WHO operational handbook on tuberculosis

Module 3: Diagnosis

Rapid diagnostics for tuberculosis detection

Third edition

Web Annex B. Critical concentrations for pretomanid and cycloserine

WHO policy statement

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Abbreviations and acronyms

7H10	Middlebrook 7H10
7H11	Middlebrook 7H11
Bdq	bedaquiline
BPaC	bedaquiline, pretomanid and clofazimine
BPaL	bedaquiline, pretomanid and linezolid
BPaLM	bedaquiline, pretomanid, linezolid and moxifloxacin
BPaMZ	bedaquiline, pretomanid moxifloxacin and pyrazinamide
BPaZ	bedaquiline, pretomanid and pyrazinamide
BPaZC	bedaquiline, pretomanid, clofazimine and pyrazinamide
CC	critical concentration
CDC	Centers for Disease Control and Prevention, USA
CI	confidence interval
C_{max}	maximum concentration
Cs	cycloserine
DR-TB	drug-resistant tuberculosis
ECOFF	epidemiological cut-off
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GTB	Global TB Programme
HR	isoniazid–rifampicin
HRZE	isoniazid–rifampicin–ethambutol–pyrazinamide
IHMT	Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal
IML Red	IML Red GmbH, Gauting, Germany
IQR	interquartile range
L	lineage
LJ	Löwenstein-Jensen
Lzd	linezolid
MDR-TB	multidrug-resistant tuberculosis
Mfx	moxifloxacin
MGIT	mycobacterial growth indicator tube
MIC	minimum inhibitory concentration
Mtb	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
MZ	moxifloxacin and pyrazinamide

NICD	National Institute for Communicable Diseases, South Africa
NITRD	National Institute of Tuberculosis and Respiratory Diseases, India
OSR	Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
Pa	pretomanid
pNWT	phenotypically non-wildtype
pWT	phenotypically wildtype
PZA	pyrazinamide
SRL	WHO TB Supranational Reference Laboratory
SRL-Germany	WHO TB Supranational Reference Laboratory, Germany (Borstel)
SRL-Sweden	WHO TB Supranational Reference Laboratory, Sweden (Stockholm)
SU	Stellenbosch University, Stellenbosch, South Africa
TAG	Technical Advisory Group
TB	tuberculosis
TTNS	time to culture negative status
Tzd	terizidone
UCL	University College London Centre for Clinical Microbiology, London, United Kingdom of Great Britain and Northern Ireland
Ultra	Xpert MTB/RIF Ultra
USA	United States of America
WHO	World Health Organization
XDR-TB	extensively drug-resistant tuberculosis

WHO policy statement

Strengthening health delivery systems is critical to achieving the global targets towards ending the tuberculosis (TB) epidemic; such strengthening includes introducing accurate diagnostic methods for detection of resistance to anti-TB agents.[1] Pretomanid and cycloserine are used to treat individuals with drug-resistant TB. However, there is no established phenotypic drug susceptibility testing method or interpretive criteria to define resistance. To address this gap, the specification of critical concentrations (CCs) for these drugs is needed, informed by epidemiological cut-off values and by pharmacokinetic, pharmacodynamic and clinical outcome data where available.

The World Health Organization (WHO) initiated a systematic search and analysis of the available evidence, which was then assessed by the WHO Technical Advisory Group (TAG) on TB Diagnostics and Laboratory Strengthening.

Following review of the evidence and advice from the TAG, WHO makes the following policy statements:

1. Two test concentrations (**0.5 and 2.0 mg/L**) should be used for pretomanid drug susceptibility testing, using the mycobacterial growth indicator tube (MGIT™) method with the following interpretation:
    - no growth at 0.5 mg/L = susceptible;
    - growth at 0.5 mg/L and no growth at 2.0 mg/L = susceptible, with a comment added to the laboratory report stating there is an interpretive uncertainty of this result and close patient follow-up is required; and
    - growth at 2.0 mg/L = resistant.
2. A CC of **16 mg/L** should be used for cycloserine drug susceptibility testing using the MGIT method.

Remarks

- *Mycobacterium tuberculosis* lineage 1 isolates frequently have minimum inhibitory concentrations (MICs) between 0.5 mg/L and 2.0 mg/L.
- For results within this range, there are interpretive difficulties, and susceptibility cannot be guaranteed.
- However, the combination regimens of bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) or bedaquiline, pretomanid and linezolid (BPaL) appear to retain clinical efficacy even when the MIC for pretomanid falls within the range of 0.5–2.0 mg/L (low availability of clinical data).
- WHO will review this recommendation when further clinical evidence of the efficacy of pretomanid-containing regimens for isolates with MICs between 0.5 mg/L and 2.0 mg/L is available.

[1] The End TB Strategy [website]. Geneva: World Health Organization; 2021 (https://www.who.int/teams/global-tuberculosis-programme/the-end-tb-strategy).

1 Background

Pretomanid (Pa), previously known as PA-824, is an anti-mycobacterial oral drug that was approved in 2019 by the United States (US) Food and Drug Administration *(1)* and recommended by the World Health Organization (WHO) as part of a 6-month all-oral regimen to treat people with pulmonary rifampicin-resistant tuberculosis (RR-TB), multidrug-resistant TB (MDR-TB) or pre-extensively drug-resistant TB (pre-XDR-TB). The use of Pa is approved in the Bdq (bedaquiline)-Pa-Lzd (linezolid)-Mfx (moxifloxacin) (BPaLM) and Bdq-Pa-Lzd (BPaL) combination regimens *(2)*. Given that Pa is a new drug, no critical concentration (CC) has been set previously. There are lineage-related differences in susceptibility to Pa among various members of the *Mycobacterium tuberculosis* complex (MTBC); most notably, lineage 1 (L1) of *M. tuberculosis* (*Mtb*) is less susceptible than lineages 2 (L2), 3 (L3), 4 (L4) and 7 (L7) *(3)*.

Cycloserine (Cs) or terizidone (Tzd) are considered equivalent oral anti-mycobacterial drugs recommended by WHO to treat MDR-TB; the two drugs are commonly used interchangeably. Tzd is formed by two molecules of Cs combined. A 2018 WHO systematic review of minimum inhibitory concentration (MIC) data identified no studies for Tzd and only a limited number of studies for Cs *(4, 5)*. As a result, the WHO CC for Löwenstein-Jensen (LJ) at 30 mg/L was withdrawn and no other CCs could be established for Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11) or Becton Dickinson BACTEC™ mycobacterial growth indicator tube (MGIT™). Hence, at present there is no WHO-endorsed phenotypic drug susceptibility testing (pDST) method for either Cs or Tzd. There is also no commercially available genotypic drug susceptibility testing (gDST) assay. WHO commissioned an update to the systematic review to evaluate whether sufficient new evidence had been published since 2018 to set a CC for one or more of the above media using the 1% proportion method.

The clinical use of these drugs calls for robust phenotypic antimicrobial susceptibility testing methods with a CC informed by epidemiological cut-off values (ECOFFs) and by pharmacokinetic (PK), pharmacodynamic (PD) and clinical outcome data where available.

WHO initiated a systematic search and analysis of the available evidence, which was then assessed by the WHO Technical Advisory Group (TAG) on TB Diagnostics and Laboratory Strengthening.

The TAG was established in 2021 *(6)*, and it oversees topics that are outside the scope of the WHO guideline development group process (Pathway A) but require critical evaluation and expert input. The scope of the TAG includes Pathway B assessments, and addressing knowledge gaps that hinder the adoption and scale-up of WHO recommendations. The goal is to help WHO to adequately address the prevailing and foreseeable challenges, and provide input into technical aspects on implementing specific TB diagnostic technologies.

The TAG comprises 24 independent experts who serve in their personal capacities, covering a spectrum of technical expertise, geographical representation and gender balance (Annex 1). Its terms of reference and brief biographies of members are available on the WHO website *(6)*.

The TAG met virtually on 5 and 6 September 2023; it reviewed the available evidence and has provided advice to WHO on setting CCs for Pa and Cs.

2 Pretomanid

2.1 Analysis of the distribution of MICs

2.1.1 Methods

A systematic review was performed to summarize the published data on MICs of Pa using the LJ, 7H10, 7H11 and MGIT methods, and to describe the wildtype MIC distribution and any associations between the MIC distribution and lineage.

To support this process, WHO issued a public call for data, appealing to national TB programmes, implementers, industry, researchers and other agencies to provide suitable evidence on Pa MICs and treatment outcomes related to lineage.

An individual patient data analysis was performed, owing to the scarcity of the data obtained through a systematic review (1 published study) that aimed to assess MIC distribution. All individual-level data were provided by the TB Alliance. Data originated from a published study by Bateson et al. (the "Bateson database") *(3)*, laboratory surveillance from India, South Africa, Tajikistan ("Paegis database"), Ukraine, the United States of America and clinical trials – namely Nix-TB *(7)*, SimpliciTB (ClinicalTrials.gov identifier: NCT03338621), STAND *(8)* and ZeNix *(9)* ("Trial database"). A total of 1365 isolates with MGIT MIC data were available across the three databases: Bateson (n=356), Paegis (n=328) and Trial (n=681).

A total of 10 laboratories provided data: the US Centers for Disease Control and Prevention; IML Red GmbH, Gauting, Germany; National Institute for Communicable Diseases, South Africa; National Institute of Tuberculosis and Respiratory Diseases, India; Supranational Reference Laboratory for TB, Borstel, Germany (SRL-Germany); Stellenbosch University, Stellenbosch, South Africa (SU); Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal (IHMT); University College London Centre for Clinical Microbiology, London, United Kingdom of Great Britain and Northern Ireland (UCL); Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy (OSR); and Supranational Reference Laboratory for TB, Stockholm, Sweden (SRL-Sweden).

Only globally important sensu stricto *Mtb* lineages (i.e. L1–L4) were included. Excluded were duplicate entries, non-*Mtb.* isolates, isolates without exact MICs and culture with evidence of mixed isolates (i.e. more than one lineage). A total of 1196 isolates were included in the analysis. However, for the sub-analysis stratified by L2-L4 and L1, isolate without lineage information were excluded.

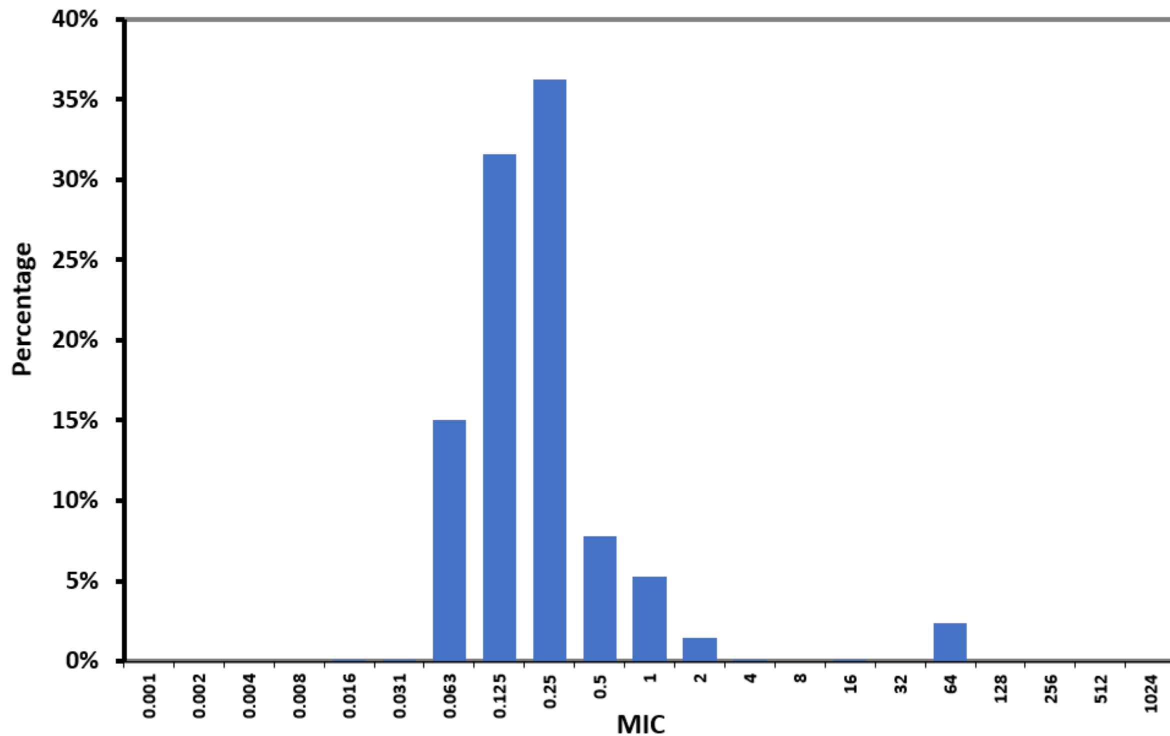
2.1.2 Results

Only one study was eligible according to the selection criteria; that study presented detailed MIC data, provided individual-level data and was included in the individual-level analysis to establish ECOFFs. The data from the only published study are labelled "Bateson" *(3)*. A second study was potentially eligible, but it presented MIC data as ranges *(10)*. The first author of the study was contacted to provide individual-level data, but was unable to provide the data.

Three laboratories (OSR, SRL-Germany and SRL-Sweden) showed a bimodal distribution; hence, data from these laboratories were excluded. Among the remaining seven laboratories with unimodal distribution, the MIC distribution of one laboratory (SU) did not have an MIC mode equal to or within one twofold dilution of the most common mode MIC observed in the other distributions; therefore, the data from SU were excluded. As a result, an aggregated MIC distribution using 1044 isolates from six laboratories was established. Fig. 1 shows the aggregated weighted MIC distribution (as relative frequencies). The aggregated weighted MIC

distribution was computed to control for imbalance in the number of isolates tested in each laboratory. This distribution was ***asymmetrical around the mode and spread across more than five dilutions***, suggesting that there may be more than one wildtype distribution with differing modes (Fig. 1). Therefore, no ECOFF was computed.

Fig. 1. Aggregated weighted distribution of *Mtb* MIC values for Pa using the MGIT method



MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration; *Mtb*: *Mycobacterium tuberculosis*; Pa: pretomanid.

A total of 707 L2, L3 or L4 isolates tested in five laboratories[1] and 157 L1 isolates tested in six laboratories[2] contributed to the analysis of MIC by lineage. For L2, L3 or L4 isolates, MIC distributions were unimodal across all five laboratories, with an MIC mode of 0.125 mg/L. Hence, it was possible to estimate an ECOFF MIC of 0.5 mg/L using the aggregated weighted data (Fig. 2).

