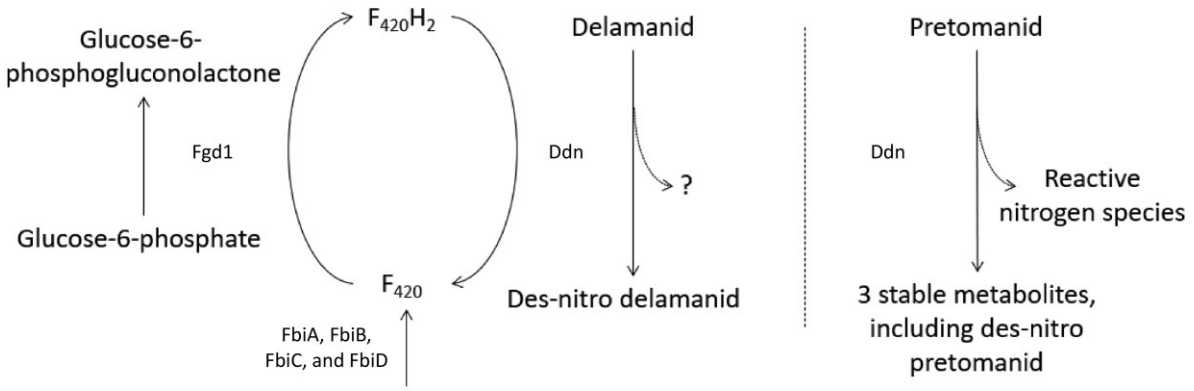


Figure 1. Schematic overview of the metabolic activation of delamanid and pretomanid by mycobacteria, adapted with permission from Liu *et al.*[23] and Rifat *et al.*[36] Delamanid and pretomanid are prodrugs that require activation by deazaflavin (F_{420})-dependent nitroreductase (Ddn). Redox cycling of deazaflavin cofactor 420, or F_{420}, is crucial in this process, which is mediated by glucose-6-phosphate dehydrogenase (Fgd1)[12,23,35,132,133] and Ddn.[10,26–28] Synthesis of F_{420} depends on FbiA, FbiB, FbiC and FbiD.[12,36–38,134] Bio-activation of delamanid by Ddn results in the formation of inactive des-nitro-imidazooxazole.[10,135] The active intermediate for delamanid has not yet been identified. Activation of pretomanid, on the other hand, generates three stable, inactive metabolites, as well as reactive nitrogen species which are responsible for respiratory poisoning by pretomanid.[26,28]

NADH dehydrogenase (*ndh*) can give rise to delamanid resistance. The authors speculate that an NAD-delamanid adduct, instead of NO, might be responsible for its anti-mycobacterial activity. Characterizing other upregulated genes during delamanid exposure could provide additional insight into its mechanism of action.[17]

Drug resistance

Studies on drug resistance suggest that delamanid and pretomanid display no cross-resistance with other currently used TB drugs, probably due to their unique mechanism of action.[10,12] That being said, by using a genetically modified *Mycobacterium smegmatis* strain, Hayashi *et al.*[34] showed that mutations in the *ndh* gene can in principle lead to resistance to isoniazid, ethambutol, and also delamanid.

Both delamanid and pretomanid have relatively high spontaneous mutation frequencies. For delamanid, the frequency of drug resistance was found to range between 1.22×10^{-5} and 6.44×10^{-6} at 16 times the MIC.[25] Spontaneous drug resistance frequencies ranging from 1.0×10^{-5} to 6.5×10^{-7} are reported for pretomanid, which are comparable to those of delamanid.[25,27,35] These frequencies are in line with resistance rates reported for isoniazid, but are higher than those reported for rifampicin in *M. tuberculosis*.[25] It could be that the relatively large target size for mutations (six non-essential genes, discussed below) foster these high frequencies, and the issue highlights the importance of combining these drugs with strong companion drugs during therapy.[36]

Mutations in the genes responsible for metabolic activation of delamanid and pretomanid (*fbiA, fbiB, fbiC, fbiD, fgd1*, and *ddn*) (Figure 1) have been associated with resistance to either drug in preclinical settings and in clinical isolates.[12,25,28,35–47] However, additional genes might be involved in delamanid resistance, as in a recent study none of the delamanid-resistant clinical isolates harboured mutations in *fbiA/B/C*, *fgd1* or *ddn*.[48] In contrast, published findings on pretomanid-resistant clinical isolates are sparse, likely because the drug only recently earned approval for clinical use.

Table 2 summarizes the findings from several studies that have investigated both delamanid and pretomanid susceptibility of *M. tuberculosis* isolates from either preclinical or clinical settings, together with an evaluation of gene mutations that coincided with drug resistance.[29,36,49] Given the similarities in the intra-bacterial metabolic pathway of delamanid and pretomanid, it is not unexpected that isolates resistant to both compounds have been identified. Out of 32 pretomanid-resistant isolates selected by Rifat *et al.*[36] from their mouse model of TB infection, 23 were resistant to delamanid as well (MIC >0.06 mg/L) and harboured mutations in *fbiA*, *fbiB*, *fbiC*, *fbiD*, *fgd*, and *ddn*. Lower levels of cross-resistance were reported in clinical isolates, with 2 out of 12 isolates being resistant to both compounds (delamanid MIC >16 mg/L, and pretomanid MIC 8 and >16 mg/L).[49] An E249K mutation in the *fbiA* gene (GAA → AAA) was found in one of these isolates, as well as a synonymous F320F mutation in *fgd1* (TTT → TTC). The particular *fgd1* mutation is, however, probably not responsible for drug resistance, as it was also observed in isolates susceptible to both drugs. Of particular interest are the isolates resistant to one drug only, while susceptibility to the other is preserved (Table 2). Isolates selected in a preclinical setting with various mutations in *fbiA*, *fbiB* or *fbiD* exhibited high-level resistance to pretomanid, while retaining susceptibility to delamanid with only modestly raised MICs to the critical value of 0.06 mg/L.[36] Apart from susceptibility testing, Lee *et al.*[29] investigated the ability of *M. tuberculosis* isolates harbouring mutations in *ddn* to activate delamanid and pretomanid. Notably, of the 46 studied *ddn* mutants, two isolates were not able to activate pretomanid, but could, however, still activate delamanid. These isolates harboured an S78Y or Y133C mutation in *ddn*, both of which are naturally occurring sequence polymorphisms. This finding suggests that mutations in *ddn* such as S78Y and Y133C might cause pretomanid resistance, while maintaining susceptibility to delamanid. Molecular docking studies indicate that the dissimilarity in the ability to activate delamanid or pretomanid might be a consequence of different binding of the compounds to Ddn.[29] The authors speculate that the chemical structure of delamanid causes steric hindrance with the deazaflavin ring of F_{420} in Ddn bound to $F_{420}H_2$. As a result, delamanid binds above F_{420} in a different orientation than pretomanid. All together, these findings imply that under certain conditions delamanid and pretomanid could replace each other in case of drug resistance to one of the two drugs.

In vitro activity

A single *in vitro* assay cannot cover the complexity of human TB infection comprising *M. tuberculosis* in various metabolic stages and residing in different niches. Hence, a variety of assays exists, each with a specific design and read-out. Although heterogeneity between assays hampers systematic comparison, here, we review studies reporting the following outcomes to get an impression of the *in vitro* activity of delamanid and pretomanid: standard *in vitro* susceptibility assays (MIC assays), drug activity against extracellular *M. tuberculosis* in different metabolic states, and activity against intracellular *M. tuberculosis* in macrophage assays. Although the MIC value is a measure of compound activity, it does not directly reflect *in vivo* efficacy as it is only one of many factors that drive pharmacokinetic (PK) and pharmacodynamic (PD) characteristics. In early stages of compound development, new drugs are often tested against replicating extracellular *M. tuberculosis*. These experiments are relatively easy to implement and allow for a quick comparison of the new compound's activity with that of already established TB drugs. Regarding metabolic states, *M. tuberculosis* is thought to be present in pulmonary lesions both as replicating and non-replicating bacteria, based on the mycobacterial growth phase.[50,51] Evaluating drug activity against non-replicating mycobacteria is relevant, because this population is more tolerant to treatment with existing TB drugs and therefore may be responsible for the prolonged TB treatment duration needed to effect cure.[51–53] Several assays have been developed that induce a non-replicating state in *M. tuberculosis*, including starvation, oxygen depletion, low pH, or by using specific strains such as the *M. tuberculosis* 18b strain which enters a non-replicating state in the absence of streptomycin.[54] We chose to also include results of the first-line drugs rifampicin and isoniazid as a reference, since it is known that rifampicin is active against

Table 2. Overview of *M. tuberculosis* isolates selected from either preclinical or clinical settings for which susceptibility to both delamanid (DLM) and pretomanid (PMD) was determined, together with an investigation of coinciding gene mutations