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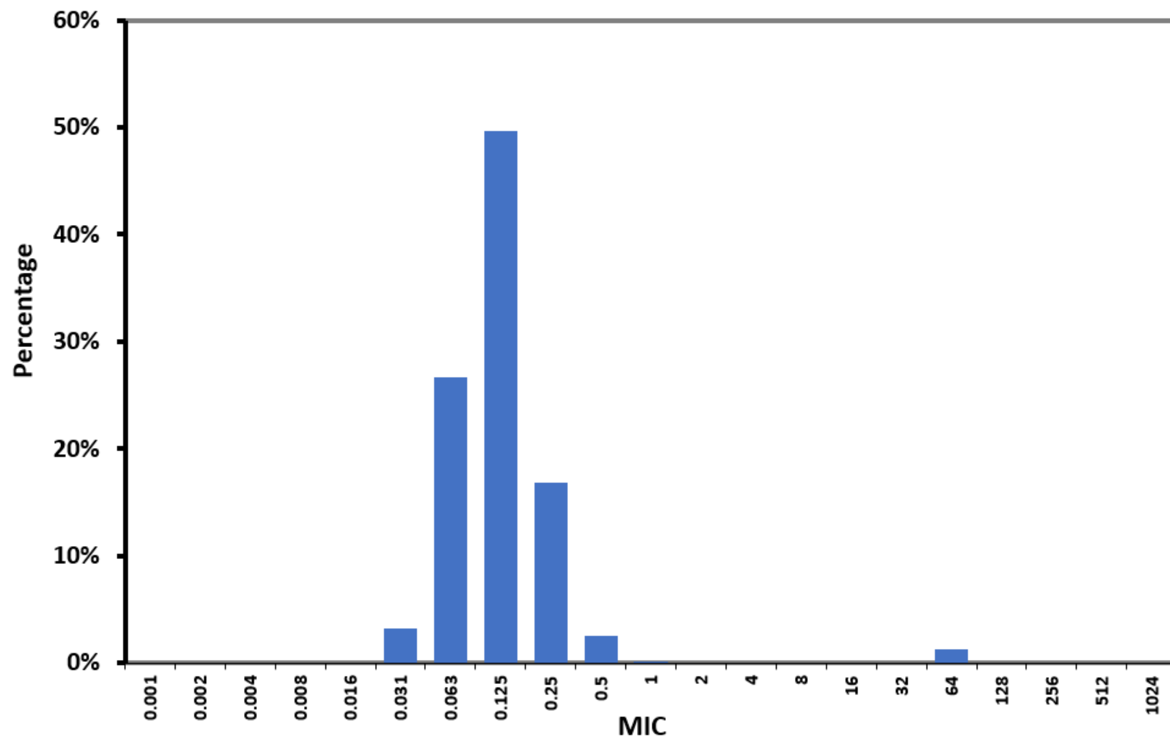
[1] SRL-Germany, IHMT, UCL, OSR and SRL-Sweden.

[2] SRL-Germany, IHMT, UCL, OSR, SRL-Sweden and SU.

Fig. 2. Aggregated weighted distribution of *Mtb* L2, L3 or L4 isolates MIC values for Pa using the MGIT method

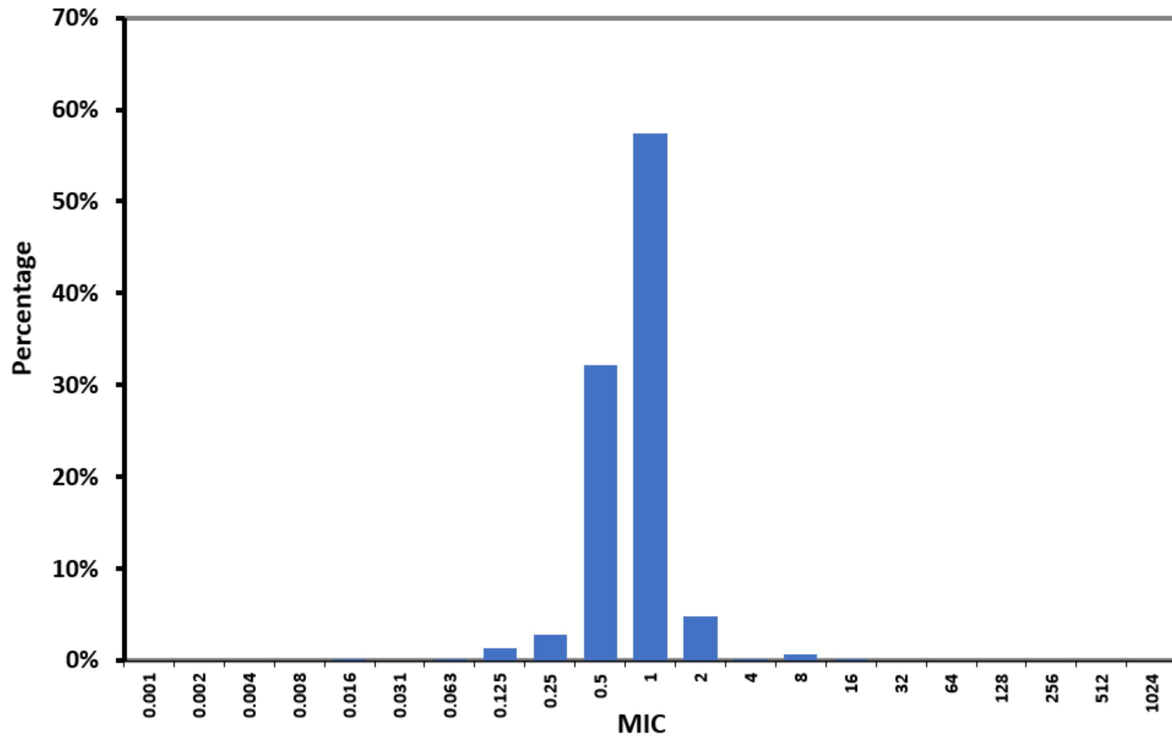


L: lineage; MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration; *Mtb*: *Mycobacterium tuberculosis*; Pa: pretomanid.

Regarding *Mtb* lineage 1 isolates except for IHMT, all laboratories tested more than 15 isolates, thus meeting the minimum European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard operating procedure (SOP) 10.1/2 criteria. The isolate tested at IHMT was excluded from the analysis. L1 MIC distribution obtained across five laboratories was unimodal (Fig. 3).

Across each of the five laboratories, the mode was equal to or within one twofold dilution of the most common mode MIC observed by other laboratories fulfilling the criteria set out by EUCAST. Thus, results from all five laboratories were included in the analysis. Because only four or the five the laboratories agreed on the same mode (according to EUCAST SOP 10.1/2 criteria, at least five laboratories should agree on the same mode) a tentative ECOFF (tECOFF) was estimated as an MIC of 2.0 mg/L using the aggregated weighted data (Fig. 3).

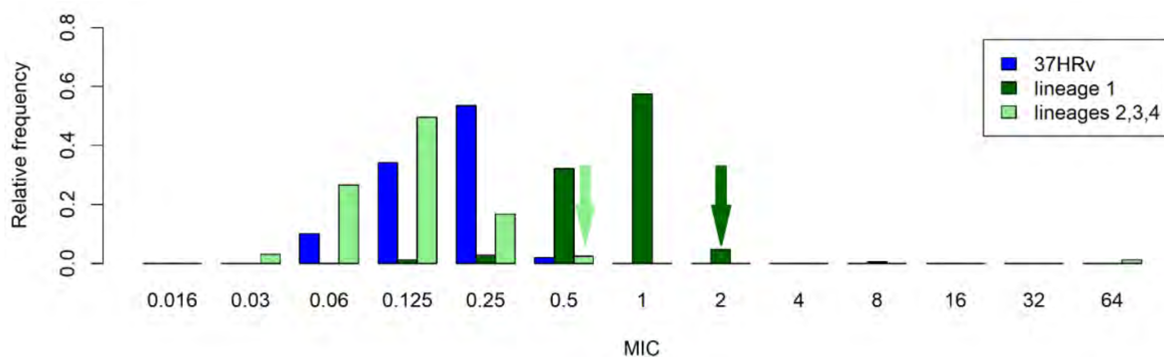
Fig. 3. Aggregated weighted distribution of *Mtb* L1 isolates MIC values for Pa using the MGIT method



L: lineage; MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration; *Mtb*: *Mycobacterium tuberculosis*; Pa: pretomanid.

The Pa MIC distributions of H37Rv isolates were comparable with isolates from *Mtb* L1 and L2, L3 or L4 (Fig. 4). The MIC distributions of H37Rv and isolates of *Mtb* L2, L3 or L4 spanned the same range of dilutions with a similar mode (H37Rv: 0.25 mg/L, *Mtb* L2, L3 or L4 isolates: 0.125 mg/L), whereas the MIC distribution of *Mtb* L1 isolates was shifted to the right with a mode of 1 mg/L.

Fig. 4. Aggregated weighted MGIT MIC distributions of clinical isolates and MGIT MIC distribution of H37Rv[a]



ECOFF: epidemiological cut-off; L: lineage; MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration.

[a] The arrows signify ECOFFs for L2, L3 or L4 and L1, respectively.

The MIC individual-level data (n=101) tested on solid 7H11 media was provided from the Institute of Tropical Medicine Antwerp. MIC distribution of all *Mtb* isolates and of L2, L3 or L4 versus L1 *Mtb* isolates generally followed a similar pattern to that seen with MGIT;

however, since only one laboratory submitted data, the data were insufficient to set an ECOFF (see more details in *Web Annex 1*).

2.2 Correlation between lineage and outcome

2.2.1 Methods

Since *Mtb* L1 isolates have a higher MGIT ECOFF (2 mg/L) than *Mtb* L2, L3 and L4 isolates (MGIT ECOFF: 0.5 mg/L), the epidemiology of TB due to L1 *Mtb* strains was reviewed. Globally, L1 accounted for 28% of TB in 2012 and 2018 *(11)*. Over 80% of the L1 global burden was in Bangladesh, India, Indonesia and the Philippines. The proportion of *Mtb* L1 may differ between drug-susceptible and drug-resistant isolates *(12-15)*. Lineage and Pa MGIT MIC information was only available for a subset of the "Trial database" (688/1029). Only 53 of 688 (7.9%) isolates for which lineage information was available belonged to L1. Given the predominance of L2, L3 and L4 TB across the trials, and the fact that *Mtb* L1 isolates have intrinsically higher MICs, results from trials investigating Pa-based regimens may not be generalizable to TB caused by L1 *Mtb* strains.

Given that countries had started introducing the BPaL/M regimen programmatically, WHO issued a public call on 17 April 2023 for data on PA MIC distributions across *Mtb* lineages and treatment outcomes. The aim of the call was to enhance the existing "Trial" data with programmatic data, to possibly allow for a more meaningful analysis. A few countries who had started implementing BPaL/M provided additional data. They included Ireland (Irish Mycobacteria Reference Laboratory, Labmed Directorate, St. James's Hospital, Dublin), Sweden (Public Health Agency of Sweden, Unit for Laboratory Surveillance of Bacterial Pathogens), Indonesia (Yayasan Riset dan Pelatihan Respirasi Indonesia, Respiratory Society of Indonesia) and India (ICMR-National Institute For Research in Tuberculosis, Chennai). In addition Médecins Sans Frontières – Netherlands (MSF) provided data from the TB-Practecal trial conducted in three countries (Belarus, South Africa and Uzbekistan).

In light of these findings, an analysis of available data was conducted to investigate whether Pa-based regimens achieved similar outcomes in participants infected with L1 strains (with wildtype MICs), compared with participants with L2, L3 or L4 strains (with wildtype MICs). In addition, the outcomes were compared in participants infected with L1 strains (with wildtype MICs), who were receiving Pa-based regimens versus standard regimens.

2.2.2 Results

Pa-based regimens: comparing outcomes in participants with L1 versus L2, L3 or L4 strains

Overall, 41 and 512 participants infected with L1 strains and L2, L3 or L4 strains received Pa-based regimens (Table 2.1).

Table 2.1. Different Pa-based regimens by lineage

	***Mtb* L1 (n=41)**	***Mtb* L2, L3 or L4 (n=464)**
BPaL	1	55
BPaL1200x26	0	35
BPaL1200x9	0	29
BPaL600x26	0	33
BPaL600x9	1	38
BPaLC	0	14

BPaLM	0	18
4BPaMZ	18	127
6BPaMZ	14	128
4Pa100MZ	2	4
4Pa200MZ	3	12
6Pa200MZ	2	3

BPaL: bedaquiline, pretomanid and linezolid; BPaMZ: bedaquiline, pretomanid, moxifloxacin and pyrazinamide; L: lineage; *Mtb*: *Mycobacterium tuberculosis*; MZ: moxifloxacin and pyrazinamide; Pa: pretomanid.

Excluding the participants who received the regimens 4Pa100MZ, 4Pa200MZ and 6Pa200MZ, a total of 527 participants received Pa-based regimens (L1: n=34; L2, L3 or L4: n=493); 18/34 (52.9%) *Mtb* L1 and 128/493 (26.0%) *Mtb* L2, L3 or L4 isolates were drug susceptible.

The favourable versus unfavourable primary outcomes were compared between participants infected with L1 strains versus L2, L3 or L4 strains. Participants for whom outcomes could not be assessed were excluded from the analysis (L1: n=3; L2, L3 or L4: n=22).

Among participants infected with L1 strains, 25/31 (80.6%, 95% confidence interval [CI]: 63.7–90.8%) had favourable treatment outcomes compared with 404/471 (85.8%, 95% CI: 82.3–88.6%) of those infected with L2, L3 or L4 strains. Although there was no significant difference in unfavourable treatment outcomes across the two groups (P=0.4302), power to detect the observed difference with the available number of participants records was extremely low (12.9%). Most of the unfavourable outcomes (n=51/73, L1: n=5; L2, L3 or L4: n=46) were due to withdrawal.

Excluding unfavourable outcomes due to non-treatment-related withdrawal from the analysis did not change the results (L1 strains 25/31 [80.6%, 95% CI: 63.7–90.8%] versus L2, L3 or L4 strains 404/457 [88.4%, 95% CI: 85.1–91.0%], P=0.2475, power=25.1%).

The time to culture negative status (TTNS) was compared among participants infected with L1 strains and L2, L3 or L4 strains. A total of 21 participants (all L2, L3 or L4) were excluded because their sputum cultures were negative between screening for inclusion in the trial and starting treatment; also, four participants from Ireland were excluded because TTNS information was not provided (all L2, L3 or L4). In addition, 24 participants could not be assessed for TTNS: five of these participants died (L1: n=1; L2, L3 or L4: n=4) and 19 were withdrawn or withdrew (L1: n=1; L2, L3 or L4: n=17); their time was censored at date of death or withdrawal. Overall, 34 participants infected with L1 strains and 468 with L2, L3 or L4 strains were included in the analysis.