Author/setting of isolation	Resistance type	Resistant to[a]	MIC (mg/L)		Gene	Mutation
			Delamanid	Pretomanid		
Rifat *et al.* (2020)[36]						
Preclinical		DLM; PMD	>16	>32	*fbiA*	Q27*
Preclinical		DLM; PMD	>16	>32	*fbiA*	D49G
Preclinical		DLM; PMD	>16	>32	*fbiA*	−G in aa 47
Preclinical		DLM; PMD	>16	>32	*fbiA*	L308P
Preclinical		DLM; PMD	>16	32	*fbiA*	Q120P
Preclinical		DLM; PMD	>16	32	*fbiA*	D286A
Preclinical		DLM; PMD	0.06–0.125	8–32	*fbiB*	L15P
Preclinical		DLM; PMD	0.125	32	*fbiB*	L173P
Preclinical		DLM; PMD	0.06–0.125	32	*fbiB*	−T in aa 684
Preclinical		DLM; PMD	>16	>32	*fbiC*	C562W
Preclinical		DLM; PMD	1	>32	*fbiC*	G194D
Preclinical		DLM; PMD	2	>32	*fbiC*	−C in aa 20
Preclinical		DLM; PMD	>16	>32	*fbiC*	K684T
Preclinical		DLM; PMD	>16	>32	*fbiC*	IS6110 ins. 85 bp upstream of *fbiC*
Preclinical		DLM; PMD	>16	>32	*fbiC*	L377P
Preclinical		DLM; PMD	>16	>32	*fbiC*	A827G
Preclinical		DLM; PMD	0.5	32	*fgd1*	K9N
Preclinical		DLM; PMD	>16	>32	*fgd1*	G191D
Preclinical		DLM; PMD	>16	≥32	*ddn*	R112W
Preclinical		DLM; PMD	>16	≥32	*ddn*	IS6110 ins. in D108
Preclinical		DLM; PMD	>16	>32	*ddn*	−G in aa 39
Preclinical		PMD	0.03	32	*fbiA*	S219G
Preclinical		PMD	0.03	16	*fbiB*	W397R
Preclinical		PMD	0.03	16–32	*fbiC*	R25G
Preclinical		PMD	0.03	16–32	*fbiC*	M776R
Preclinical		PMD	0.06	>32	*fbiD*	G147C
Preclinical		PMD	0.06	>32	*fbiD*	A132V
Preclinical		PMD	0.06	>32	*fbiD*	−ATC in aa 129
Preclinical		PMD	0.03–0.06	>32	*fbiD*	R25S
Preclinical		PMD	0.06	>32	*fbiD*	A198P
Preclinical		PMD	0.06	>32	*fbiD*	C152R
Preclinical		PMD	<0.03	>32	*fbiD*	A68E
Wen *et al.* (2019)[49]						
Clinical	XDR	DLM; PMD	>16	8	[b]	[b]
Clinical	XDR	DLM; PMD	>16	>16	*fgd1*	F320F
					fbiA	E249K
Clinical	MDR	DLM	16	0.063	*fgd1*	F320F
Clinical	MDR	DLM	>16	0.031	[b]	[b]
Clinical	MDR	DLM	0.5	0.063	*fgd1*	F320F
Clinical	MDR	DLM	>16	0.063	*fgd1*	F320F
Clinical	XDR	DLM	>16	≤0.016	*fgd1*	F320F
Clinical	XDR	PMD	≤0.016	>16	[b]	[b]
Clinical	MDR	None	≤0.016	0.13	*fgd1*	F320F
Clinical	MDR	None	≤0.016	0.25	*fgd1*	F320F
Clinical	MDR	None	≤0.016	0.5	*fgd1*	F320F
Clinical	XDR	None	≤0.016	0.25	*fgd1*	F320F
Lee *et al.* (2020)[29]						
Clinical		DLM; PMD	32	256	*ddn*	S78Y

Rifat *et al.*[36] determined the MIC by broth macrodilution assay, Wen *et al.*[49] by microplate Alamar blue assay (MABA) and Lee *et al.*[29] by resazurin assay.

[a]The clinical breakpoint for susceptibility to delamanid is ≤0.06 mg/L, as set by the EUCAST[73]; EUCAST clinical breakpoints for pretomanid are awaited. In this Table, 1 mg/L is used as the cut-off value for susceptibility to pretomanid.[70]

[b]No mutations were found in *ddn*, *fgd1*, *fbiA*, *fbiB*, or *fbiC*.

both replicating and non-replicating *M. tuberculosis*,[55] while isoniazid only targets replicating bacilli.[56]

Delamanid

The MIC distribution for delamanid against clinical *M. tuberculosis* strains as reported by the EUCAST shows that MICs mostly range between ≤0.002 to 0.03 mg/L.[57] Depending on the method used, the majority of isolates have an MIC of 0.004 mg/L or 0.008 mg/L as tested by agar dilution or MGIT 960, respectively. This is in agreement with various articles reporting MICs ≤0.025 mg/L against both drug-susceptible and drug-resistant *M. tuberculosis* strains.[10,11,45,48,49,58,59] EUCAST sets the clinical breakpoint for strain susceptibility to delamanid at MIC ≤0.06 mg/L.[60]

Table 3 summarizes findings on the *in vitro* activity of delamanid against replicating extracellular *M. tuberculosis*. Saliu *et al.*[61] compared the activity of delamanid with that of rifampicin against clinical *M. tuberculosis* isolates tolerant to isoniazid, meaning that these isolates grew better than the laboratory H37Rv strain in the presence of 0.1 mg/L isoniazid as measured by $^{14}CO_2$ production. The authors found that against these isolates, killing rates of delamanid at 1 mg/L were comparable to those of rifampicin at 2 mg/L over 14 days of drug exposure.[61] Dalton *et al.*[62] showed that delamanid significantly reduced the mycobacterial numbers as measured by relative light units (RLU) after 3 days of drug exposure.

Information on *in vitro* activity of delamanid against non-replicating bacilli is sparse (Table 4). In a study by Upton *et al.*,[63] the non-replicating state was induced by oxygen depletion.[63] The authors found that delamanid at 4.4 μM was sufficient to reduce colony forming units (cfu) by 99% after 10 days of exposure. As *M. tuberculosis* can be present intracellularly in pulmonary lesions, Matsumoto *et al.*[10] used infected macrophages differentiated from human THP-1 monocytes to assess delamanid activity against intracellular *M. tuberculosis*. Delamanid showed strong and concentration-dependent activity, which at 0.1 mg/L was similar to that of rifampicin at 3 mg/L.

Only a few studies describe the *in vitro* activity of delamanid-containing TB drug combinations. Matsumoto *et al.*[10] investigated potential synergistic activity of delamanid and first-line TB drugs against 27 clinical *M. tuberculosis* isolates by chequerboard analysis. There was no interaction observed between delamanid and rifampicin (FIC indices between >0.5 and 0.75) for the majority of isolates (88.9%). This also accounted for the interaction between delamanid and isoniazid (44.4% FIC index >0.5–0.75, 18.5% FIC index >0.75–1.0, 37% FIC index >1.0–4.0). Also using a chequerboard assay, Chandramohan *et al.*[64] demonstrated either an additive or synergistic effect between delamanid and bedaquiline or moxifloxacin, depending on the *M. tuberculosis* strain being drug-susceptible, monoresistant to isoniazid or rifampicin, MDR or XDR. However, it should be pointed out that the results of chequerboard assays should be interpreted with utmost care, as it is not clear how well these artificial *in vitro* assays translate to *in vivo* results for *M. tuberculosis*.

Pretomanid

Pretomanid was only recently approved as a TB drug, and therefore, the evaluation of clinical breakpoints is currently ongoing.[65] Pretomanid activity has been assessed against drug-susceptible, MDR and XDR *M. tuberculosis* strains, with reported MICs of 0.015–1 mg/L.[12,49,66–69] Pending the EUCAST clinical breakpoints, the EMA proposed 1 mg/L as the critical concentration when using the MGIT system for drug susceptibility testing.[70] In addition, *M. tuberculosis* isolates with pretomanid resistance-associated gene mutations have an MIC above this critical concentration.[26,35] Based on the few studies that assessed the MIC of both delamanid and pretomanid, the reported values for delamanid (0.001–0.024 mg/L) were lower than those for pretomanid (0.012–0.200 mg/L).[10,49,63]

Pretomanid activity against replicating *M. tuberculosis* is summarized in Table 3. Sala *et al.*[71] demonstrated that pretomanid (3 mg/L) killed replicating *M. tuberculosis* (using the 18b strain exposed to streptomycin, which allows for the strain to replicate) to the same extent as isoniazid (0.5 mg/L) and rifampicin (10 mg/L) after 7 days of drug exposure. Using an H37Rv *M. tuberculosis* strain, Piccaro *et al.*[55] showed that the activity of pretomanid (2 mg/L) was comparable to that of isoniazid (2 mg/L), though it was inferior to the activity of rifampicin (8 mg/L). In a study by Dalton *et al.*,[62] 3 days of pretomanid exposure kept the *M. tuberculosis* load at a stable level, whereas the untreated

Table 3. Summary of *in vitro* activity of delamanid and pretomanid against replicating, extracellular *M. tuberculosis*

Author	*M. tuberculosis* strain	Drug treatment (dose)	Treatment duration	Read-out	Outcome
Saliu *et al.* (2007)[61]	Clinical INH-tolerant strains	DLM (1 mg/L)	14 days	Growth Index	Killing rates of DLM were comparable to those of RIF (2 mg/L).
Dalton *et al.* (2017)[62]	Bioluminescently-labelled H37Rv	DLM; PMD	3 days	RLU	DLM significantly reduced RLU. RLU levels stayed stable during PMD and RIF exposure.
Sala *et al.* (2010)[71]	18b, exposed to streptomycin	PMD (3 mg/L)	7 days	cfu	PMD bactericidal activity was comparable with that of INH (0.5 mg/L) and RIF (10 mg/L).
Piccaro *et al.* (2013)[55]	H37Rv	PMD (2 mg/L)	7 days	cfu	PMD reduced cfu counts to a comparable extent as INH (2 mg/L), but to a lesser extent than RIF (8 mg/L).

INH, isoniazid; DLM, delamanid; RIF, rifampicin; PMD, pretomanid; RLU, relative light units; cfu, colony forming units.

Table 4. Summary of *in vitro* activity of delamanid and pretomanid against non-replicating, extracellular *M. tuberculosis*.

Author	*M. tuberculosis* strain	Induction non-replicating state	Drug treatment (dose)	Treatment duration	Read-out	Outcome
Upton *et al.* (2015)[63]	H37Rv	Oxygen depletion	DLM (4.4 μM); PMD (17.4 μM)	10 days	cfu	DLM at 4.4 μM, and PMD at 17.4 μM reduced cfu by 99%.
Lenaerts *et al.* (2005)[66]	H37Rv	Oxygen depletion	PMD (2, 10, 50 mg/L)	4 days	cfu	PMD showed dose-dependent bactericidal activity. At 50 mg/L, PMD activity was higher than that of INH at 50 mg/L, and was comparable to RIF at 2 mg/L, but inferior to RIF at 10 or 50 mg/L.
Hu *et al.* (2008)[72]	H37Rv	Starvation, oxygen depletion	PMD (0.31–20 mg/L)	4–7 days	cfu	PMD showed dose-dependent bactericidal activity. At ≤1.25 mg/L, PMD was only minimally active. Mycobacterial elimination was observed at ≥10–20 mg/L.
Sala *et al.* (2010)[71]	18b strain	No exposure to streptomycin	PMD (3 mg/L)	7 days	cfu	PMD activity was higher against non-replicating than fast-replicating *M. tuberculosis*. PMD and RIF (10 mg/L) were equally active and PMD activity was superior to INH (0.5 mg/L).
Stover *et al.* (2000)[12]	Bioluminescently-labelled H37Rv	Oxygen depletion	PMD (10 mg/L)	7 days	RLU	PMD was active against non-replicating mycobacteria. PMD activity (10 mg/L) was comparable to MTZ (10 mg/L), and superior to INH (10 mg/L).
Papadopoulou *et al.* (2007)[75]	Bioluminescently-labelled H37Rv	Oxygen depletion	PMD (6.4–12.8 mg/L)	10 days	Luminescent signal/cfu	PMD at 6.4–12.8 mg/L, and RIF at 2.5 mg/L were sufficient to kill ≥90% of *M. tuberculosis*. This activity was superior to INH (>100 mg/L).
Piccaro *et al.* (2013)[55]	H37Rv	Oxygen depletion	PMD (2 mg/L)	7–21 days	cfu	PMD showed time-dependent bactericidal activity, which was inferior to RIF (8 mg/L) and superior to INH (2 mg/L).
Somasundaram *et al.* (2013)[76]	H37Rv	Oxygen depletion	PMD (3, 12.5 mg/L)	2–21 days	cfu	PMD (12.5 mg/L) resulted in mycobacterial elimination at day 21, which was superior to RIF (1 mg/L). Bactericidal activity of PMD at 3 mg/L was comparable to RIF at 1 mg/L.
Iacobino *et al.* (2016)[74]	H37Rv	Starvation, oxygen depletion, low pH	PMD		cfu	PMD reduced cfu counts by ≥2 $\log_{10}$, which was similar to RIF and superior to INH.
Early *et al.* (2019)[73]	H37Rv	Low pH	PMD	7 days	cfu	PMD (12 μM) reduced cfu by ≥2 $\log_{10}$, similar to RIF (75 μM), whereas INH showed no activity.

DLM, delamanid; PMD, pretomanid; cfu, colony forming units; INH, isoniazid; RIF, rifampicin; MTZ, metronidazole.

control showed a significant increase in mycobacterial load. In comparison, the activity of delamanid in this assay was relatively higher, leading to a reduction in the mycobacterial load.

Activity of pretomanid against non-replicating *M. tuberculosis* was more elaborately studied than for delamanid (Table 4). Its activity was shown be concentration dependent.[66,72] In an experimental set-up using the 18b *M. tuberculosis* strain (in a non-replicating state in the absence of streptomycin), pretomanid appeared to be more active against non-replicating compared with replicating *M. tuberculosis*, reducing the mycobacterial load by 4.5 $\log_{10}$ cfu/mL versus 2 $\log_{10}$ cfu/mL after 7 days of drug exposure, respectively.[71] This observation matches the finding that reactive nitrogen species released upon activation of pretomanid have a greater impact on the ATP synthesis under anaerobic conditions.[16,33] Stover *et al.*[12] aimed to induce a non-replicating state in *M. tuberculosis* by microaerophilic culture conditions. In this assay, the bactericidal activity of 7 days exposure to pretomanid (10 mg/L) was comparable to that of the structurally related metronidazole (10 mg/L), and superior to that of isoniazid (10 mg/L). Lenaerts *et al.*[66] also observed a higher bactericidal activity of pretomanid (50 mg/L) compared to isoniazid (50 mg/L) following 4 days of drug exposure in an oxygen depletion assay. In various experimental set-ups, bactericidal activity of pretomanid against non-replicating *M. tuberculosis* matched the activity of rifampicin.[66,71,73–76] Upton *et al.*[63] evaluated the bactericidal activity of both delamanid and pretomanid against non-replicating *M. tuberculosis* in an oxygen depletion assay. The authors found that 17.4 μM pretomanid was sufficient to reduce cfu by 99% after 10 days of exposure, while for delamanid a concentration of 4.4 μM was sufficient to achieve this goal.[63] Published information on pretomanid activity against intracellular bacilli is rather limited. In a whole blood culture assay, Wallis *et al.*[77] demonstrated modest concentration-dependent bactericidal activity of pretomanid at 0–2 mg/L. In another assay, using *M. tuberculosis*-infected THP-1 cells, pretomanid at 0.1–1 mg/L led to a similar reduction in mycobacterial numbers as isoniazid at 0.3–3 mg/L. However, the intracellular activity of pretomanid was inferior to that of delamanid and rifampicin in this study.[10]

The *in vitro* activity of drug combinations was more extensively studied for pretomanid than for delamanid. Whereas delamanid combined with bedaquiline showed *in vitro* synergy,[64] additive or antagonistic effects have been reported when pretomanid was combined with bedaquiline,[77,78] although it should be noted that different experimental designs were used in these studies, hampering comparison of the outcomes. The interaction between pretomanid and bedaquiline is of interest, since several new and promising drug regimens contain these two drugs (ClinicalTrials registration no. NCT03338621, NCT03086486, NCT02589782). An additive effect was found when pretomanid was combined with linezolid.[78] The combination of pretomanid and moxifloxacin was shown to be additive or synergistic against actively replicating or non-replicating *M. tuberculosis*, respectively.[78–80] In this context, Drusano *et al.*[80] showed that the addition of bedaquiline to the combination of pretomanid and moxifloxacin achieved eradication of actively replicating *M. tuberculosis* one week sooner compared with the two-drug combination. Using a modified chequerboard assay, López-Gavín *et al.*[81] demonstrated that a combination of pretomanid, clofazimine, and moxifloxacin was active against drug-susceptible and MDR clinical isolates, with the activity of the drugs being additive. In a recent study using a hollow fibre infection model, the performance of the combination of pretomanid, moxifloxacin and pyrazinamide was equal to that of the standard regimen consisting of isoniazid, rifampicin and pyrazinamide (HRZ) against both replicating, non-replicating and intracellular *M. tuberculosis*.[82] Lastly, Piccaro *et al.*[55] reported that when pretomanid was combined with rifampicin, moxifloxacin, and amikacin, *M. tuberculosis* was efficiently killed within 14 days in aerobic as well as hypoxic conditions, but no comparison was made with the standard regimen. Again, when interpreting these data, it is important to bear in mind the limitations regarding the translational value of these highly simplified *in vitro* drug combination assays.

Pharmacokinetics

The efficacy of a drug depends on its PD and its PK profile. By combining PK with a microbiological parameter, PK/PD indices can be determined (e.g. AUC_{0-24}/MIC or $\%T_{>MIC}$), which can be used to optimize dosing schedules.[83] Knowledge on what doses in animals reach exposures (or ideally driving PK/PD indices) that match exposures reached in humans at clinically approved doses assists in interpreting drug activity and efficacy results in animal studies and translating these to humans. Furthermore, animal studies can shed light on drug distribution, drug metabolism, and drug clearance.

Delamanid

Animal studies have shown that following oral administration of delamanid, the drug is widely distributed among various organs.[84,85] After treating rats with a single oral dose of radioactively labelled ^{14}C-delamanid (3 mg/kg), radioactivity was detected in the lungs, central nervous system, eyeball, placenta, fetus, and breastmilk.[84] Penetration of the blood–brain barrier was confirmed by Tucker *et al.*[85] in a rabbit model of tuberculous meningitis. Delamanid was detected in cerebrospinal fluid, albeit at lower concentrations than in plasma. Brain tissue concentrations, on the other hand, were found to be 5-fold higher than those in plasma.[85] Results from both studies suggest that delamanid could be of value in treating extra-pulmonary TB, including TB meningitis, but further studies are required.