The median TTNS was 43 days (95% CI of the median: 42–43 days, interquartile range [IQR]: 27–57 days) for L2, L3 or L4 strains and 29 days (95% CI of the median: 29–36 days, IQR: 22–37 days) for L1 strains. The P-value of the logrank test was 0.1825. Since participants with L1 strains were more likely to have drug-susceptible TB, a Cox's proportional hazards regression was performed comparing TTNS between lineages adjusting for the resistance status. The adjusted hazard ratio was 1.31 (95% CI: 0.90–1.90), with a P-value of 0.164. Therefore, the TTNS seems comparable between the two groups; however, the group infected with L1 TB strains had a small sample size (see *Web Annex 1* for more details).

Outcomes in participants infected with L1 strains receiving standard versus Pa-based regimens

A total of 51 participants were included in this analysis, of whom 41 were treated with a Pa-based regimen (Table 2.1) and 10 with a standard regimen (2HRZE/4HR). Of the 41

participants treated with a Pa-based regimen, 25 had drug-susceptible TB, 14 had mono-resistance to rifampicin or isoniazid, and two had MDR-TB. Of the 10 participants treated with standard regimens, all had drug-susceptible TB. Participants for whom outcomes could not be assessed were excluded (Pa-based regimen: n=6; standard regimen: n=2) leaving a total of 43 participants in the analysis (Pa-based regimen: n=35; standard regimen: n=8).

Among participants receiving a standard regimen, 8/8 (100%, 95% CI: 67.6–100%) had a favourable outcome compared with 29/35 (82.9%, 95% CI: 67.3–91.9%) among those receiving a Pa-based regimen (P=0.58). Given the very small number of participants receiving a standard regimen, the power to detect a difference was almost null.

The TTNS was compared between participants receiving a standard regimen and those receiving a Pa-based regimen. Two participants, both receiving a Pa-based regimen, were censored (one died and one was withdrawn); their time was censored at date of death or withdrawal. Thus, the analysis included 10 participants treated with a standard regimen and 41 participants treated with Pa-based regimens.

The median TTNS was 67 days (95% CI of the median: 36–NA days, IQR: 36–120 days) for the standard regimen and 29 days (95% CI of the median: 29–36 days, IQR: 28–43 days) for the Pa-based regimen. The P-value of the logrank test was 0.02. Adjustment for confounding was not possible owing to the small sample size.

2.3 A systematic review of PK and PK/PDs of Pa

2.3.1 Methods

The review was conducted in accordance with the principles outlined in the PRISMA statement *(16)*. The search of databases was performed on 14 August 2023, without date restriction. Title and abstract screening, as well as full text screening, was performed by two reviewers independently. In case of differences, consensus was reached through discussion. Criteria for selection of PK variability were studies with a prospective, observational or retrospective design.

Criteria for selection of PK/PD studies were in vitro (hollow fibre infection model) animal and human studies investigating the relationship between drug dose, concentration and microbiological response. It was important that the study design allowed for the effect of the drug of interest to be assessed. This could be as either monotherapy or combination therapy (where the drug was administered at various dosages or exposures). For better interpretation of the microbiological response, the MIC had to be assessed.

In total, 502 articles were retrieved from PubMed and Web of Science. After the removal of 128 duplicates, 374 articles underwent abstract and title screening, resulting in 61 articles for full text screening. After the exclusion of 24 non-relevant articles, 37 articles were included in the final assessment. A total of five in vitro studies, 14 in vivo studies, nine human studies and nine modelling studies were included (see more details in *Web Annex 2*).

2.3.2 Results

Pa has a clear exposure effect relationship (i.e. the percentage time [T] the concentration of an antibiotic remains above the MIC [%T>MIC]), and the exposure to the drug is highly dependent on concomitant food intake. Drug–drug interactions with rifamycins can reduce the exposure substantially. Thus, drug exposure in routine care is expected to be variable. The impact of variable drug exposure on treatment response depends on the MIC for Pa, but also on companion drugs in the regimen. A combination of Bdq, Mfx and pyrazinamide (PZA)

seems favourable, and may help to compensate for its limited role against nonreplicating bacteria in lung lesions.

With regards to PK/PD markers, the most significant predictors of Pa efficacy were %T/MIC and area under the curve (AUC)/MIC based on the free drug. Preclinical models coupled with model simulations for human-equivalent doses (200 mg, 400 mg daily) were able to show attainment of as high as 100% T/MIC at an MIC of less than 0.1 mcg/mL. Similarly, high target attainment of T/MIC (92–99%) was achieved for a Pa dose of 100–200 mg daily in Phase 2 studies for the observed MIC of less than 0.1 mcg/mL.

PK/PD targets established in a mouse model show that free drug T greater than an MIC of 22%, 48% and 77% were associated with bacteriostasis, a 1-log kill and 80% of the maximum observed effect (EC_{80}), respectively. For programmatic care, the exposure of Pa should be sufficient to achieve at least kill in 90% of the population (T>MIC48%). Pa maximum concentration (C_{max}) in people with TB on 200 mg/daily as part of BPaZC (Bdq, Pa, PZA and Cs), BPaZ (Bdq, Pa and PZA) or BPaC (Bdq, Pa and Cs) was about 4 mg/L (up to 6 mg/L). Based on protein binding and a free drug fraction of about 15% (variable 5–15%), the free drug will be about 0.6 mg/L (up to 0.9 mg/L in some people). However, because this assumption is based on the C_{max} timepoint, it is unclear whether this concentration of more than 0.5 mg/L would be achieved for about 50% of time at the site of infection. It is likely that Pa MIC tested as part of combination regimens will show an additive or synergistic effect, meaning that the target of T>MIC48% may become more achievable, and an ability to kill 90% of the bacterial population will be maintained. This means that stasis based on Pa alone can be expected in less than 10% of the population, provided that strong companion drugs are included in the regimen (e.g. Bdq, Lzd and Mfx) and the isolate is susceptible to those drugs (see more details in *Web Annex 2*).

3 Cycloserine

3.1 Analysis of the distribution of MICs

3.1.1 Methods

A PubMed search without date restrictions was conducted on 6 June 2023 using intentionally broad search terms, because the titles or abstracts of papers do not necessarily mention MIC data. In addition, MIC data were solicited from the WHO Supranational Reference Laboratory Network and directly from key researchers (as identified through the literature search and a public call for data by WHO). Only studies that had not already been considered in the 2018 review were considered further.

Studies that met the following criteria were included in the review:

- the MICs for at least one of the anti-TB compounds of interest (with at least three concentrations tested per drug) were determined using the proportion method with a critical proportion of 1%, using LJ, 7H10, 7H11 or MGIT;
- the drug concentrations tested were clearly defined (i.e. to assess potential truncations of the MIC results);
- the number of isolates tested at each concentration was given (i.e. to evaluate the shape of the MIC distributions and determine the mode of the distributions); and
- the MIC data were available for at least 10 isolates per drug.

A total of 741 studies were identified, of which 162 had not been considered in 2018 and were reviewed for this report. Of these 162 studies, 17 met all inclusion criteria for Cs, compared with just six in 2018, and were further stratified by culture medium (LJ=1, 7H10=4, 7H11=2 and MGIT=11). No studies met all inclusion criteria for Tzd.

MIC data from different media were analysed separately to take into account systematic differences between media *(17, 18)*. All mutations in the coding or upstream regions of genes associated with resistance to Cs (*ald*, *alr* and *cycA*) were included, where known. Strains without mutations or only synonymous mutations were reported as genotypically wildtype (gWT). Frameshifts in *ald* were assumed to confer a loss of function phenotype, in the same way as WHO does for other nonessential resistance genes *(19, 20)*.

3.1.2 Results

No new data were identified for LJ and 7H10 media compared with the 2018 review; hence, no CC could be set. For 7H11, all data were from a single laboratory; thus, the evidence was insufficient to set a CC.

For MGIT, the available data were stratified for phenotypically wildtype (pWT) isolates and for phenotypically non-wildtype (pNWT) isolates.

Data from nine novel laboratories for pWT isolates were identified in this review (Table 3.1) *(21-26)*.[1,2] The results from Dyuzhik differed from the remaining studies because a bimodal MIC distribution was reported. It is plausible that isolates with high MICs are genuinely pNWT because Dyuzhik included predominantly MDR-TB isolates and also focused on isolates from people with suspected relapses or chronic TB to enrich for pNWT isolates. More details on particular studies can be found in *Web Annex 3*.

Table 3.1. Cs MICs for pWT isolates in MGIT[a]

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	DCS MIC (mg/L) 0.5	1	2	4	8	10	15	16	20	30	40
6) Zhu 2023	6		1	9	H37Rv ATCC 27294					**9**							
	6	clinical	159	159	MDR-TB			2	35	**71**	50			1			
7) Wu 2022	7		1	3	H37Rv ATCC 27294					**3**							
	7	clinical	117	117	at least MDR	gWT		11	35	**44**	24			3			
8) Robledo	8		1	30	H37Rv BCCM 500735					**16**	14						
	8	clinical	30	30	pan-S					2	**16**			12			
	8	clinical	29	29	at least MDR		1				**12**			16			
	8		1	5	H37Rv BCCM 500735	gWT parent				2	**3**						
9) Gonçalves 2014	9		1	1	H37Rv ATCC 27294					**1**							
	9	clinical	10	10	pan-S						**10**						
	9	clinical	32	32	different levels of R					2	**29**			1			
10) Dyuzhik 2016 & 2017	10		1	2	H37R ATCC 25618					2							
	10	clinical	226	226	mostly at least R to INH					7	37	**70**	56		14	31	11
11) Jou	11		1	4	H37Rv ATCC 27294					3	1						
	11	clinical	1	1	pan-S	gWT				1							
	11	clinical	78	78	different levels of R	gWT				25	**46**			7			
12) Augustynowicz-Kopeć	12		1	1	H37Rv ATCC 27294						**1**						
	12	clinical	4	4	pan-S					1	**3**						
	12	clinical	11	11	different levels of R						**9**			2			
13) Werngren	13		1	15	H37Rv ATCC 27294					2	**13**						
	13		1	1										1			
	13	clinical	1	1	pan-S									1			
	13	clinical	5	5	different levels of R						4			1			
14) Nakatani 2017	14		1	1	H37Rv ATCC 27294					**1**							
	14	clinical	4	4	different levels of R	gWT								**4**			
15) Evangelopoulos 2019	15		1	1	H37Rv Pasteur	gWT parent					**1**						

Cs: cycloserine; gWT: genotypically wildtype; MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration; pWT: phenotypically wildtype.

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[1] Augustynowicz-Kopeć E, personal communication, 2023; Jou R, Liu K-H, Wu S-H and Chan H-H, personal communication, 2023; Robledo J, Hurtado UA, Alvarez N, Realpe T and Osorio A, personal communication, 2023; Werngren J, Mansjö M, Mengshoel AH, Groenheit R and Schön T, personal communication, 2023.

[2] The results published as Dyuzhik et al. 2016 represent a subset of results included in the thesis by Dyuzhik 2017. The latter results were subsequently included in this report.

[a] The new studies identified compared with the 2018 review are shown in **bold**, truncated values are highlighted in red and shadowed cells represent MICs tested within a particular study.

Three laboratories reported the MICs for *alr* in vitro mutants (Table 3.2). In addition, 10 unique *alr* mutations were reported in clinical isolates from three laboratories *(25, 27)*. Only four isolates from two laboratories with an *ald* mutation were identified, with MICs of 8–32 mg/L *(25)*.[1] One study reported two double mutants with the same *ald* frameshift and *alr* R379C with MICs of 4 mg/L or less, and 16 mg/L.[2] Details on studies can be found in *Web Annex 3*.

Table 3.2. Cs MICs for mutated isolates in MGIT

						DCS MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Genotypic results**	**4**	**8**	**16**	**32**	**64**	**Comment**
15) Evangelopoulos 2019	15		1	1	*alr* large upstream deletion					1	One mutant deposited as BCCM 501135.
15) Evangelopoulos 2019	15		2	2	*alr* g-57t (E6*)					2	One mutant deposited as BCCM 501137.
11) Jou	11		1	1	*alr* g-57t (E6*)					1	Deposited as ATCC 35826.
8) Robledo	8		1	1	*alr* g-57t (E6*)			1			
8) Robledo	8	in vitro mutants	3	4	*alr* c-14t (T20M)		1	2	1		
8) Robledo	8		1	1	*alr* c-8t (S22L)			1			
8) Robledo	8		1	1	*alr* R296W (R320W)			1			
8) Robledo	8		12	12	*alr* D320N (D344N)				12		
15) Evangelopoulos 2019	15		8	8	*alr* D320N (D344N)					8	On mutant deposited as BCCM 501136. Proposed resistant control strain.
7) Wu 2022	7		1	1	*alr* a-188c (a-116c)			1			
11) Jou	11		1	1	*alr* t-71g (M1G)	1					
11) Jou	11		1	1	*alr* g-24a (G17R)		1				
11) Jou	11		1	1	*alr* c-14t (T20M)				1		
7) Wu 2022	7		1	1	*alr* c-8t (S22L)				1		
11) Jou	11		7	7	*alr* Q6R (Q30R)	6	1				
7) Wu 2022	7		2	2	*alr* L89R (L113R)				1	1	
11) Jou	11		1	1	*alr* L89R (L113R)				1		
11) Jou	11	clinical	1	1	*alr* R219S (R243S)				1		
11) Jou	11		1	1	*alr* S237N (S261N)			1			
14) Nakatani 2017	14		3	3	*alr* M319T (M343T)					3	
11) Jou	11		2	2	*alr* M319T (M343T)				1	1	
7) Wu 2022	7		1	1	*alr* M319T (M343T)					1	
7) Wu 2022	7		1	1	*ald* LoF				1		
7) Wu 2022	7		1	1	*ald* E32G				1		
11) Jou	11		1	1	*ald* E118K			1			
11) Jou	11		1	1	*ald* A184T		1				
11) Jou	11		2	2	*ald* LoF & *alr* R379C (R403C)	1		1			

Cs: cycloserine; MGIT: mycobacterial growth indicator tube; MIC: minimum inhibitory concentration.

The new studies identified compared with the 2018 review are shown in **bold**, truncated values are highlighted in red and shadowed cells represent MICs tested within a particular study.

3.2 A systematic review of PK/PDs of Cs

3.2.1 Methods

This systematic review was an update of an earlier report that was written to inform a technical report on the PK/PD of medicines used in the treatment of drug-resistant TB (DR-TB), prepared by WHO *(5)*. The search of databases was performed on 14 August 2023, with date restriction.

Criteria for selection of PK variability were studies with a prospective, observational or retrospective design. Criteria for selection of PK/PD studies were in vitro (hollow fibre infection model), animal and human studies investigating the relationship between drug dose, concentration and microbiological response. It was important that the study design allowed for the effect of the drug of interest to be assessed, either as monotherapy or as combination therapy (where the drug was administered at various dosages or exposures). For better interpretation of the microbiological response the MIC had to be assessed.