Delamanid is highly protein bound (>97%).[86] It is thought that plasma albumin is mainly responsible for metabolizing delamanid,[86,87] with the formation of M1 (DM-6705) as the major metabolite. Hepatic cytochrome P450 (CYP) enzymes are assumed to play a role in the subsequent degradation of M1 into another seven metabolites.[87] No interaction between delamanid and CYP isoforms was observed,[10,88] and delamanid metabolites were found to inhibit some CYP isoforms only at considerably higher concentrations than observed in human plasma.[88] These results imply that drug–drug interactions with compounds that are metabolized by CYP enzymes, including antiretroviral drugs, are unlikely. However, this subject is being further assessed in clinical studies.[89]

Studies in mice, rats, guinea pigs, rabbits and dogs have been performed to shed light on the pharmacokinetic profile of

delamanid (Table 5). The delamanid dose currently approved for clinical use is 100 mg twice a day, taken with food.[90] In a randomized, placebo-controlled, multinational clinical trial, Gler *et al.*[91] found an AUC of 7.925 μg·h/mL in patients treated with delamanid 100 mg twice daily for 56 days. A slightly lower AUC_{0-24} of 3.40 μg·h/mL was reported by Mallikaarjun *et al.*[92] in humans for delamanid at the daily dose of 200 mg. Several dosing strategies in various animal studies resulted in AUC values similar to those in humans (Table 5). In mice, 2.5, 3, and 10 mg/kg at single oral administration and 2.5 mg/kg orally administered for 4 weeks in a combination regimen with bedaquiline and linezolid led to AUC values between 3.58 and 11.55 μg·h/mL.[10,87,92,93] Likewise, in rats, 3 and 10 mg/kg at single drug administration generated exposures of 7.9418 (AUC_{0-480}) and 5.68 (AUC_{0-96}) μg·h/mL, respectively.[87,94] In guinea pigs, a single dose of delamanid at 10 mg/kg resulted in a relatively low AUC_{0-48} of 2.32 μg·h/mL, while this was 9.45 μg·h/mL for a dose of 100 mg/kg.[95] Also in rabbits and dogs delamanid exposures matching clinical exposures were shown following a single oral dose of delamanid at 5 mg/kg[85] and 10 mg/kg,[87] respectively. Using a murine chronic TB infection model, Mallikaarjun *et al.*[92] found that the PK/PD driver for delamanid activity was described best by the AUC_{0-24}/MIC (Pearson's correlation coefficient = 0.97), and to a lesser extent by the $\%T_{>MIC}$ (Spearman correlation coefficient = 0.53). In that study, an AUC_{0-24}/MIC of 252 was determined to achieve 80% of the maximum activity of the drug in the mouse model. Based on the results from two human Early Bactericidal Activity studies, a mean AUC_{0-24}/MIC of 393 was established at a dose of 200 mg after 14 days of treatment.[92]

Pretomanid

Like delamanid, pretomanid is widely distributed among various organs. After a single oral administration of 40 mg/kg in rats, pretomanid was detectable in liver, heart, lung, spleen, kidney, stomach and intestine.[96] Pretomanid was shown to effectively cross the blood–brain barrier as well.[96–99] In rats, plasma concentrations were shown to be 5-fold higher than brain tissue concentrations, and 2.5-fold higher than lung tissue concentrations. However, this might be different for multiple dose administrations.[99]

In human plasma, 94% of pretomanid is protein bound.[72] Dogra *et al.*[31] found that after incubation of pretomanid with supernatant of human liver homogenates several minor metabolites could be identified, but not the des-nitro metabolite that is formed upon bio-activation of pretomanid by mycobacterial Ddn. Hence, while mycobacteria can activate pretomanid by des-nitrification, this process does not occur with human liver supernatant.[31] Preclinical[100,101] and clinical[102] studies have indicated that exposure to pretomanid is altered when co-administered with several other drugs. Together, these results imply that, compared with delamanid, albumin metabolism plays a smaller role for pretomanid, and that pretomanid is at least partly metabolized in the liver.[87] However, the exact metabolic pathway of pretomanid is yet to be unravelled, and mechanisms that underlie drug–drug interactions (e.g. CYP isoenzymes and drug transporters) require further study.

Results of various animal PK studies for pretomanid are summarized in Table 6. The methods of these studies are quite heterogeneous, using different animal species (mice, rats or guinea pigs), dose levels, treatment durations, routes of administration, and treatment combinations. In humans, the currently approved dose in the clinic is 200 mg once a day to be taken with food.[70] Human clinical trials have reported AUC_{0-t} values corresponding to this dosing regimen of 28.087 μg·h/mL (single dose administration, in fasted state, monotherapy), 51.643 μg·h/mL (single dose administration, in fed state, monotherapy),[103] 30.2 μg·h/mL (7 days treatment, monotherapy), 60.487 μg·h/mL (14 days treatment, combination regimen with bedaquiline, pyrazinamide and clofazimine), 61.534 μg·h/mL (14 days treatment, combination regimen with bedaquiline and clofazimine), and 76.292 μg·h/mL (14 days treatment, combination regimen with bedaquiline and pyrazinamide).[104] As can be seen in Table 6, similar drug exposure in mice was reached after administration of a single oral dose of 25 mg/kg.[105] A single oral dose administration of 54 mg/kg[106] and 4 weeks of daily oral treatment with 100 mg/kg in combination with either bedaquiline, moxifloxacin and pyrazinamide, or with bedaquiline and linezolid[107] resulted in AUC_{0-24} of 127.5, 104.2 and 99.13 μg·h/mL, respectively, which were slightly higher than the exposures reached in humans. However, at 100 mg/kg, another mouse study demonstrated higher AUC_{0-24} values ranging between 327.6 and 424.0 μg·h/mL.[108] In that study, pretomanid was either administered alone or within a combination regimen, and was given once or for 2 months.[108] None of the rat studies showed AUC values that equal human exposures.[96,99–101] In guinea pigs, a single oral administration of 50 mg/kg, and 7 day treatment with 25 mg/kg or 50 mg/kg administered twice daily, resulted in AUC values in the range of those observed in humans at the approved clinical dose.[109]

According to Ahmad *et al.*,[110] pretomanid activity was best described by the free drug $\%T_{>MIC}$ ($R^2 = 0.87$), followed by free drug AUC/MIC ($R^2 = 0.60$). In the same study, simulated $\%T_{>MIC}$ values in humans at a pretomanid dose of 200 mg were predicted to be 100%, assuming an MIC of 0.03125 mg/L. Such high $\%T_{>MIC}$ values were also reported in the clinical study by Diacon *et al.*[104] Although pretomanid at a dose of 25 mg/kg in mice resulted in exposure (AUC) comparable to exposure in humans, this dose led to lower plasma $\%T_{>MIC}$ values than observed in clinical studies.[105] Since both $\%T_{>MIC}$ and AUC/MIC are thought to be important drivers of efficacy for pretomanid, once daily dosing with 25 to 100 mg/kg has been used in mice in attempts to model $\%T_{>MIC}$ and AUC/MIC that are similar to those observed in patients. In conclusion, determining the appropriate dosing regimen in animal models that mimics both the AUC values and $\%T_{>MIC}$ encountered in the clinic is challenging. Ongoing mouse studies are exploring a lower dose of pretomanid at 50 mg/kg or lower, administered twice a day, in order to reflect the clinical drug exposure more accurately.

Doses used for delamanid in animal models are generally lower than those for pretomanid (Tables 5 and 6), while the indicated daily dose in humans is equal for both drugs (200 mg). In humans, drug exposures corresponding to this clinical dose are lower for delamanid than for pretomanid.[92,103,104,111,112] In mice, on the other hand, drug exposures following administration of delamanid or pretomanid at 25–30 mg/kg seem to be in the same range (AUC_{0-24}: 35.84 and 50.9 μg·h/mL, respectively).[87,105] Hence, it seems reasonable that delamanid is dosed

Table 5. Overview of pharmacokinetic parameters of delamanid evaluated in various animal studies

Reference	Animal model	Infected	Dose (mg/kg)	Single drug or combination	Treatment duration	Route of drug administration	Sample	Methods	T_{max} (h)	$T_{½}$ (h)	C_{max} (mg/L)	AUC time span	AUC (μg·h/mL)
Mallikaarjun *et al.* (2020)[92]	Mice, SLC:ICR	No	0.625	Single drug	Single-dose	Oral gavage	Plasma	HPLC-MS/MS			0.100	0–24	1.188
	Mice, SLC:ICR	No	2.5	Single drug	Single-dose	Oral gavage	Plasma	HPLC-MS/MS			0.297	0–24	3.581
	Mice, SLC:ICR	No	10	Single drug	Single-dose	Oral gavage	Plasma	HPLC-MS/MS			1.012	0–24	11.547
Matsumoto *et al.* (2006)[10]	Mice	Yes	2.5	Single drug	Single dose	Oral	Plasma	LC-ESI-MS/MS	6	7.6	0.297	0–24	4.13
Pieterman *et al.* (2021)[93]	Mice, BALB/c	Yes	2.5	BDQ (25) + LZD (100)	4 weeks	Oral gavage	Plasma	LC-MS/MS	0.75		0.864–1.080	0–24	11.234
Sasahara *et al.* (2015)[87]	Mice, ICR	No	3	Single drug	Single dose	Oral	Plasma	LC-MS/MS	2	7.2	0.4787	0–480	5.536
												0–∞	6.1508
	Mice, ICR	No	30	Single drug	Single dose	Oral	Plasma	LC-MS/MS			2.3141	0–24	35.8403
	Mice, ICR	No	30	Single drug	13 weeks	Oral	Plasma	LC-MS/MS			2.9209	0–24	36.5094
Ramirez *et al.* (2021)[94]	Rats, Sprague Dawley	No	10	Single drug	Single-dose	Oral gavage	Plasma	HPLC	3.4		0.256	0–96	5.68
Shibata *et al.* (2017)[84]	Rats, Sprague-Dawley; males, non-fasted	No	3	Single drug	Single dose	Oral	Blood	Radioactivity of ^{14}C-labelled delamanid	8	82.3	0.5818	0–168	19.4
												0–∞	22.8
	Rats, Sprague-Dawley; males, fasted	No	3	Single drug	Single dose	Oral	Blood	Radioactivity of ^{14}C-labelled delamanid	6.3	49.5	0.7351	0–168	19.6
												0–∞	20.9
	Rats, Sprague-Dawley; females, non-fasted	No	3	Single drug	Single dose	Oral	Blood	Radioactivity of ^{14}C-labelled delamanid	8	57.2	0.643	0–168	20.3
												0–∞	22.3
	Rats, Sprague-Dawley; females, fasted	No	3	Single drug	Single dose	Oral	Blood	Radioactivity of ^{14}C-labelled delamanid	5	59.8	0.8149	0–168	19.7
												0–∞	21.3
Sasahara *et al.* (2015)[87]	Rats, Sprague-Dawley	No	3	Single drug	Single dose	Oral	Plasma	LC-MS/MS	4	5.1	0.6005	0–480	7.9418
												0–∞	7.9698
	Rats, Sprague-Dawley	No	30	Single drug	Single dose	Oral	Plasma	LC-MS/MS			2.6953	0–24	36.6397
	Rats, Sprague-Dawley	No	30	Single drug	26 weeks	Oral	Plasma	LC-MS/MS			1.7992	0–24	34.2379
Chen *et al.* (2017)[95]	Guinea pigs	No	10	Single drug	Single dose	Oral	Plasma	HPLC-ESI-MS/MS			0.21	0–48	2.32
		No	100	Single drug	Single dose	Oral	Plasma	HPLC-ESI-MS/MS			0.53	0–48	9.45

Continued

Table 5. *Continued*

Reference	Animal model	Infected	Dose (mg/kg)	Single drug or combination	Treatment duration	Route of drug administration	Sample	Methods	T_{max} (h)	$T_{½}$ (h)	C_{max} (mg/L)	AUC time span	AUC (μg·h/mL)
Tucker *et al.* (2019)[85]	Rabbits, New Zealand White	Yes	5	Single drug	Single-dose	Oral gavage	Plasma	HPLC-MS/MS	12	13.9	0.2558	0–24	3.8112
												0–48	4.229
												0–∞	4.2553
	Rabbits, New Zealand White	No	5	Single drug	Single-dose	Oral gavage	Plasma	HPLC-MS/MS	12	14.1	0.1956	0–24	2.7856
												0–48	3.4613
												0–∞	3.5545
Sasahara *et al.* (2015)[87]	Dogs	No	10	Single drug	Single dose	Oral	Plasma	LC-MS/MS	8	18.4	0.3578	0–768	10.628
												0–∞	10.9275
	Dogs, beagle	No	30	Single drug	Single dose	Oral	Plasma	LC-MS/MS			0.3831		
	Dogs, beagle	No	30	Single drug	39 weeks	Oral	Plasma	LC-MS/MS			1.4007	0–24	21.7692

T_{max}, time until the highest concentration is reached; $T_{½}$, half-life time, time until the initial drug concentration is halved; C_{max}, highest concentration reached.

at lower levels than pretomanid in animal studies, in order to mimic exposures in humans at clinically approved doses.

In vivo activity

Animal models (mostly mouse models) are used to study the treatment response in a setting that approximates the complex environment encountered in TB-infected humans.[113] Numerous mouse TB models have been developed that differ in inoculation route and dose, incubation period, treatment duration, outcome assessment, and mouse strain.[113,114] Treatment outcome can be evaluated immediately after treatment completion (bactericidal activity) or a few months later to determine whether mice are cured nor not, which is defined by the absence of relapse (sterilizing activity).[113] However, most mouse strains develop cellular granulomas upon TB infection, instead of the necrotizing, caseous lesions observed in human pulmonary TB.[115] To study drug efficacy in the context of such necrotic lesions, other mouse strains (e.g. C3HeB/FeJ) or other animals (e.g. guinea pigs, rabbits or NHP) can be used.[116] In this section, studies on delamanid will be discussed first followed by pretomanid, after which the compounds will be compared. For drug combinations, only combinations that have been assessed for both delamanid and pretomanid are considered in this review.

Delamanid

An overview of results from different animal models evaluating the treatment response of delamanid is presented in Table 7. Multiple mouse models have demonstrated bactericidal activity of delamanid at doses as low as 0.313 mg/kg (range of tested doses: 0.078–100 mg/kg).[10,11,63,93,117–120] Dose-dependency of delamanid activity was shown in three studies.[10,11,120] Depending on the model, delamanid showed similar or higher bactericidal activity than rifampicin,[10,11,118] and activity of delamanid was shown to be equal in both immunocompromised and immunocompetent mice.[10] Two mouse studies demonstrated bactericidal activity of delamanid in animals presenting with hypoxic lesions.[95,117] Gengenbacher *et al.*[117] used *Nos2*$^{-/-}$ mice that develop hypoxic lung lesions upon dermal injection with *M. tuberculosis*. In this model, lung cfu counts significantly decreased after treatment with delamanid at 1 mg/kg for 3 weeks. Using a guinea pig TB model, Chen *et al.*[95] showed strong bactericidal activity of delamanid (100 mg/kg) administered for 8 weeks, as no cfu could be retrieved from the lung homogenates after treatment. This activity was similar to that of the standard HRZ-regimen.[95] The potential role for delamanid in the treatment of latent TB is unknown as no preclinical studies investigating this have been published at this current time.

Drug combination regimens containing delamanid have been studied to a lesser extent *in vivo* than combinations containing pretomanid (Table 8). Two combination regimens were studied for both compounds although not in the same experiment: (i) rifampicin and pyrazinamide together with delamanid (RDZ) [10,95] or pretomanid (RPaZ),[106,108] and (ii) bedaquiline and linezolid either combined with delamanid (BDL)[93] or pretomanid (BPaL).[107,121–123] Unfortunately, no head-to-head comparisons of delamanid- or pretomanid-containing drug combinations have been published to date. The RDZ regimen (delamanid at

Table 6. Overview of pharmacokinetic parameters of pretomanid evaluated in various animal studies

Reference	Animal model	Infected	Dose (mg/kg)	Treatment combination	Treatment duration	Route of drug administration	Sample	Methods	Pretomanid T_{max} (h)	$T_{½}$ (h)	C_{max} (mg/L)	AUC time span	AUC (μg·h/mL)
Lakshminarayana *et al.* (2014)[105]	Mice, CD-1	No	25	No		Oral	Plasma	LC-MS/MS	2	2.7	6	0–24	50.9
			10	No		Intravenous	Plasma	LC-MS/MS		1.6			
Nuermberger *et al.* (2006)[108]	Mice, BALB/c	Yes	100	No	Single dose	Oral gavage	Serum	HPLC	4.7	12.8	21.4	0–24	327.6
	Mice, BALB/c	Yes	100	No	2 months	Oral gavage	Serum	HPLC	1.3	18.3	25	0–24	396.8
	Mice, BALB/c	Yes	100	RIF (10) + INH (25) + PZA (150)	Single dose	Oral gavage	Serum	HPLC	11.0	11.3	20.4	0–24	370.5
	Mice, BALB/c	Yes	100	RIF (10) + INH (25) + PZA (150)	2 months	Oral gavage	Serum	HPLC	3.3	9.7	27.7	0–24	424
Tasneen *et al.* (2008)[106]	Mice, BALB/c		54		Single dose	Oral	Serum				15.1	0–∞	127.5
Ahmad *et al.* (2011)[110]	Mice, BALB/c	No	3–1458	Single drug	Single dose	Esophagal gavage	Serum	HPLC	4	4–6			
Mudde *et al.* (2021)[107]	Mice, BALB/c	Yes	100	BDQ (25) + MXF (100) + PZA (150)	4 weeks	Oral	Serum	LC-MS/MS			6.89–7.03	0–24	104.2
	Mice, BALB/c	Yes	100	BDQ (25) + LZD (100)	4 weeks	Oral	Serum	LC-MS/MS			7.70–9.50	0–24	99.13
Wang *et al.* (2018)[101]	Rats, Sprague-Dawley	No	20	Single drug	Single dose	Oral	Plasma	LC-MS/MS	5	5.6	3.87	0–36	2678.74
												0–∞	2787.23
Wang *et al.* (2015)[96]	Rats, Sprague-Dawley	No	20	Single drug	Single dose	Oral	Plasma	LC-MS/MS	6	8.3	3.48	0–36	3291.9
												0–∞	3552.7
	Rats, Sprague-Dawley	No	40	Single drug	Single dose	Oral	Plasma	LC-MS/MS	6	6.2	7.98	0–36	5850.9
												0–∞	6007.9
	Rats, Sprague-Dawley	No	80	Single drug	Single dose	Oral	Plasma	LC-MS/MS	6	7.4	15.29	0–36	12445.1
												0–∞	13072.1
Bratkowska *et al.* (2015)[99]	Rats, Sprague Dawley	No	20	Single drug		Oral	Plasma	LC-MS/MS	6		0.63	0–∞	3.7248
	Rats, Sprague Dawley	No	20	Single drug		Intraperitoneal	Plasma	LC-MS/MS	0.25		1.15	0–∞	3.9885