[1] Smirnova T, personal communication, 2023.

[2] Jou R, Liu K-H, Wu S-H and Chan H-H, personal communication, 2023.

In total, 192 articles were retrieved from PubMed and Web of Science on 14 August 2023 covering the period since the previous report *(5)*. After the removal of 62 duplicates, 130 articles underwent abstract and title screening, resulting in 24 articles for full text screening. After the exclusion of five non-relevant articles, 19 articles were included in the final assessment. Review of the references of the included articles resulted in one additional article to be included for final analysis. A total of one in vitro study, one in vivo study and 17 human studies were included (see more details in *Web Annex 4*).

3.2.2 Results

Since the release of the WHO technical report on the PK/PD of medicines used in the treatment of DR-TB, a range of studies have been conducted *(4)*. This was helpful to overcome the evidence gap shown in the previous systematic review of Cs, which found no preclinical studies and very few human studies, with sparse PK sampling *(28)*.

The current review provides a more detailed understanding of the PK and PK/PD of Cs and its potential implications for drug dosing as part of programmatic care. Of the six studies developing a population PK model, five found that a one-compartment model with first-order absorption described the data well. A lag time or transition compartment was used to account for any delay in absorption of bioconversion from Tzd to Cs. Important factors associated with drug exposure were renal function and body size; these factors were found in larger sized studies. Smoking could be an additional factor influencing drug exposure because it increased the nonrenal clearance route of Cs. Given that nonrenal clearance accounts for 30% of the total clearance, it is not clear whether dose adjustments need to be made for smoking status. Overall, the PK of Cs is consistent between studies, as demonstrated by comparable structures for the population PK models; however, variability in exposure to Cs among individuals is significant, prompting therapeutic drug monitoring in some studies.

The in vitro study by Deshpande et al. *(29)* is the only preclinical study that investigated the relationship between drug exposure and microbiological response. Overall, T>MIC was able to predict microbiological response; also, with an increasing percentage T>MIC the effect of Cs increased from stasis (20%), bactericidal (30%), 80% of maximum kill (64%) to prevention of acquired resistance (100%). Two human studies investigated the PK/PD relationship. One of those studies ranked drug exposures, and showed that people with higher exposure responded better to treatment *(30)*. The second study collected information on drug exposure, treatment response and pathogen susceptibility in a large prospective cohort *(26)*. Using classification and regression tree analysis, the authors found that treatment response was determined by T>MIC 33.2%. This study can be considered a clinical validation of the preclinical PK/PD study performed by Deshpande et al. *(29)*; it demonstrated that, as for other TB medicines, the PK/PD parameters are comparable between in vitro, in vivo and human studies.

The relationship with T>MIC established by Deshpande et al. *(29)* encouraged many investigators to assess target attainment. For such assessment, the ratio between drug exposure and pathogen susceptibility (i.e. MIC) is important; therefore, most clinical studies used local PK data and MIC distributions. Investigators used T>MIC30% and T>MIC64% to determine whether more than 90% of the population would achieve either one or both of these PK/PD targets using simulated dosages of 250–1000 mg (ranging from once to four times daily in different studies). A few studies used more traditional targets to assess target attainment using a C_{max} of 20–35 mg/L or included the MIC value in the assessment by stating C_{max}>MIC≥1. Overall, there is a clear trend that higher dosages (500 mg daily) are required to attain the therapeutic target of T>MIC30% for MIC at 16 mg/L. The target for maximum kill can only

be attained at lower MIC values (≤8). Given that a substantial number of people display a C_{max} concentration of more than 35 mg/L at higher dosages, side-effects will increase *(31)*. Although various PK/PD targets have been developed, ranging from statis to prevention of acquired resistance, the question remains of which target will be used to select the dose for programmatic treatment. Aiming for stasis (T>MIC20%) does not seem to make sense from an efficacy point of view, whereas aiming for prevention of acquired resistance (T>MIC100%) would result in too many side-effects. When setting a breakpoint based on maximum kill (T>MIC64%), the MIC would probably be much lower than the ECOFF; hence, setting a breakpoint based on T>MIC30% makes more sense from the points of view of both ECOFF and treatment tolerability *(32)*.

To conclude, Cs is a drug with substantial PK variability and a narrow therapeutic window. With a concentration effect relationship (T>MIC) supported by preclinical and human data, PK/PD considerations can help when deciding on the dose most likely to be beneficial for the treatment of people with MDR-TB. It makes sense to consider a target of T>MIC30%, because aiming for higher targets (T>MIC64–100%) will probably require high dosages that will not be well tolerated. It is highly likely that a daily dose of 750 mg (250+500) or 500 mg daily will achieve T>MIC30% in cases with MIC at or below 16 mg/L (see more details in *Web Annex 4*).

4 WHO statement for pretomanid and cycloserine

Following review of the evidence and advice from the TAG, WHO makes the following policy statements:

3. Two test concentrations (**0.5 and 2.0 mg/L**) should be used for pretomanid drug susceptibility testing, using the mycobacterial growth indicator tube (MGIT™) method with the following interpretation:
   - no growth at 0.5 mg/L = susceptible;
   - growth at 0.5 mg/L and no growth at 2.0 mg/L = susceptible, with a comment added to the laboratory report stating there is an interpretive uncertainty of this result and close patient follow-up is required; and
   - growth at 2.0 mg/L = resistant.
4. A CC of **16 mg/L** should be used for cycloserine drug susceptibility testing using the MGIT method.

Remarks

- *Mycobacterium tuberculosis* lineage 1 isolates frequently have minimum inhibitory concentrations (MICs) between 0.5 mg/L and 2.0 mg/L.
- For results within this range, there are interpretive difficulties, and susceptibility cannot be guaranteed.
- However, the combination regimens of bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) or bedaquiline, pretomanid and linezolid (BPaL) appear to retain clinical efficacy even when the MIC for pretomanid falls within the range of 0.5–2.0 mg/L (low availability of clinical data).
- WHO will review this recommendation when further clinical evidence of the efficacy of pretomanid-containing regimens for isolates with MICs between 0.5 mg/L and 2.0 mg/L is available.

5 Implementation considerations

5.1 Implementation considerations for Pa

- Preferably, the two concentrations should be tested simultaneously**.** Sequential testing on the same isolate can be considered based on the local frequency of L1 isolates or isolates with MICs of 0.5 mg/L or more.
- For isolates with an MIC in the range 0.5–2.0 mg/L, recommendations are:
  - continue a BPaLM/BPaL regimen; and
  - continue follow-up – if there is a poor bacteriological or clinical response, consider a change in treatment regimen *(33)*.

5.2 Implementation considerations for Cs

- CC for Cs may be used as a surrogate for Tzd resistance.
- Given the known heat instability of Cs, stock solutions should be stored at between –80 °C and –60 °C (not at –20 °C) for up to 1 year and they should not be refrozen following use.

6 Further research

6.1 Further research for Pa and Cs

Further research topics for Pa and Cs overall include:

- further investigation and characterization of new and known molecular mechanisms of resistance for Pa and Cs, and resolution of uncertainty in the annotation of genes associated with resistance;
- further investigation and establishment of the breakpoints for drug susceptibility testing for Pa and Cs with media other than MGIT, including broth microdilution;
- therapeutic drug monitoring tests to guide approaches to dose adjustment in patients experiencing toxicity; and
- temporal trends in MIC through routine surveillance.

6.1.1 Further research for Pa

Further research topics for Pa specifically include:

- population representative sampling to understand the geographic distribution of L1 disaggregated by rifampicin status;
- operational research evaluating clinical outcomes of individuals with L1 and isolates with MICs of 0.5 mg/L or more among those on BPaL, BPaLM and other investigational regimens;
- operational studies, research studies and routine surveillance on resistance disaggregated by lineage (strongly desired: matched phenotypic and sequencing data); and
- PK/PD studies on L1 and isolates with MICs of 0.5 mg/L or more to inform future drug susceptibility testing criteria.

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Conflict identified	Nil
Conclusion	No conflict of interest

POTENTIAL CONFLICT OF INTEREST	
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Conflict identified	**(1a) Employment**: Employment with FIND, the global alliance for diagnostics, but not closely involved in any recent diagnostic evaluations at FIND.
Conclusion	Non-significant
Expert name	Farzana Ismail, South Africa
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: XDR Cartridge evaluation (Cepheid). Funds provided to research unit within the National Institute for Communicable Diseases in the amount of US$ 140 000; and bedaquiline post-marketing surveillance and emerging resistance (Janssen). Fund provided to the research unit in the amount of US$ 300 000.

POTENTIAL CONFLICT OF INTEREST	
	(2b) Non-monetary support valued at more than US$ 1000 overall: Activity (?) on latent TB infection in health care workers. Consumables and personnel were provided by Qiagen. This interest is still ongoing. Sponsorship to the International Union of TB and Lung Disease conference 2018 (Janssen). This included flight (to The Hague), accommodation and conference registration fee.
Conclusion	Significant: laboratory was involved in evidence generation and analysis to inform the current meeting.
Expert name	Madhukar Pai, Canada
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: 1. Two ongoing grants from the Bill & Melinda Gates Foundation (none related to TB). 2. A grant from FIND: Tuberculosis diagnostics in conjunction with development of new regimens to fight TB and DR-TB. This grant is to support FIND by conducting market analyses of TB tests; uptake of TB tests; systematic reviews of TB diagnostics; product landscapes; and secondary analyses of data (e.g. TB biomarker database). The work now also involves COVID-19 diagnostics. No specific product evaluation is included.
Conclusion	Non-significant
Expert name	Thomas Shinnick, United States of America
Conflict identified	**(1b) Consulting, including service as a technical or other adviser**: As an independent consultant, [the participant] received contracts and travel support from WHO, FIND, USAID for work related to laboratory strengthening and developing global guidance documents.
Conclusion	Non-significant
Expert name	Sadia Shakoor, Pakistan
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: Co-investigator of projects for which the expert's institution (Aga Khan University) has received funding support from Janssen. Research: The Bedaquiline

POTENTIAL CONFLICT OF INTEREST	
	DREAM programme and Bedaquiline EQA project. The funding covered 5% salary support for this expert from 2018 to 2020.
Conclusion	Non-significant
Expert name	Daniela Maria Cirillo, Italy
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: The expert participated in the 2020 advisory board (Biomérieux) for which they received €1000 in financial gain (personal?). This engagement ended in 2020. The expert has also participated in the evaluation of diagnostic assays; for the evaluation of blood stability for VIDAS, the research unit in their institution received €11 200 from Biomérieux; and for the evaluation of the XDR test prototype for Cepheid and FIND, the research unit received €14 295 80 in 2018.
Conclusion	Significant: laboratory was involved in evidence generation and analysis to inform the current meeting.
Expert name	Irina Lyadova, Russian Federation
Conflict identified	**(2b) Non-monetary support valued at more than US$ 1000 overall**: The expert was a lecturer at the "Recent advances in treatment and diagnosis of drug-resistant TB" in the Global Public Health meeting, sponsored by Johnson & Johnson. Travel expenses were covered. **(4a) Patents, trademarks, or copyrights**: Russian patents on TB diagnostics in 2012 and 2013, linked to the Central TB research institute where the expert worked. The patents belong to the expert's employer. This interest ceased in 2018.
Conclusion	Non-significant
Expert name	Christopher Coulter, Australia
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: Research support from FIND to conduct LOD studies on TB molecular tests (Cepheid Xpert® MTB/XDR; Bioneer). The monetary value of the contract was just over AUD 40 000 with 60% of the contract to fund the labour to do the studies and the balance consumables. The interest ceased in 2019.
Conclusion	Non-significant

POTENTIAL CONFLICT OF INTEREST	
Expert name	Mark Nicol, Australia
Conflict identified	**(2a) Research support, including grants, collaborations, sponsorships, and other funding**: Research support from NIH, Wellcome Trust, Bill & Melinda Gates Foundation, FIND, United Kingdom MRC and EDCTP to evaluate novel TB diagnostics (Xpert MTB/RIF; Xpert MTB/RIF Ultra; Epistem GeneDrive; BD MAX MDR-TB; Truenat TB; Determine TB-LAM; SILVAMP TB-LAM). No funding from commercial entities. Research grants belonged to the University of Cape Town and the University of Western Australia. Significant research funding (several million dollars). However, no personal income or income to family members. These activities are ongoing. The estimated total grant funding for this research programme would be in the order of US$ 10 million. **(4a) Patents, trademarks, or copyrights**: Provisional patent for novel method for extracting mycobacterial DNA from sputum. This patent is jointly owned by the University of Cape Town and the expert. This interest is ongoing.
Conclusion	Non-significant

AUD: Australian dollars; DNA: deoxyribonucleic acid; DR-TB: drug-resistant TB; DST: drug susceptibility testing; EDCTP: European & Developing Countries Clinical Trials Partnership; FIND: Foundation for Innovative New Diagnostics; LOD: limit of detection; MRC: Medical Research Council; NIH: National Institutes of Health; POC: point of care; R2D2: Rapid Research in Diagnostics Development; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TB: tuberculosis; United Kingdom: United Kingdom of Great Britain and Northern Ireland; USAID: United States Agency for International Development; WHO: World Health Organization.

Annex 3. Pretomanid minimum inhibitory concentrations distributions across lineages and possible impact on clinical outcomes: a systematic review and individual-level data analysis

Report for the World Health Organization, Global TB Program

v3.0, August 2023

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Figure 18. Time to sustained negative mycobacterial culture by treatment participants with L1 TB

Abbreviations list

BDQ	Bedaquiline
CDC	Centers for Disease Control and Prevention, USA
CI	Confidence interval
ECOFF	Epidemiological Cut-Off
IML Red	IML Red GmbH, Gauting, Germany
IHMT	Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal
IQR	Interquartile range
ITM	Institute of Tropical Medicine Antwerp
L	Lineage
LJ	Löwenstein-Jensen
LZD	Linezolid
MIC	Minimal inhibitory concentrations
MGIT	Mycobacterial Growth Indicator Tube™
MDR	Multi-drug resistant
MTBC	*Mycobacterium tuberculosis* complex
Mtb	*Mycobacterium tuberculosis*
MFX	Moxifloxacin
NICD	National Institute for Communicable Diseases, South Africa
NITRD	National Institute of Tuberculosis and Respiratory Diseases, India
OSR	Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
PA	Pretomanid
RR	Rifampicin resistant
SRL-Germany	Supranational Reference Laboratory for TB, Borstel, Germany
SRL-Sweden	Supranational Reference Laboratory for TB, Stockholm, Sweden
SU	Stellenbosch University, Stellenbosch, South Africa
TTS	Time to culture negative status
UCL	University College London, Clinical Microbiology, London, UK
7H10	Middlebrook 7H10
7H11	Middlebrook 7H11

1. Introduction

Pretomanid (PA), previously known as PA-824, is a new anti-mycobacterial oral drug approved in 2019 by the US Food and Drug.[1] PA is one of three or one of four drugs in the new 6-month regimen recommended by WHO to treat people with pulmonary rifampicin (RR), multi-drug resistant (MDR) or pre-XDR (extensively resistant) TB. The use of PA is approved in the BDQ (bedaquiline)-PA-LZD (linezolid)-MFX (moxifloxacin) (BPaLM) and BDQ-PA-LZD (BPaL) combined regimens.[2] Its clinical use calls for a robust phenotypic antimicrobial susceptibility testing method with a breakpoint informed by epidemiological cut-off values (ECOFFs) and clinical outcome data.