Continued

Table 6. *Continued*

Reference	Animal model	Infected	Dose (mg/kg)	Treatment combination	Treatment duration	Route of drug administration	Sample	Methods	Pretomanid T_{max} (h)	$T_{½}$ (h)	C_{max} (mg/L)	AUC time span	AUC (μg·h/mL)
Wang *et al.* (2014)[100]	Rats, Sprague-Dawley		20	Single drug	Single dose	Oral	Plasma	LC-MS/MS	6		3.485	0–36	3297.503
												0–∞	3558.315
	Rats, Sprague-Dawley		20	MXF (40) + PZA (160)	Single dose	Oral	Plasma	LC-MS/MS	4.6		6.388	0–36	4851.288
												0–∞	5052.658
Sung *et al.* (2009)[136]	Guinea pigs	No	20			Intravenous	Plasma	HPLC	0.11	1.91	9.19	0–24	26.54
	Guinea pigs	No	40			Oral gavage	Plasma	HPLC	4.00	2.43	4.14	0–24	25.77
	Guinea pigs	No	20			Insufflation	Plasma	HPLC	4.33	2.83	2.01	0–24	14.80
	Guinea pigs	No	40			Insufflation	Plasma	HPLC	3.25	4.38	3.42	0–24	32.34
	Guinea pigs	No	60			Insufflation	Plasma	HPLC	3.60	5.91	4.58	0–32	50.96
Dutta *et al.* (2013)[109]	Guinea pigs	No	12.5	Single drug	Single dose	Oral	Serum	HPLC	2.65	1.94	1.68	0–∞	11.19
	Guinea pigs	No	25 (BID)	Single drug	7 days	Oral	Serum	HPLC	2.25	4.7	2.99	0–∞	42.19
	Guinea pigs	No	50	Single drug	Single dose	Oral	Serum	HPLC	2.66	3.16	5.84	0–∞	39.79
	Guinea pigs	No	50 (BID)	Single drug	7 days	Oral	Serum	HPLC	7	2.16	5.79	0–∞	70.95

T_{max}, time until the highest concentration is reached; $T_{½}$, half-life time, time until the initial drug concentration is halved; C_{max}, highest concentration reached; BID, bis in die, i.e. twice a day.

2.5 mg/kg) showed promising bactericidal activity in a mouse TB model, reaching culture-negativity at least 2 months faster than the standard regimen consisting of isoniazid, rifampicin, pyrazinamide and ethambutol (HRZE).[10] Similar results of the RDZ regimen (delamanid at 100 mg/kg) were found by Chen *et al.*[95] in a guinea pig TB model. Delamanid combined with bedaquiline and linezolid was recently evaluated by Pieterman *et al.*[93] Mice were infected with *M. tuberculosis* of the Beijing genotype via intratracheal instillation. Two weeks later, treatment was started with BDL (delamanid at 2.5 mg/kg) via oral gavage for 2 to 6 months. The mycobacterial load in the lungs was assessed both directly following treatment completion, and three months later to evaluate whether the infection had relapsed or not. Treatment with BDL was highly effective. Of the 15 mice treated with BDL for 4 months or longer, only 1 mouse relapsed. In the HRZE-group on the other hand, relapse rates were much higher, and after 6 months of treatment there were still bacteria in 1 out of 3 mice that could be cultured from the lungs.

Pretomanid

The *in vivo* bactericidal activity of pretomanid as a monotherapy has been evaluated in various animal studies (Table 7). In mice, pretomanid showed bactericidal activity at dose levels of 12.5–20 mg/kg or higher (range of tested doses: 1.25–600 mg/kg).[10,12,66,71,108,119,120,124,125] In several studies, the activity of pretomanid (40–100 mg/kg) was similar to that of isoniazid (25 mg/kg) [12,66,124] and rifampicin (20 mg/kg).[66] The rank order in activity was slightly different in two mouse models of latent TB infection using BCG-immunized mice,[125,126] with pretomanid (50 mg/kg) showing less activity than rifampicin (10 mg/kg), although the activity was similar to that of isoniazid (10 mg/kg). Pretomanid's promising activity in animals presenting with hypoxic pulmonary lesions was demonstrated in various animal models, including a *Nos2*$^{-/-}$ mouse model,[117] a C3HeB/FeJ mouse model,[126] and two guinea pig models.[12,127] The ability of pretomanid as monotherapy to cure latent TB was evaluated in one murine study using BCG-vaccinated C3HeB/FeJ mice.[126] In all mice (15/15), the infection relapsed after 4 months of treatment with only pretomanid (50 mg/kg). The same outcome was observed for isoniazid (10 mg/kg), while rifampicin (10 mg/kg) performed better with a relapse rate of 33%. Selection of resistant colonies was, however, not part of the published study.

Five studies have evaluated the bactericidal activity of both delamanid and pretomanid.[10,63,117,119,120] Again, limited information is available where both compounds are evaluated side by side in the same model, and in the same experiment. Interestingly, in all five studies, the bactericidal activity of delamanid was superior to that of pretomanid. Delamanid led to higher load reductions than pretomanid at equal dose levels,[10,63,119] or required lower dose levels than pretomanid to achieve a comparable load reduction.[10,117,120] However, comparing the bactericidal activity of the compounds in the light of drug exposure rather than dose levels adds nuance to the presumed superiority of delamanid. The clinically approved dosing regimen of delamanid (100 mg, twice a day) is reported to result in AUC values between 3.40 and 10.673 μg·h/mL.[92,112] Higher AUC values of 28.087 to 76.292 μg·h/mL were reported for pretomanid in clinical studies (200 mg, once a day), with %$T_{>MIC}$

Table 7. Summary of treatment activity of delamanid and pretomanid as a monotherapy in various animal models of tuberculosis

Author	Animal (inoculation route)	*M. tuberculosis* strain	Time until start of treatment	Drug treatment (dose, mg/kg)	Treatment duration	Route of drug administration	Drug exposure	Outcome
Gengenbacher *et al.* (2017)[117]	*Nos2*$^{-/-}$ mice (intradermal)	H37Rv	42 days (control) or 56 days (hypoxic lung lesions)	DLM (1); PMD (75)	70–84 days	Oral	NA	DLM and PMD were both active against non-replicating and replicating bacilli, and had comparable bactericidal activity in hypoxic necrotic lesions.
Tasneen *et al.* (2015)[120]	BALB/c mice (aerosol)	H37Rv	13–14 days	DLM (3–100); PMD (10–600)	2–8 weeks	Oral	NA	DLM and PMD showed time-dependent and dose-dependent bactericidal activity. DLM was approximately 10-fold more active than PMD.
Upton *et al.* (2015)[63]	BALB/c mice (aerosol)	Erdman	10 days	DLM; PMD (100)	3 weeks	Oral	NA	DLM was significantly more active than PMD in this model of acute infection. DLM led to a 1 $\log_{10}$ reduction in lung cfu. PMD inhibited mycobacterial growth, but did not reduce lung cfu.
	BALB/c mice (aerosol)	Erdman	70 days	DLM; PMD (100)	3 weeks	Oral	NA	DLM was significantly more active than PMD in this model of chronic infection. DLM led to a 2 to 3 $\log_{10}$ reduction in lung cfu. PMD led to a 2 $\log_{10}$ reduction in lung cfu.
Kmentova *et al.* (2010)[119]	BALB/c mice		70 days	DLM; PMD (100)	3 weeks	Oral	NA	DLM was 10-fold more active than PMD, with 3 $\log_{10}$ versus 2 $\log_{10}$ reduction in lung cfu, respectively.
Matsumoto *et al.* (2006)[10]	ICR mice (intravenous)	Kurono	4 weeks	DLM (0.156–40); PMD (1.25–40)	4 weeks	Oral	AUC_{0-24} = 4.13 μg·h/mL (single dose of 2.5 mg/kg DLM)	DLM led to a dose-dependent reduction in lung cfu. For PMD, RIF and INH higher doses were needed to equal the load reduction by DLM.
	BALB/c (nude) mice (intravenous)	Kurono	1 day	DLM (0.313–10)	10 days	Oral	AUC_{0-24} = 4.13 μg·h/mL (single dose of 2.5 mg/kg)	DLM led to a dose-dependent reduction in lung cfu, which was equal in immunodeficient and immunocompetent mice.
Sasaki *et al.* (2006)[11]	ICR mice (intravenous)	Kurono	1 day	DLM (0.5–10)	10 days	Oral	NA	DLM led to a 2.5 $\log_{10}$ to >4.4 $\log_{10}$ reduction in lung cfu, which was superior to RIF (5 mg/kg).
	ICR mice (intravenous)	Kurono	1 day	DLM (0.078–2.5)	28 days	Oral	NA	DLM led to a dose-dependent reduction in lung cfu. DLM activity (0.313 mg/kg) was similar to RIF (5 mg/kg).

Continued

Table 7. *Continued*

Author	Animal (inoculation route)	*M. tuberculosis* strain	Time until start of treatment	Drug treatment (dose, mg/kg)	Treatment duration	Route of drug administration	Drug exposure	Outcome
Hariguchi *et al.* (2020)[118]	ICR mice (intratracheal inoculation)	Kurono	4 weeks	DLM (2.5)	4 weeks	Oral	NA	DLM led to a significant 1.5 $\log_{10}$ reduction of lung cfu, which was similar to RIF (5 mg/kg)
Pieterman *et al.* (2021)[93]	BALB/c mice (intratracheal instillation)	Beijing	2 weeks	DLM (1.25, 2.5 or 5)	3 weeks	Oral	AUC_{0-24} = 11.234 μg·h/mL (4 weeks treatment, dose 2.5 mg/kg, combined with BDQ 25 mg/kg + LZD 100 mg/kg)	DLM led to a 2 $\log_{10}$ reduction in lung cfu for all tested doses.
Chen *et al.* (2017)[95]	Guinea pig (intratracheal inoculation)	Kurono	4 weeks	DLM (100)	4 or 8 weeks	Oral	AUC_{0-24} = 9.45 μg·h/mL (single dose of 100 mg/kg)	DLM led to a 3 $\log_{10}$ reduction in lung cfu after 4 weeks of exposure. No cfu were retrieved after 8 weeks of exposure. DLM showed bactericidal activity in hypoxic lesions.
Stover *et al.* (2000)[12]	BALB/c mice (intravenous)	H37Rv	4 days	PMD (25, 50, or 100)	10 days	Oral	NA	PMD led to a dose-dependent reduction in lung cfu. PMD activity (25 mg/kg) was similar to INH activity (25 mg/kg).
Tyagi *et al.* (2005)[124]	BALB/c mice (aerosol)	H37Rv	20 days (initial phase), 8 weeks (continuation phase)	PMD (3.125–200)	4–16 weeks	Oral	NA	PMD activity (100 mg/kg) was comparable to that of INH (25 mg/kg). PMD was active during both the initial and continuation phase of therapy.
Lenaerts *et al.* (2005)[66]	C57BL/6 mice (aerosol)	Erdman	19 days	PMD (50, 100, or 300)	9 days	Oral	NA	PMD showed dose-dependent activity. PMD activity (100 mg/kg) was similar to that of RIF (20 mg/kg) and INH (25 mg/kg).
	C57BL/6 mice (aerosol)	Erdman	19 days	PMD (100)	12 weeks	Oral	NA	PMD (100 mg/kg) was as active as INH (25 mg/kg).
Lakshminarayana *et al.* (2014)[105]	BALB/c mice (intranasal)	H37Rv	4 weeks	PMD (25 or 100)	4 weeks	Oral	AUC_{0-24} = 50.9 μg·h/mL (dose 25 mg/kg)	At 25 mg/kg PMD led to a 1.48 $\log_{10}$ reduction in lung cfu, and to a 2.3 $\log_{10}$ reduction at 100 mg/kg.
Nuermberger *et al.* (2006)[108]	BALB/c mice (aerosol)	H37Rv	19 days	PMD (100)	2 months	Oral	AUC_{0-24} = 396.8 μg·h/mL (2 months treatment, dose 100 mg/kg)	PMD led to a 2 $\log_{10}$ reduction in lung cfu.

Tasneen *et al.* (2008)[106]	BALB/c	H37Rv	2 weeks	PMD (100)	2 months	Oral	$AUC_{0-\infty}$ = 127.5 μg·h/mL (single dose of 54 mg/kg)	PMD led to a 2.7 log_{10} reduction in lung cfu, which was slightly inferior to the 3.1 log_{10} reduction by RIF (10 mg/kg).
Sala *et al.* (2010)[71]	BALB/c mice (intravenous)	18b without streptomycin	4 weeks	PMD (100)	8 weeks	Oral	NA	PMD led to a 1.5 log_{10} reduction in lung cfu, which was superior to INH (25 mg/kg), but inferior to RIF (10 mg/kg).
Lanoix *et al.* (2014)[125]	BCG-immunized-BALB/c mice (aerosol)	H37Rv	6 weeks	PMD (50)	8 weeks	Oral	NA	PMD led to a 1 log_{10} reduction in lung cfu, which was similar to INH (10 mg/kg), but inferior to RIF (10 mg/kg)
	BCG-immunized-C3HeB/FeJ mice (aerosol)	H37Rv	6 weeks	PMD (50)	8 weeks	Oral	NA	PMD led to a 0.75 log_{10} reduction in lung cfu, which was similar to INH (10 mg/kg), but inferior to RIF (10 mg/kg)
Dutta *et al.* (2014)[126]	BCG-immunized-C3HeB/FeJ mice (aerosol)	H37Rv	6 weeks	PMD (50)	1–4 months	Oral	NA	PMD led to a 2.7 log_{10} reduction in lung cfu, which was comparable to INH (10 mg/kg), but inferior to RIF (10 mg/kg). The relapse rate of PMD (assessed 3 months after completion of a 4-month treatment duration) was 100%, which was equal to INH, and higher than RIF (33%).
Stover *et al.* (2000)[12]	Guinea pig (aerosol)	H37Rv	4 weeks	PMD (40)	4 weeks	Oral	NA	PMD led to a 1 log_{10} reduction in lung cfu, which was comparable to INH (25 mg/kg).
Garcia-Contreras *et al.* (2010)[127]	Guinea pig (aerosol)	H37Rv	4 weeks	PMD (inhaled: 180 or 360 mg; oral: 40 mg/kg)	4 weeks	Inhaled or oral	NA	PMD led to a significant reduction of the mycobacterial load. Higher PMD activity was observed for oral administration versus inhaled doses.

DLM, delamanid; PMD, pretomanid; NA, not assessed; cfu, colony forming units; RIF, rifampicin; INH, isoniazid; BDQ, bedaquiline; LZD, linezolid.

Table 8. Summary of treatment efficacy of delamanid and pretomanid within various drug combination regimens in animal models of tuberculosis

Author	Animal (infection route)	*M. tuberculosis* strain	Incubation period until start of treatment	Drug combination (dose in mg/kg)	Treatment duration	Route of drug administration	Exposure to DLM or PMD	Outcome	
								Bactericidal activity	Relapse rates
Matsumoto *et al.* (2006)[10]	ICR mice (intratracheal instillation)	Kurono	28 days	2 months RIF (5) + DLM (2.5) + PZA (100) and 2 months RIF (5) + DLM (2.5)	4 months	Oral	AUC_{0-24} = 4.13 μg·h/mL (monotherapy, single dose of 2.5 mg/kg)	Faster culture-negativity (by at least 2 months) in the lungs compared to the standard regimen (HRZE).	NA
Chen *et al.* (2017)[95]	Guinea pigs (intratracheal instillation)	Kurono	4 weeks	RIF (25) + DLM (100) + PZA (150)	4 or 8 weeks	Oral	AUC_{0-24} = 9.45 μg·h/mL (monotherapy, single dose of 100 mg/kg)	Culture-negativity in the lungs was reached after 4 weeks of treatment versus 8 weeks for the standard regimen (HRZ).	NA
Nuermberger *et al.* (2006)[108]	BALB/c mice (aerosol)	H37Rv	19 days	2 months RIF (10) + PMD (100) + PZA (150) and 4 months RIF (10) + PMD (100)	6 months	Oral	AUC_{0-24} = 396.8 μg·h/mL (monotherapy, 2 months treatment, dose 100 mg/kg)	Culture-negativity in the lungs was reached after 4 months of treatment versus 6 months for the standard regimen (HRZ). This difference was not statistically significant.	Relapse rates were comparable to those of the standard HRZ-regimen (2/19 versus 0/46, respectively).
Tasneen *et al.* (2008)[106]	BALB/c mice (aerosol)	H37Rv	2 weeks	RIF (10) + PMD (12.5/25/50/100) + PZA (150)	2, 4, 5, or 6 months	Oral	$AUC_{0-\infty}$ = 127.5 μg·h/mL (monotherapy, single dose of 54 mg/kg)	PMD at 50 and 100 mg/kg increased activity of RIF + PZA in a dose-dependent manner. Culture-negativity in the lungs was reached after 2 months of treatment (PMD 100 mg/kg).	No relapse was seen after 4 months of treatment versus a relapse rate of 15% for the regimen (HRZ).
Pieterman *et al.* (2021)[93]	BALB/c mice (intratracheal instillation)	Beijing	2 weeks	BDQ (25) + DLM (2.5) + LZD (100)	2–6 months	Oral	AUC_{0-24} = 11.234 μg·h/mL (4 weeks treatment, dose 2.5 mg/kg, BDL combination)	Culture negativity in the lungs was reached after 2 months of treatment versus 20 weeks for the standard regimen (HRZE).	No relapse was seen after treatment duration of 4 months or longer (except for 1 mouse, treated for 5 months). HRZE-treated mice still relapsed after 6 months of treatment (1/3 mice).
Tasneen *et al.* (2016)[128]	BALB/c mice (aerosol)	H37Rv	13–14 days	BDQ (25) + PMD (50) + LZD (100)	2–4 months	Oral	NA	Two and 3 months of treatment led to a significantly lower mycobacterial load in the lungs compared to the standard regimen (HRZ).	No relapse was seen after 3 months of treatment. Infection still relapsed in HRZ-treated mice after 4 months of treatment.
Xu *et al.* (2019)[123]	BALB/c mice (aerosol)	H37Rv	13 days	BDQ (25) + PMD (100) + LZD (100)	1–4 months	Oral	NA	Addition of PMD to BDQ + LZD led to a higher mycobacterial load reduction when	After 2 months of treatment with BPaL, infection relapsed in 7/15 mice. No relapse

								administered for 1 and 2 months and prevented the emergence of BDQ resistance.	was seen after 3 months of treatment.
Bigelow *et al.* (2020)[121]	BALB/c mice (aerosol)	H37Rv or HN878	2 weeks	BDQ (25) + PMD (50 or 100) + different LZD dosing strategies (45 or 90)	1–3 months	Oral	NA	BDQ + PMD with different dosing strategies for LZD resulted in a higher mycobacterial load reduction compared to the standard regimen (HRZE). LZD's contribution to BDQ + PMD + LZD regimens was dependent on the *M. tuberculosis* strain.	Relapse rates were highly variable between the different LZD dosing strategies, LZD (90 mg/kg) dosed every other day leading to the highest relapse rate (11/15 mice) and LZD (90 mg/kg) dosed daily to the lowest relapse rates (1/15 mice)
Xu *et al.* (2021)[122]	BALB/c mice (aerosol)	H37Rv	2 weeks	BDQ (25) + PMD (100) + LZD (100)	1 month	Oral	NA	Lung cfu were reduced by approximately 2 $\log_{10}$.	NA
Mudde *et al.* (2021)[107]	BALB/c mice (intratracheal instillation)	Beijing	2 weeks	BDQ (25) + PMD (100) + LZD (100)	6–13 weeks	Oral	AUC_{0-24} = 104 and 99.13 μg·h/mL (4 weeks treatment, dose 100 mg/kg, BPaMZ combination or BPaL combination, respectively)	NA	Mice treated for the maximum duration of 13 weeks still showed relapse (3/3 mice).
Tasneen *et al.* (2021)[137]	BALB/c mice (aerosol)	HN878 (Beijing subfamily)	7 weeks	BDQ (25) + PMD (100) + LZD (100)	1 or 2 months	Oral	NA	1 month of BPaL treatment led to a 3.87 $\log_{10}$ reduction in lung cfu.	After 2 months of treatment with BPaL, infection relapsed in 7/15 mice. After 3 months of treatment with HRZE, infection relapsed in 9/15 mice.