This report summarises the evidence from:

1. a systematic review of the literature: to describe the distribution of minimal inhibitory concentrations (MICs) of PA
2. an individual level analysis of *Mycobacterium* tuberculosis (*Mtb*) isolates to determine PA ECOFFs overall and by lineage (L)
3. an individual level analysis of patient outcome data: comparing outcomes in participants receiving a PA-based regimen with L1 TB vs L2, L3, or L4 TB
4. an individual level analysis of patient outcome data: comparing outcomes in participants with L1 TB either receiving a PA-based regimen or a standard regimen

2. Systematic review

2.1. Aims and objectives

The aim of this systematic review was to summarise the published data on MICs of PA using the Löwenstein-Jensen (LJ), Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11), and the Becton Dickinson Mycobacterial Growth Indicator Tube™ (MGIT) and describe any associations between the MIC distribution and lineage.

2.2. Methods

The review was conducted in accordance with the principles outlined in the PRISMA statement.[3]

Laboratory studies, clinical trials, cross-sectional studies, cohort studies, data from routine surveillance were eligible.

To describe the PA MIC studies had to fulfil the following inclusion criteria:

1. The MICs for PA with at least three concentrations tested was determined using the proportion method with a critical proportion of 1%, using LJ, 7H10, 7H11 or MGIT.
2. The PA concentrations tested were clearly defined (i.e. to assess potential truncations of the MIC results).

3. The number of isolates tested at each concentration were provided (i.e. to evaluate the shape of the MIC distributions and determine the mode of the distributions).
4. The MIC data were available for at least 10 isolates per laboratory.
5. For studies that reported only MIC ranges (i.e. did not meet the third criterion), we tried to obtain raw data from the corresponding authors and/or their co-authors.

Review articles, case reports, commentaries, editorials, modelling studies, other studies which did not report on primary data were excluded.

Only *Mycobacterium tuberculosis* complex (MTBC) isolates were eligible to be included in the analysis regardless of resistance profile. The outcome of interest was PA MIC. Possible explanatory variables used to determine difference in MIC distributions included lineage, country of origin and resistance pattern.

The following databases were searched with no restriction for time or language.

- MEDLINE
- EMBASE
- Web of Science
- Cochrane Library

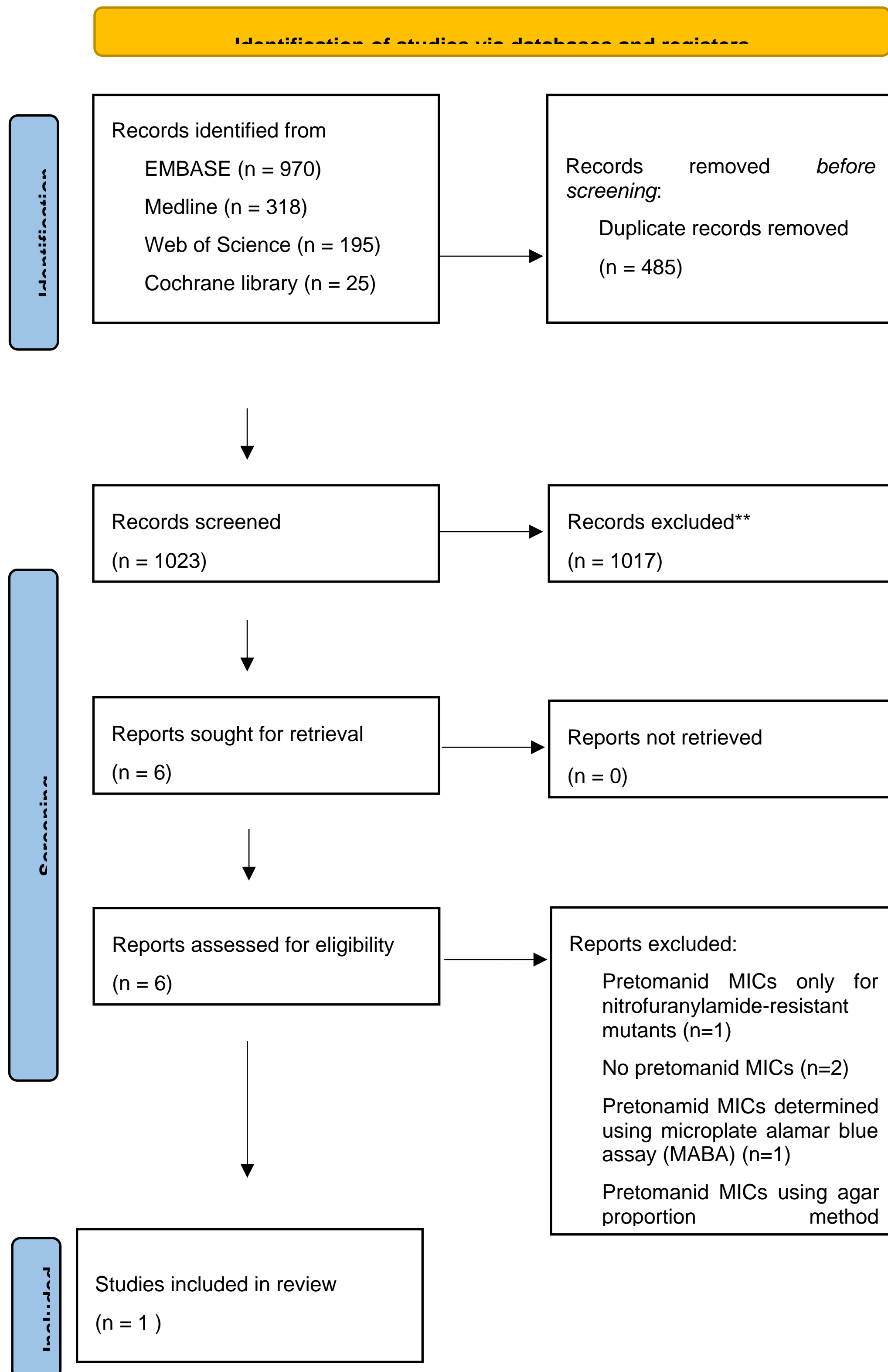
We used a search strategy combing "Pretomanid" or "PA-824" with search terms for "tuberculosis".

2.3.Results

2.3.1. Search yield

A total of 1508 articles were identified during the database search of which only six were retained for full text review (Figure 1). No additional studies were identified through reference review and searching pre-prints prior to peer-review. Only one study was eligible according to the selection criteria.[4] A second study was potentially eligible, but MIC data was presented as ranges.[5] The first author of the study was contacted for individual level data. Individual level data of this study was not available.

Figure 1. PRISMA diagram of the included studies



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

2.3.2. Description of studies

The one eligible study which presented detailed MIC data provided individual level data and hence is included in the individual level analysis to establish ECOFFs (see below). The data from this study is labelled 'Bateson'.

3. Individual level analysis – mic

3.1.MGIT system

3.1.1. Methods

3.1.1.1. Data

All individual level data was provided by the TB Alliance. Data originated from a published study by Bateson et al. ('Bateson database')[4], laboratory surveillance from US, India, South Africa, Ukraine, Tajikistan ('Paegis database') and clinical trials namely Nix-TB[6], SimpliciTB (ClinicalTrials.gov Identifier: NCT03338621), STAND[7], ZeNix[8] ('Trial database'). Table 1 summarises the variables which were available across the different databases. The treatment and outcome information were not used for the analysis of MIC distribution.

Table 1. Available variables

Variable	**Bateson**	**Paegis**	**Trials**
Isolate ID	x	x	x
Test Laboratory*	x		
Country of Isolation		x	x
Trial	NA	NA	x
Resistance category**	x		x
MTBC member or lineage	x		For a subset of isolates
MIC (mg/L)	x	x	For a subset of isolates
Treatment and outcome information			x

*Names of laboratories were provided as a separate information from the TB Alliance, **different definitions of resistance were used across the different a databases and trials.

The following laboratories provided data: Centers for Disease Control and Prevention, USA (CDC), IML Red GmbH, Gauting, Germany (IML Red), National Institute for Communicable Diseases, South Africa (NICD), National Institute of Tuberculosis and Respiratory Diseases, India (NITRD), Supranational Reference Laboratory for TB, Borstel, Germany (SRL-Germany), Stellenbosch University, Stellenbosch, South Africa (SU), Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal (IHMT), University College London Centre for Clinical Microbiology, London, UK (UCL), Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy (OSR), Supranational Reference Laboratory for TB, Stockholm, Sweden (SRL-Sweden).

For quality control (QC) the H37Rv PA MIC was determined for every batch of MTBC isolates tested as part of the Bateson and Paegis sample set. If the QC H37Rv MIC was outside the acceptable range (0.06 to 0.5 mg/L) the whole batch of MTBC isolates had to be retested. All laboratories involved in surveillance (Paegis) were pre-qualified. This meant they had to first pass an external quality control challenge with a panel of MTBC isolates with varying PA MICs. UCL had similar QC measures when testing the ZeNix/SimpliciTB trial isolates. Therefore, the majority (or all) H37Rv PA MICs were within the 0.06 to 0.5 mg/L range. All laboratories that had performed MIC testing as part of the TB Alliance datasets provided data of repeated MIC testing of H37Rv isolates for quality assurance. H37Rv distributions were plotted for each laboratory.

3.2.1.2. Analysis

Only *M. tuberculosis* (*M.tb*) isolates were included in the analysis. MTBC L5, L6, L7, and L8 isolates were excluded as well as other MTBC sub-species (e.g. *M. bovis*) and *M. canettii*. Any data from mixed cultures were excluded. Data was deduplicated to ensure only unique isolates were included. Initially the MIC distribution for all *Mtb* isolates for which exact MIC data were available was investigated. Given the findings from a recently published study by Bateson et al [4] showing a shift of the MIC distribution towards the right for *Mtb* L1 compared to *Mtb* L2, L3 or L4 isolates we investigated the MIC distribution stratified by lineage. In a second steps ECOFFs were determined for *Mtb* L1 and *Mtb* L2, L3 or L4 isolate separately (for those isolates with available lineage information).

The aggregated analysis followed the EUCAST SOP 10.1. As suggested by the guidelines, the accepted MIC distribution were aggregated, by weighting them in order to contribute equally to the aggregated distribution despite the unbalance in the number of isolates. This was achieved by using weights equal to the inverse of the number of isolates for each laboratory. When the data did not fulfil all but most of the EUCAST SOP 10.1 criteria (which are the same as for SOP 10.2), a tentative ECOFF (tECOFF) was computed. All the analyses were performed using R 3.6.2 (http://www.R-project.org/) and the ECOFFinder program version 2.1 (available at EUCAST website).

In the plots, the MICs were log2 transformed [log2(MIC)] to verify the normality assumption of the distribution.

3.1.2. Results

3.1.2.1. MIC distribution of H37Rv

A total of 10 laboratories provided data on the MIC distribution of H37Rv ranging from 5 (IHMT, US) to 61 (UCL) repeat MIC measurements (Figures 2 and 3). The mode was -2 (MIC=0.25mg/L), -3 (MIC=0.125mg/L) and -4 (MIC=0.06mg/L) for 5 (CDC, NICD, NITRD, UCL, SRL-Germany) 3 (SU, SRL-Sweden, IHMT) and 2 (IML Red, OSR) laboratories, respectively.

Figure 2. MGIT MIC distribution of H37Rv by laboratory

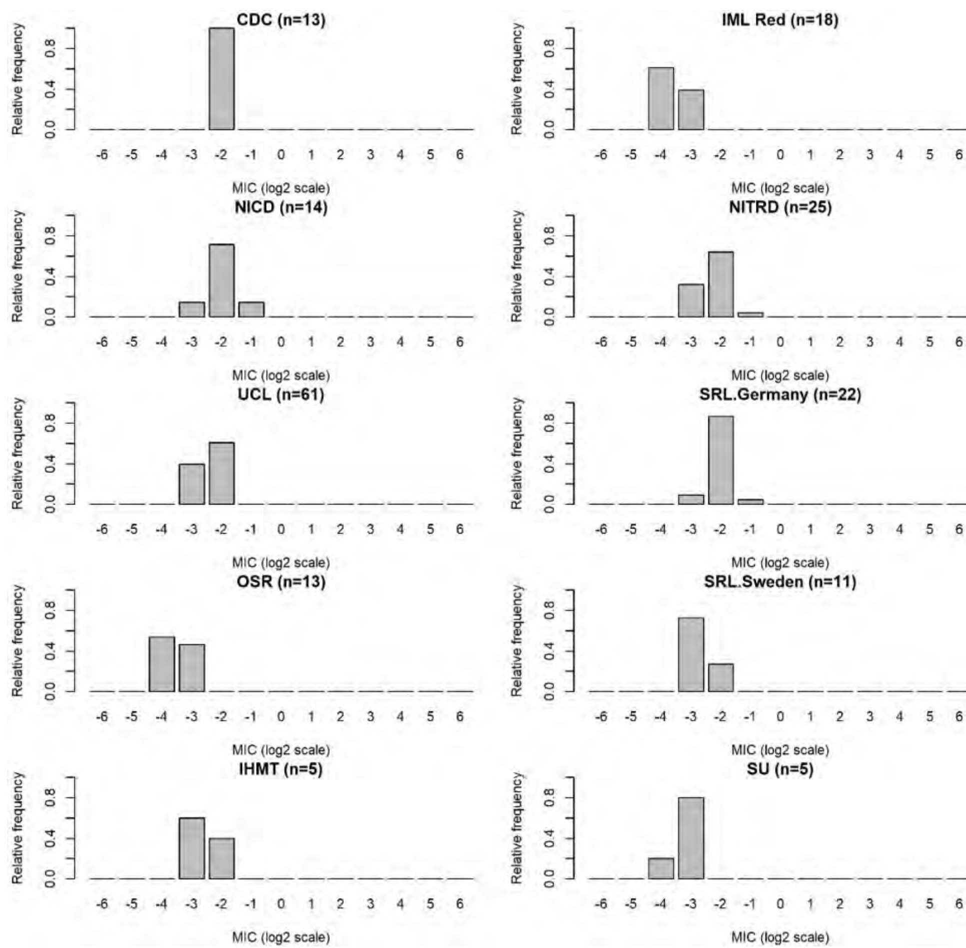
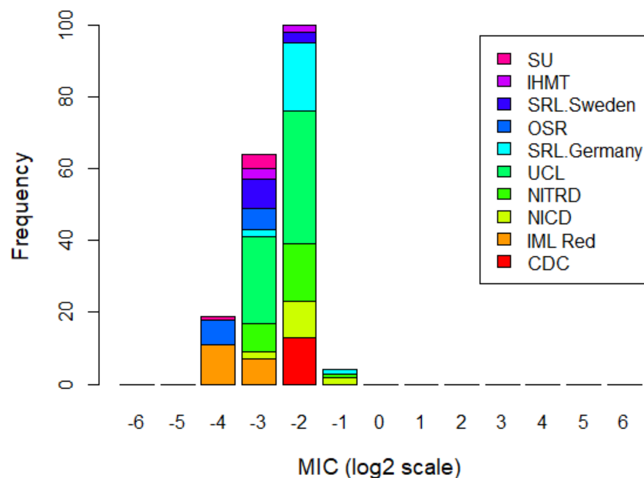