DLM, delamanid; PMD, pretomanid; RIF, rifampicin; PZA, pyrazinamide; HRZE, isoniazid + rifampicin + pyrazinamide + ethambutol; HRZ, isoniazid + rifampicin + pyrazinamide; BDQ, bedaquiline; LZD, linezolid; BDL, bedaquiline + delamanid + linezolid; BPaMZ, bedaquiline + pretomanid + moxifloxacin + pyrazinamide; BPaL, bedaquiline, pretomanid, linezolid.

(an important driver of efficacy) up to 100%.[103,104] In mice, AUC values similar to the ones measured in patients were found for delamanid at dose levels of 2.5 to 10 mg/kg (Table 5).[10,87,92,93] Although pretomanid is often dosed at 100 mg/kg in mice,[12,63,66,71,105,106,108,119] in order to model the $\%T_{>MIC}$ achieved in patients, lower dose levels of pretomanid (25 to 54 mg/kg) lead to AUC values more closely reflecting AUC values reported in humans (Table 6).[105,106] Matsumoto *et al.*[10] reported that in their mouse model of TB infection, delamanid at 2.5 mg/kg showed similar bactericidal activity to pretomanid at 20 mg/kg, as both dose levels reduced the mycobacterial burden in the lungs by 1.9 $\log_{10}$ cfu after 4 weeks of treatment. In line with these results, Tasneen *et al.*[120] observed in their mouse TB model that 8 weeks of treatment with either delamanid at 2.5 mg/kg or pretomanid at 30 mg/kg resulted in a 1.6 $\log_{10}$ reduction in lung cfu counts. Taken together, these results indicate that when the compounds are compared at dose levels equivalent to those in humans based on AUC, their bactericidal activity is quite similar.

As to the performance of pretomanid in combination with other TB drugs, the combination of pretomanid, rifampicin and pyrazinamide (RPaZ) demonstrated higher bactericidal activity than the standard HRZ regimen in two mouse TB models (Table 8).[106,108] Relapse rates, however, did not seem to differ considerably between the two regimens.[106,108] Pretomanid combined with bedaquiline and linezolid (BPaL) performed better than the standard regimen in various mouse TB models, in terms of bactericidal activity and relapse rates.[107,121,122,128] BPaL and BDL were studied in two separate studies using the same experimental set-up, except that treatment with BDL lasted 8 to 24 weeks, while this was 6 to 13 weeks for BPaL. For both drug regimens, at least 2 to 2.5 months of treatment were needed to prevent relapse in some of the mice.[93,107]

Discussion

With this review, we aimed to provide an overview of preclinical data on the nitroimidazoles delamanid and pretomanid. Both compounds have contributed considerably to the change of the TB treatment landscape during the last decade, and are expected to further impact the improvement of TB treatment in the coming years. Although both compounds belong to the same drug class and share many similarities, we identified several differences between the drugs, shaping the context in which results from preclinical research on delamanid and pretomanid could inform clinical studies.

Based on what is known in the published literature, the mode of action of delamanid and pretomanid seems to differ slightly. Both compounds affect mycolic acid synthesis. Pretomanid only inhibits synthesis of ketomycolates [12] and not methoxymycolates, whereas delamanid inhibits the synthesis of both these classes.[9,10] Although both compounds intervene in aerobic respiration, pretomanid activity generates the formation of reactive nitrogen species,[16,28] whereas an NAD–delamanid adduct is thought to contribute to the antimycobacterial activity of delamanid.[34] Apart from the nitroimidazole ring, delamanid and pretomanid have distinct chemical structures (Table 1). However, the structural components that are thought to be involved in the antimycobacterial activity of delamanid and pretomanid are shared between the two drugs.[28,34]

Of particular interest is the finding that certain *M. tuberculosis* isolates with preserved susceptibility to delamanid, are resistant to pretomanid (or the other way around).[29,36,49] Here, the different chemical structure of the compounds could play a role in terms of the binding orientation to Ddn. Lee *et al.*[29] demonstrated that the dual methoxy and phenoxy-methyl substituents on the C6 position of the oxazole ring cause delamanid to bind differently to Ddn than pretomanid, which contains an oxazine ring with only a single substituent at the equivalent position. As such, certain mutations in *ddn* could result in pretomanid resistance while retaining the ability to activate delamanid. The fact that drug resistance has been found in *M. tuberculosis* isolates from patients who have not been treated with delamanid or pretomanid, implies that resistance to these drugs might arise due to genetic drift.[29] Indeed, the genes associated with delamanid and pretomanid resistance are genetically diverse. Various gene mutations might result in drug resistance, while at the same time several genetic variances have been reported that were not associated with drug resistance.[39,44,46,48,68] Therefore, it is not easy to pinpoint specific mutations that indicate under what circumstances delamanid and pretomanid can replace each other in the case of drug resistance or drug intolerance. Drug susceptibility testing before and during TB treatment could be performed to overcome this problem and adapt treatment regimens accordingly.

One critical hiatus in our comparison of preclinical studies is the limited amount of available head-to-head data where both compounds are tested in the same assay or model, in the same laboratory at the same time. In order to have an accurate preclinical comparison, more one-on-one preclinical studies will have to be conducted. In addition, we acknowledge the fact that the complexity of human TB infection is not easily captured in *in vitro* assays and *in vivo* preclinical models. Therefore, the preclinical performance of compounds is an informative approximation of their effect in humans. The comparison of results between different *in vitro* assays is further complicated by the great variety in experimental design, including differences in treatment duration, metabolic state of *M. tuberculosis*, methods of inducing a non-replicating state, and treatment dosing. Each of these variables could considerably impact the results on drug activity. This also accounts for *in vivo* TB models, with differences in animals used, inoculation dose, route of infection, incubation time, treatment dose, treatment duration, and outcome assessment. However, we also regard this plethora of assays and testing models as an advantage as every tool will provide more, and often complementary, information on both compounds under various conditions.

When comparing delamanid and pretomanid in terms of bactericidal activity *in vitro*, delamanid is more potent than pretomanid, with lower MIC values (0.001–0.024 mg/L versus 0.012–0.200 mg/L, respectively, based on head-to-head comparisons)[10,49,63] and delamanid effects higher mycobacterial load reductions *in vitro*, at lower drug concentrations than pretomanid (Tables 3 and 4). In various mouse models of TB infection, delamanid reduced the mycobacterial load in the lungs of mice to a greater extent than pretomanid when the drugs were administered at equal doses (Table 7),[10,63,119] and comparable load reductions were established when delamanid was dosed at lower levels than pretomanid.[10,117,120] However, it is more informative to compare the activity of delamanid and pretomanid after administration to mice at dose levels that result in

drug exposures similar to those achieved at the approved clinical dose. For delamanid, this would be 2.5 to 10 mg/kg in mice, as corresponding AUC values are in the same range as AUC values reported in humans at its clinically approved dose.[10,87,92,93,112] For pretomanid, administration of 25 to 54 mg/kg in mice was reported to result in human-equivalent dose exposures, based on AUC, although 25 mg/kg was reported to result in lower % $T_{>MIC}$ lower than achieved in the clinic.[103–106] In fact, in two different mouse TB models, the activity of delamanid at 2.5 mg/kg was shown to be similar to pretomanid at 20 mg/kg and 30 mg/kg.[10,120] Considering that for delamanid AUC_{0-24}/MIC was found to be the main driver of activity [92] and that its activity is dose-dependent in tested concentrations up to 100 mg/kg in mice,[10,120] one might speculate that if higher drug exposures could be safely reached in the clinic, delamanid might be expected to do better than at its currently approved clinical dose. This may also hold true for pretomanid, for which $\%T_{>MIC}$ is the most important driving PK/PD index (and this is already >90% at the approved clinical dose), but for which AUC/MIC is also an important driver of efficacy.[104,110]

As to delamanid-containing and pretomanid-containing regimens, no head-to-head comparisons have yet been published. In animal studies, the drug combinations where delamanid or pretomanid replaced isoniazid in the standard regimen, as well as the relatively new BPaL and BPaMZ regimens, showed higher activity and achieved cure following a shorter treatment period than the HRZE regimen. Especially for pretomanid, many other drug combinations have been assessed *in vivo*,[123,129–131] whereas delamanid-containing TB regimens were studied to a lesser extent. Studying the substitution of pretomanid for delamanid in future studies on the efficacy of novel TB drug regimens would be worthwhile, as delamanid might be a valuable alternative to pretomanid (or vice versa) in the case of drug resistance, intolerance or significant drug–drug interactions.

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Transparency declarations

None to declare.

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