Figure 3. Aggregated MGIT MIC distribution of H37Rv



3.1.2.2. MIC distribution for *Mtb* isolates overall

A total of 1365 isolates with MGIT MIC data were available across the three databases: Paegis (n=328) Bateson (n=356) and Trial (n=681). The Bateson and Trial database contained 117 duplicates. For two of these isolates the Bateson database contained an exact MIC, while the Trial database did not, thus the MICs in Bateson database were used. From the remaining 1248

unique isolates a total of 52 isolates were excluded leaving 1196 isolates for analysis. Reasons for exclusion were: i) non *Mtb* (*M. canettii* n=21, *M. bovis* n=2, *M. bovis* BCG n=1, *M. caprae* n=1, M. *microti* n=1, *M. pinnipedii* n=1); ii) non lineage 1,2,3,4 (lineage 5 n=2, lineage 6 n=3, lineage 7 n=3), mixed cultures (lineages 2 and 4 n=4, lineages 1 and 3 n=1, lineages 3 and 4 n=1) and isolates without exact MICs (≤0.004 n=1; ≤0.008: n=1; >8 n=1; >16 n=7; >32 n=1) (Table 2). The number of isolates tested by laboratory ranged from 20 (IHMT) to 699 (UCL) (Table 3).

Table 2. Isolates without exact MIC

Dataset or trial	Laboratory	Country	lineage	MIC (mg/L)
Bateson	SRL-Germany		1.1.1.1	>32
Bateson/Trials-STAND	UCL		2.2.1	>16
Bateson	UCL		1.1.3	>8
Paegis	CDC	United States		>16
Paegis	NICD	South Africa		>16
Paegis	NICD	South Africa		>16
Trials-SimpliciTB	UCL	Tanzania	3	<=0.004
Trials- SimpliciTB	UCL	Tanzania	4.2.2.2	<=0.008
Trials-Zenix	UCL	South Africa	2.2.1	>16
Trials-Zenix	UCL	Russia	2.2.1	>16
Trials-Zenix	UCL	Russia	2.2.1	>16

Table 3. Number of isolates per laboratory

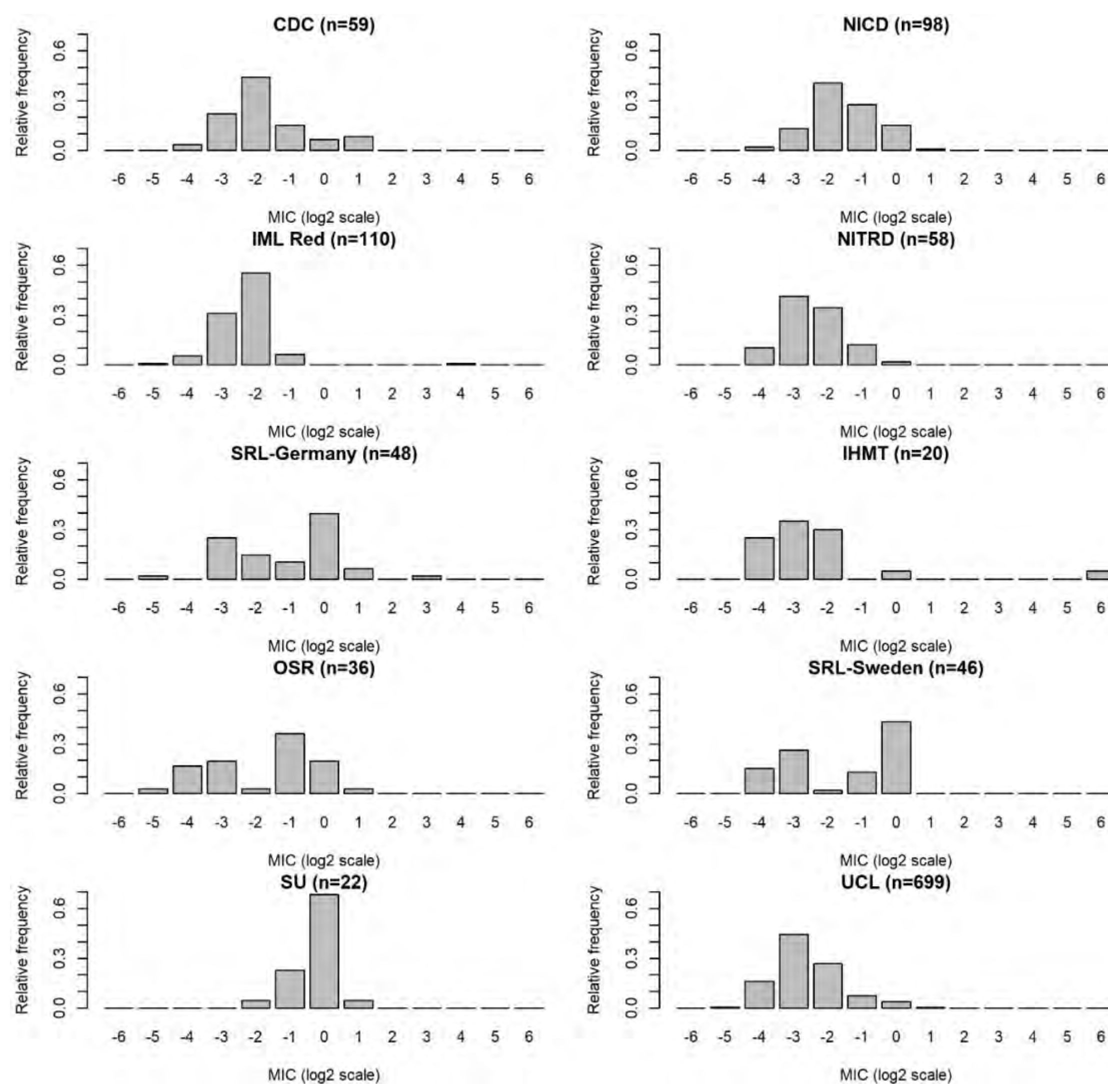
Laboratory	Number of isolates	Database
CDC	59	Paegis (United States)
IML Red	110	Paegis (Tajikistan, Ukraine)
NICD	98	Paegis (South Africa)
NITRD	58	Paegis (India)
SRL-Germany	48	Bateson
SU	22	Bateson
IHMT	20	Bateson
UCL	699	Bateson & Trial
OSR	36	Bateson
SRL-Sweden	46	Bateson

Three laboratories (SRL-Germany, SRL-Sweden, OSR) showed a bimodal distribution in log2 scale (Figure 4). Among the seven laboratories with a unimodal distribution the mode was -3 (MIC=0.125mg/L) (n=1, UCL), between -3 (MIC=0.125mg/L) and -2 (MIC=0.25mg/L) (n=2, NITRD, IHMT), -2 (MIC=0.25mg/L) (n=3, CDC, IML Red, NICD) and 0 (MIC=1mg/L) (n=1, SU).

Data from the three laboratories with bimodal distributions were excluded from the analysis (see 3.3.1 in EUCAST SOP 10.1/2). Among the remaining seven laboratories with unimodal distribution, the MIC distribution of one laboratory (SU) did not have a MIC mode equal to or within one two-fold dilution of the most common mode MIC observed in the other distributions. Therefore, the data from SU were excluded (3.3.6 in EUCAST SOP 10.1/2).

Further analysis was conducted on the remaining isolates (n=1044) from six laboratories, thus complying with the criteria set by EUCAST SOP 10.1/2. EUCAST SOP 10.1/2 sets out the following minimum criteria to determine ECOFFs: i) 5 laboratories; ii) 15 isolates tested per laboratory and iii) and 100 isolates.

Figure 4. MGIT MIC distribution of all *Mtb* isolates by laboratory



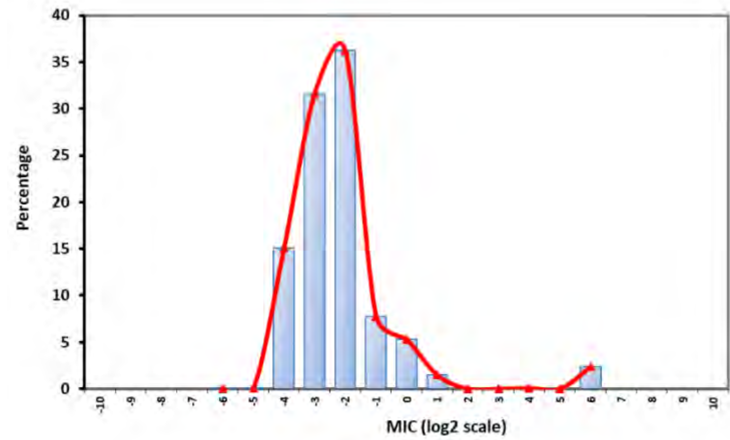
An aggregated MIC distribution using 1044 isolates from six laboratories was established. The table in Figure 5 shows the aggregated MIC distribution (as absolute and relative frequencies) and the aggregated weighted MIC distribution (as relative frequencies). The aggregated

weighted MIC distribution was computed to control for imbalance in the number of isolates tested in each laboratory (Appendix 1 EUCAST SOP 10.1).

The aggregated weighted distribution was ***asymmetrical around the mode and spread across more than 5 dilutions*** suggesting there may be more than one wildtype distribution with differing modes (Figure 5) (see 3.5.2 and 3.5.3 of the EUCAST SOP 10.2). Therefore, no ECOFF was computed.

Figure 5. Aggregated MGIT MIC distribution of all *Mtb* isolates

MIC (mg/L)	log2MIC	Unweighted		Weighted
		N	%	%
0.016	-6	1	0.0958%	0.0020%
0.03	-5	6	0.5747%	0.0885%
0.06	-4	134	12.8352%	15.0535%
0.125	-3	400	38.3142%	31.6108%
0.25	-2	339	32.4713%	36.2207%
0.5	-1	103	9.8659%	7.7821%
1	0	48	4.5977%	5.3028%
2	1	9	0.8621%	1.4742%
4	2	1	0.0958%	0.0020%
8	3	0	0.0000%	0.0000%
16	4	2	0.1916%	0.0807%
32	5	0	0.0000%	0.0000%
64	6	1	0.0958%	2.3829%



3.1.2.3. MIC distribution by lineage

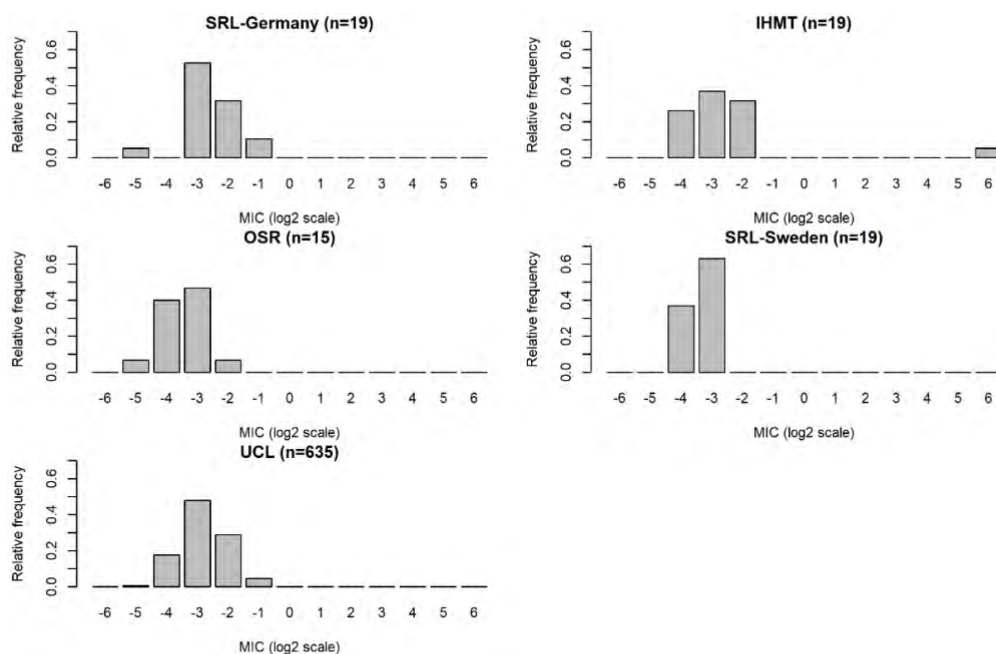
3.1.2.3.1. Mtb L2, L3 or L4 isolates

A total of 707 L2, L3 or L4 isolates tested in five laboratories contributed to this analysis. All laboratories tested at least 15 isolates (Table 4). MIC distributions (in log2 scale) were unimodal across all five laboratories with the same MIC mode of -3 (MIC=0.125mg/L) allowing to estimate an ECOFF using the aggregated weighted data (Figure 6).

Table 4. Number of *Mtb* L2, L3 or L4 isolates per laboratory

Laboratory	Number of isolates	Database
SRL-Germany	19	Bateson
IHMT	19	Bateson
UCL	635	Bateson & Trial
OSR	15	Bateson
SRL-Sweden	19	Bateson

Figure 6. MGIT MIC distribution for *Mtb* L2, L3 or L4 isolates by laboratory



Aggregated unweighted and weighted MIC distributions (as absolute and relative frequencies) were calculated (Figure 7). The MIC distribution was symmetrical and within 5 dilutions. The estimated distribution, for computing the ECOFF, well fitted the distribution of the data, since the normality assumption on the $\log_2$ scale was satisfied. The ECOFF at 99.0% and 99.9% was 0.5mg/L (Figure 8).

Stratified analysis by drug resistance status of *Mtb* L2, L3 or L4 isolates was performed to investigate differences in ECOFF, 332 were classified as drug susceptible and 375 as drug resistant by the respective study. Arguable drug resistant isolates may not represent the wildtype population. Hence focusing on the drug susceptible isolates may provide a more accurate ECOFF. Only UCL contributed ≥ 15 MIC data points for each drug resistance

category. This dataset therefore did not fulfil the minimum EUCAST criteria. The 99.0% and 99.9% was 0.25mg/L and 0.5mg/L, respectively, for drug susceptible isolates (data not shown), similar to the finding of the combined analysis.

Figure 7. Aggregated MGIT MIC distribution for *Mtb* L2, L3 or L4 isolates

MIC (mg/L)	log2MIC	Unweighted		Weighted
		N	%	%
0.016	-6	0	0.0000%	0.0000%
0.03	-5	7	0.9901%	3.1958%
0.06	-4	130	18.3876%	26.6147%
0.125	-3	340	48.0905%	49.6150%
0.25	-2	196	27.7228%	16.8656%
0.5	-1	31	4.3847%	2.4817%
1	0	2	0.2829%	0.0022%
2	1	0	0.0000%	0.0000%
4	2	0	0.0000%	0.0000%
8	3	0	0.0000%	0.0000%
16	4	0	0.0000%	0.0000%
32	5	0	0.0000%	0.0000%
64	6	1	0.1414%	1.2250%

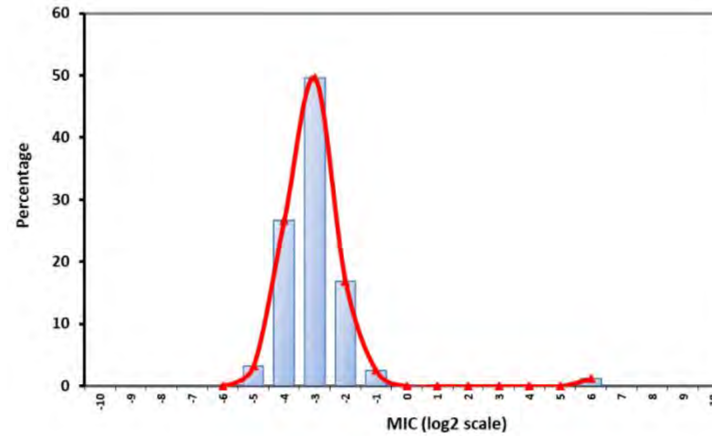
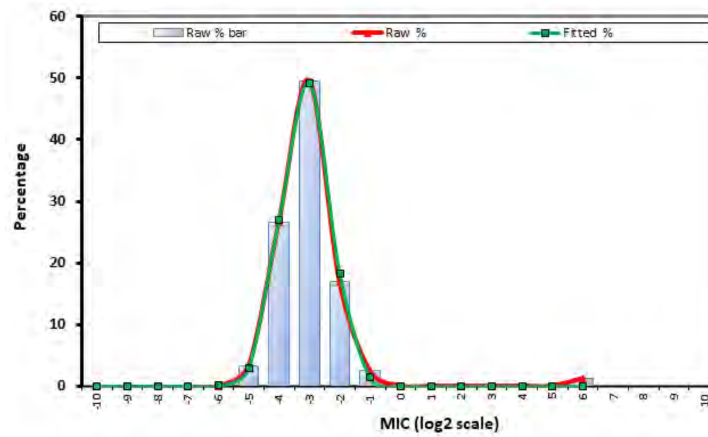


Figure 8. Computed MGIT ECOFF for *Mtb* L2, L3, or L4 isolates with the ECOFFinder program v. 2.1

Selected Subset	≤ 32	Dil Range
Modal MIC	0.125	5
Log_2MIC Mode	-3	
Max Log_2MIC	6	
Selected Log_2 Mean	-3.6231	=0.081 µg/mL
Selected Log_2 SD	0.7347	
CV	54.4%	

Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	0.1876	0.25	3.7%	18.5%
ECOFF 97.5%	0.2202	0.25	3.7%	18.5%
ECOFF 99.0%	0.2654	0.5	1.2%	1.3%
ECOFF 99.5%	0.3013	0.5	1.2%	1.3%
ECOFF 99.9%	0.3916	0.5	1.2%	1.3%



3.1.2.3.2.   Mtb L1 isolates

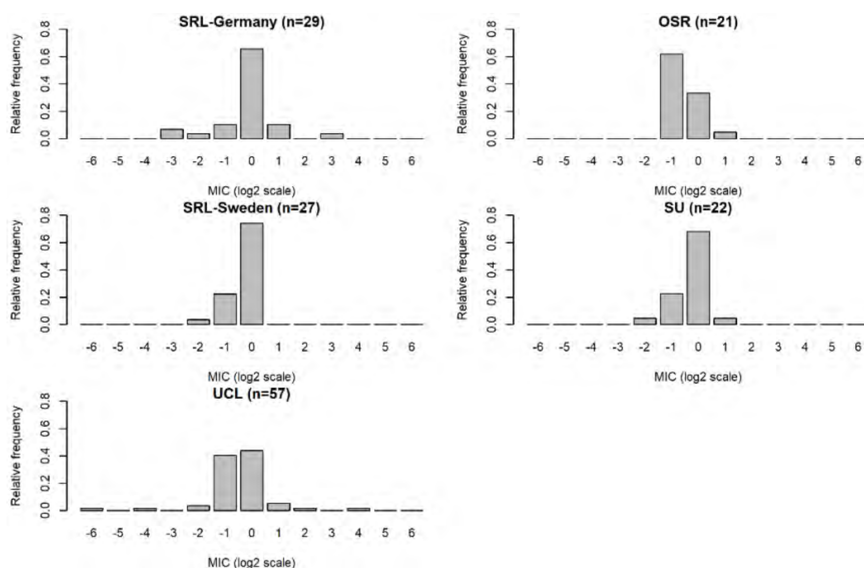
Data on 157 L1 isolates tested in six laboratories were available (Table 5). Except for IHTM all laboratories tested more than 15 isolates the minimum EUCAST SOP 10.1/2 criteria. The isolate tested at IHTM was excluded from the analysis.

Table 5: Number of *Mtb* L1 isolates per laboratory

Laboratory	Number of isolates	Database
SRL-Germany	29	Bateson
SU	22	Bateson
IHMT	1	Bateson
UCL	57	Bateson & Trial
OSR	21	Bateson
SRL-Sweden	27	Bateson

MIC distributions obtained in all five laboratories were unimodal (Figure 9). MIC modes in $\log_2$ scale were 0 (MIC=1mg/L) (n=3, SRL-Germany, SU, SRL-Sweden), between 0 (MIC=1mg/L) and -1 (MIC=0.5mg/L) (n=1, UCL) and -1 (MIC=0.5mg/L) (n=1, OSR). Across all five laboratories the mode was equal to or within one two-fold dilution of the most common mode MIC observed in the other distributions fulfilling the criteria set out by 3.3.6 in EUCAST SOP 10.1/2. Thus, all laboratories were included in the analysis. However, given that only 4/5 laboratories agreed on the same mode (while the minimum should be five) a tentative ECOFF (tECOFF) was estimated (see 3.2.8 and 3.2.9 in EUCAST SOP 10.1/2).

Figure 9. MGIT MIC distribution for *Mtb* L1 isolates by laboratory



Aggregated unweighted and weighted MIC distribution (as absolute and relative frequencies) and the aggregated weighted MIC distribution were calculated. The distribution was symmetrical and within 4 or 5 dilutions (Figure 10). The estimated distribution, for computing

the tECOFF, well fitted the distribution of the data The tECOFF at 99.0% and 99.9% was 2mg/L (Figure 11). We did not perform an analysis stratified by resistance category because of small samples size.

Figure 10. Aggregated MGIT MIC distribution for *Mtb* L1 isolates

MIC (mg/L)	log2MIC	Unweighted		Weighted
		N	%	%
0.016	-6	1	0.6410%	0.1690%
0.03	-5	0	0.0000%	0.0000%
0.06	-4	1	0.6410%	0.1690%
0.125	-3	2	1.2821%	1.3057%
0.25	-2	5	3.2051%	2.8783%
0.5	-1	50	32.0513%	32.2206%
1	0	86	55.1282%	57.4218%
2	1	8	5.1282%	4.8448%
4	2	1	0.6410%	0.1690%
8	3	1	0.6410%	0.6528%
16	4	1	0.6410%	0.1690%
32	5	0	0.0000%	0.0000%
64	6	0	0.0000%	0.0000%

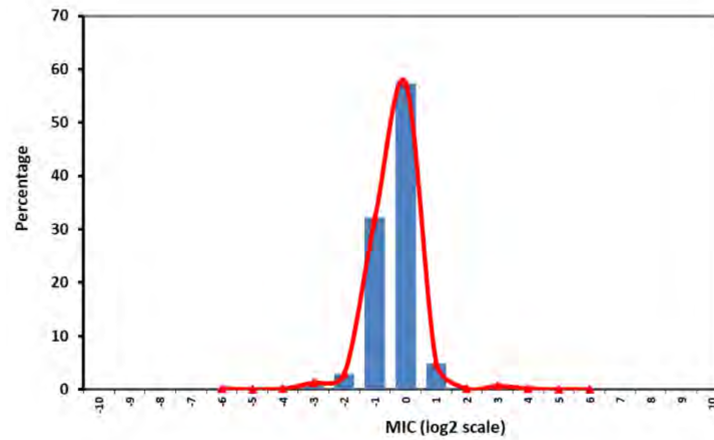
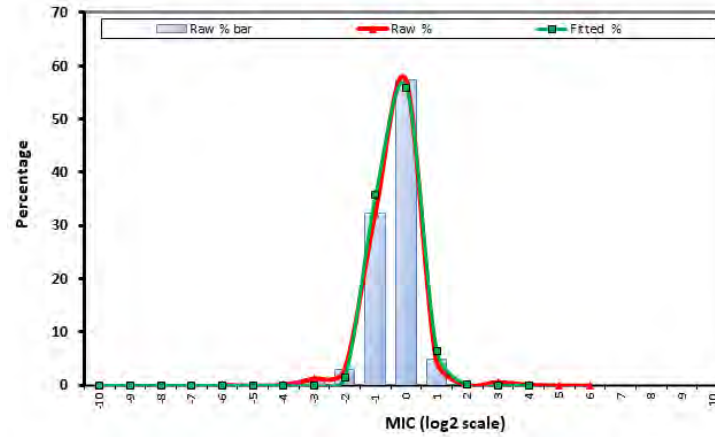


Figure 11. Computed MGIT tECOFF for *Mtb* L1 isolates with the ECOFFinder program v. 2.1

Selected Subset	≤ 8	Dil Range
Modal MIC	1	4
Log_2MIC Mode	0	
Max Log_2MIC	4	
Selected Log_2 Mean	-0.8271	=0.564 µg/mL
Selected Log_2 SD	0.5431	
CV	39.0%	

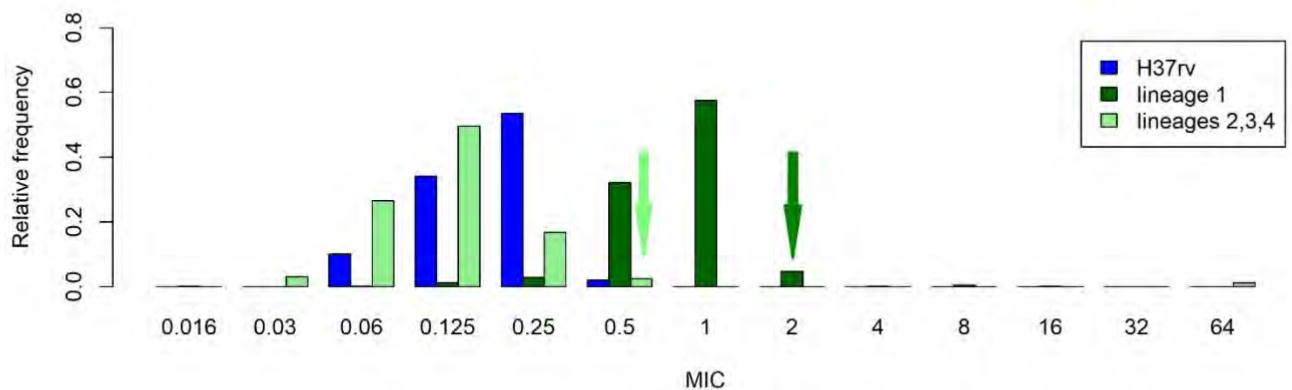
Selected Values	Exact	R'd-up	%Obs>	%@ECOFF
ECOFF 95.0%	1.0469	2	1.0%	6.3%
ECOFF 97.5%	1.1788	2	1.0%	6.3%
ECOFF 99.0%	1.3531	2	1.0%	6.3%
ECOFF 99.5%	1.4863	2	1.0%	6.3%
ECOFF 99.9%	1.8039	2	1.0%	6.3%



3.2. Comparison of lineage and H37Rv MIC distributions (MGIT)

The PA MIC distributions of H37Rv isolates were compared with *Mtb* L2, L3 or L4 and *Mtb* L1 isolates (Figure 12). The MIC distributions of H37Rv and *Mtb* L2, L3 or L4 isolates spanned the same range of dilutions with a similar mode (H37Rv: 0.25mg/L, *Mtb* L2, L3 or L4 isolates: 0.125mg/L), while the MIC distribution of *Mtb* L1 isolates was shifted to the right with a mode of 1mg/L.

Figure 12. Aggregated weighted MGIT MIC distributions of clinical isolates and MGIT MIC distribution of H37Rv



3.3. Solid media 7H11

3.3.1. Methods

3.3.1.1. Data

All individual level data (n=101) was provided from the Institute of Tropical Medicine Antwerp (ITM). Isolates were tested using a solid media (7H11) method at the ITM laboratory. MIC data and lineage data were provided.

3.3.1.2. Analysis

Only *Mtb* isolates were included in the analysis. *Mtb* L 5, 6, 7 and 8, isolates were excluded. Any data from mixed cultures were excluded. Since the data did not fulfil any of the EUCAST SOP 10.1/2 criteria, we described the MIC distributions for i) all isolates and ii) stratified by lineage, but did not attempt to compute an ECOFF.

3.3.2. Results

3.3.2.1. MIC distribution for *Mtb* isolates overall

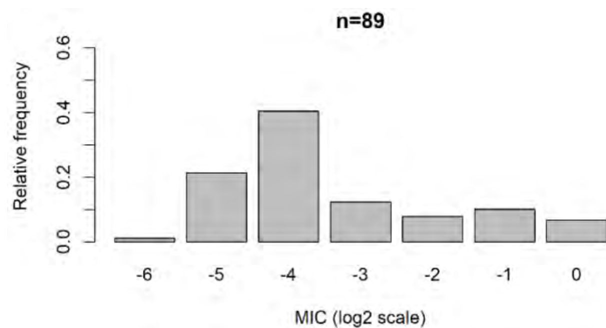
Data was available on 6 repeat MIC tests for H37Rv: the MIC was 0.125mg/L (n=5) and 0.06mg/L (n=1).

7H11 MIC data were available for 101 isolates, of those 10 isolates were excluded because they belonged to lineages 5,6,7, and 8. An additional 2 lineage 4 isolates were excluded because no exact MIC value was available (isolate ID 2004-00851 and 2004-02926 with MICs≤0.016 mg/L) resulting in a total of 89 isolates tested in a single laboratory.

The MIC distribution (in log2 scale) was unimodal but asymmetric around the mode -4 (MIC=0.06mg/L) and encompassed more than 5 dilutions (see 3.5.2 and 3.5.3 of the EUCAST SOP 10.1/2). In addition, the distribution had a long right sided tail with a possible second peak at -1 (MIC=0.5mg/L) (Figure 13) (see 3.3.1 of the EUCAST SOP 10.1/2).

Figure 13. 7H11 MIC distribution for all *Mtb* isolates

MIC (mg/L)	log2MIC	N	%
0.016	-6	1	1.1236%
0.03	-5	19	21.3483%
0.06	-4	36	40.4494%
0.125	-3	11	12.3596%
0.25	-2	7	7.8652%
0.5	-1	9	10.1124%
1	0	6	6.7416%



3.3.2.2. <u>MIC distribution by lineage</u>

Data on 70 L2, L3 or L4 *Mtb* isolates were available. The MIC distribution (in log2 scale) was slightly asymmetric around the mode -6 (MIC=0.06mg/L) and spread across <5 dilutions (Figure 14).

Data on 19 L1 *Mtb* isolates were available. The MIC distribution (in log2 scale) was symmetric around the mode -1 (MIC=0.5mg/L) (Figure 15).

While more data from laboratories is needed to determine an ECOFF for 7H11, there is a clear difference in distributions between *Mtb* L1 and *Mtb* L2, L3 or L4 isolates similar to the results obtained in the MGIT system.

Figure 14. 7H11 MIC distribution for *Mtb* L2, L3 or L4 isolates

MIC (mg/L)	log2MIC	N	%
0.016	-6	1	1.4286%
0.03	-5	19	27.1429%
0.06	-4	36	51.4286%
0.125	-3	11	15.7143%
0.25	-2	3	4.2857%
0.5	-1	0	0.0000%
1	0	0	0.0000%

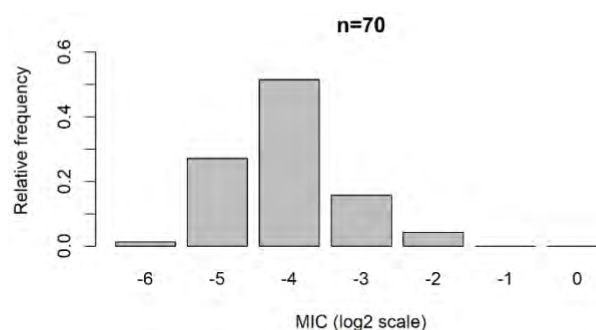
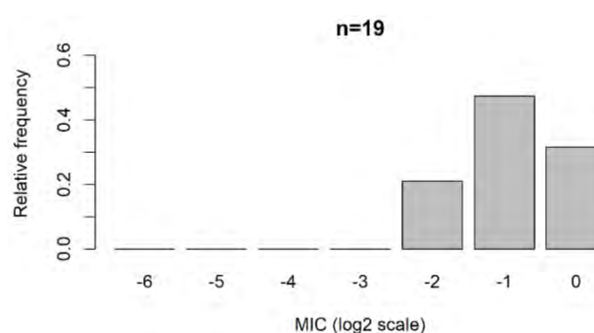


Figure 15. 7H11 MIC distribution for *Mtb* L1 isolates

MIC (mg/L)	log2MIC	N	%
0.016	-6	0	0.0000%
0.03	-5	0	0.0000%
0.06	-4	0	0.0000%
0.125	-3	0	0.0000%
0.25	-2	4	21.0526%
0.5	-1	9	47.3684%
1	0	6	31.5790%



4. Outcome analysis by lineage and regimen

From above analysis it is clear that *Mtb* L1 isolates have a higher ECOFF (MGIT: tECOFF 2 mg/L) than *Mtb* L2, L3 and L4 isolates (ECOFF MGIT: 0.5 mg/L). Globally L1 accounts for 28% of TB in 2012 and 2018.[9] Over 80% of the L1 global burden was in India, the Philippines, Indonesia and Bangladesh. The proportion of *Mtb* L1 may differ between drug susceptible and drug resistant isolates.[10-13] Lineage and PA MGIT MIC information was only available for a subset of the 'Trial' data (688/1029). Only 53/688 (7.9%) of the isolates for which lineage information was available belonged to L1. Given the predominance of L2, L3 and L4 TB across the trials and the fact that *Mtb* L1 isolates have intrinsically higher MICs, results from trials investigating PA-based regimens may not be generalisable to L1 TB.

Given that countries had started introducing the BPaL/M regimen programmatically WHO issued a public call on the 17.04.2023 for data on PA MIC distributions across *Mtb* lineages and treatment outcomes. The aim of the call was to enhance the existing 'Trial' data with programmatic data to possibly allow for a more meaningful analysis. Few countries who had started implementing BPaL/M provided additional data. This included Ireland (Irish Mycobacteria Reference Laboratory, Labmed Directorate, St. James's hospital, Dublin), Sweden (Public Health Agency of Sweden, Unit for Laboratory Surveillance of Bacterial Pathogens), Indonesia (Yayasan Riset dan Pelatihan Respirasi Indonesia, Respiratory Society

of Indonesia) and India (ICMR-National Institute For Research in Tuberculosis, Chennai). In addition Médecins Sans Frontières – Netherlands (MSF) provided data from the TB-Practecal trial conducted in three countries (South Africa, Belarus, Uzbekistan). The minimum variables needed for data to be included in the treatment outcome analysis was: regimen type, treatment outcome, lineage and Pa MGIT MIC. India supplied aggregated MIC data by lineage confirming the ECOFF analysis performed as part of this report. Indonesia supplied data on regimen, treatment outcomes and lineage, but did not have Pa MIC data. Hence data from India and Indonesia were not included in the treatment outcomes analysis (see Table 7). The number of L1 *Mtb* isolates across the different dataset were sequencing results were available was: Sweden 1/17, Ireland 0/10, Indonesia 1/43, India 114/221 and MSF 0/67. The MSF dataset had eleven persons with mixed culture (L2 and L4).

To investigate whether the increased MIC of L1 *Mtb* isolates was clinically significant we conducted an analysis of available data to investigate whether PA-based regimens revealed similar results in participants with L1 TB (with wildtype MICs) compared to participants with L2, L3 or L4 TB (with wildtype MICs). We further compared outcomes in participants with L1 TB (with wildtype MICs) receiving PA-based regimens versus standard regimens.

It is important to note that the analyses which was conducted used data from clinical trials and some post-implementation data with very different "PA-based" regimens. Differences included the addition of MXF and clofazimine, dosing (for MXF and LZD) and duration of treatment. This is a major limitation of the analysis. Furthermore, the exclusion of records due to missing lineage and PA MIC information resulted in a highly biased sample set. The sample size was small. Thus, we could not adjust for confounding. Importantly we could not adjust for resistance to other drugs in the regimen. However, we decided to perform the analysis to provide an example of such an analysis pooling data across different trials or observational studies.

In addition, we performed a samples size calculation for:

- a non-inferiority trial comparing a PA-based WHO recommended regimen with a standard regimen in L1 TB
- an observational study comparing outcomes in L1 MDR-TB with L2, L3 or L4 MDR-TB receiving PA-based WHO recommended regimen

4.1. Methods

4.1.1. Data

The 'Trials' database included 681 participants with available MGIT PA MIC data. We excluded records without lineage information (n=7), with mixed cultures (n=6) and those with PA resistant isolates (n=8) (Table 6). A total of 660 records of the 'Trials' database were included in the analysis. Table 7 summarizes the additional data submitted to WHO in response to the public call the total number of participants and reasons for exclusions. For specific analysis additional information was required and hence the number of records may have varied by analysis dependent on the availability of such information.

4.1.2. Analysis

Comparisons of the proportion of the unfavourable versus favourable primary outcomes between two groups were performed with the Fisher's exact test. Participants categorized as

unassessable for the primary outcome were excluded from the analysis. An analysis detailing the reasons for being "unassessable" is provided in the result section.

For the analysis of the time to negative culture status (TTNS), participants without positive culture between Screening and Week 4 were excluded. The TTNS was analysed as a time-to-event outcome and participants who did not reach a negative status because of death or withdrawn were censored. For these participants, the time was computed up to death or withdrawal. The Kaplan-Meier estimator was used to estimate the survival curve and to derive descriptive statistics of the outcome. The log-rank test was employed for comparing the TTNS-free survival between two groups, while the Cox's proportional hazards regression for comparisons accounting also for the resistance status.

In all the analyses, the significant level was set to 0.05. All the analyses were performed using R 3.6.2 (http://www.R-project.org/).

The sample size calculations were performed for comparing the proportion of the unfavourable primary outcomes with the Fisher's exact test in different settings which are explained in the results section. The computations were performed with the PASS 2021 (Power Analysis and Sample Size Software (2021). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass).

Table 6. PA-resistant isolates in the 'Trials' databaseTrial

Trial	Subject ID	Country	TB type	Lineage	PA MIC (mg/L)
STAND	0601-004	Ukraine	DS	2.2.1	>16
Zenix	1004021	South Africa	PRE-XDR	2.2.1	>16
Zenix	1206003	Russia	XDR	2.2.1	1
Zenix	1206020	Russia	PRE-XDR	2.2.1	>16
Zenix	1207004	Russia	PRE-XDR	2.2.1	>16
Zenix	1207013	Russia	XDR	2.2.1	1
SimpliciTB	1403013S	Tanzania	DS	1.1.2	16
SimpliciTB	1701014R	Philippines	DR^	1.2.1.2.1	4

DR^ = Mono-resistance to rifampicin or isoniazid, or resistance to both rifampicin and isoniazid

Table 7. Additional databases submitted in response to the WHO public call and reasons for exclusion of records

Data	Total records	Excluded records	Resistance pattern unknow	No lineage information	Mixed cultures	Not L1, L2, L3 or L4	No Pa MIC	Incomplete treatment outcome	With PO	With TTNS
MSF	473	409	397	395	11	0	13	0	64	60
India	221	221	221	0	0	0	221	221	0	0
Ireland	10	6	0	0	0	0	1	5	4	0
Indonesia	51	51	7	8	0	0	51	0	0	0
Sweden	18	18	18	0	0	1	0	18	0	0

*A record could have been excluded for multiple reasons, PO = Primary outcome, TTNS = time to culture negative status

4.2.Results

The mode of the *Mtb* L1 MIC distribution of the isolates included in the analysis. was between 0.5mg/L and 1mg/L, while the mode of the *Mtb* L2,3 and 4 MIC distribution was 0.125mg/L (Table 8).

Table 8. MGIT MIC distribution by lineage of isolates included in the outcome analysis

MIC (mg/L)	***Mtb* L1**	***Mtb L2*, L3 or L4**
	N	**N**
<=0.004	0	1
<=0.008	0	1
<=0.06	0	2
<=0.25	0	4
0.008	0	0
0.016	1	0
0.03	0	4
0.06	1	107
0.125	0	299
0.25	2	222
0.5	23	37
1	23	0
2	1	0
4	0	0
8	0	0
16	0	0
>16	0	0

4.2.1. PA-based regimens: comparing outcomes in participants with L1 vs. L2, L3 or L4 TB

Overall, 41 and 512 participants with L1 TB and L2, L3 or L4 TB received PA-based regimens (Table 9). Participants received different PA-based regimens (Table 10) and in the following analysis we excluded 26 patients (L1: n=7, L2, L3 or L4: n=19) who received 4Pa100MZ, 4Pa200MZ and 6Pa200MZ.

4.2.2.1. Clinical primary outcome

Excluding the patients who received the regimens 4Pa100MZ, 4Pa200MZ and 6Pa200MZ, a total of 527 patients received PA-based regimens (L1: n=34, L2, L3 or L4: n=493), 18/34 (52.9%) *Mtb* L1 and 128/493 (26.0%) *Mtb* L2, L3 or L4 isolates were drug susceptible (Table 8).

We compared favourable vs unfavourable primary outcomes between participants with L1 TB versus L2, L3 or L4 TB. Participants with unassessable outcomes were excluded from the analysis (L1: n=3, L2, L3 or L4: n=22). Unassessable outcomes were due to:

- non-TB related death during follow-up, without TB failure or relapse (n=1)
- withdrawal during follow-up with negative mycobacterial cultures during follow-up (n=1)
- late exclusion, because of randomization failure (n=15)
- pregnancy during follow-up (n=1)
- loss to follow-up (n=6)
- not in modified intention-to-treat (mITT) population (n=1).

Among participants with L1 TB 25/31 (80.6%, 95% CI: 63.7%-90.8%) had favourable treatment outcomes compared to 404/471 (85.8%, 95% CI: 82.3%-88.6%) with L2, L3 or L4 TB. While there was no significant difference in unfavourable treatment outcomes across the two groups (p=0.4302), power to detect the observed difference with the available number of participants records was extremely low (12.9%).

The majority of unfavourable outcomes (n=51/73, L1: n=5, L2, L3 or L4: n=46) were due to withdrawal. Reasons for withdrawal were as follows:

a) adherence issues (n=5)
b) adverse event during treatment (n=31)
c) investigator-initiated without further information (n=7)
d) participant-initiated without further information (n=7)
e) treatment failure (n=1)

Excluding unfavourable outcomes due to non-treatment related withdrawal (reasons c), and d)) from the analysis did not change the results (L1 TB 25/31 (80.6%, 95% CI: 63.7%-90.8%) vs L2, L3 or L4 TB 404/457 (88.4%, 95% CI: 85.1%-91.0%), p=0.2475, power=25.1%).

Table 9. Baseline characteristics of participants by lineage

	Characteristics	*Mtb* L1		*Mtb L*2, L3 or L4	
		N (51)		N (677)	
Treatment	Standard regimen	10		165	
	PA-based regimen	41 [34§]		512 [493§]	
Drug resistance category	DS	35		291	
	DR^	14		128	
	MDR*	0		1	
	MDR	2		70	
	Pre-XDR	0		91	
	XDR	0		96	
		Standard treatment	PA-based regimen	Standard treatment	PA-based regimen
	DS	10	25 [18§]	145	146 [128§]
	DR^	0	14 [14§]	0	128 [128§]
	MDR*	0	0	0	1 [0§]
	MDR	0	2 [2§]	13	57 [57§]
	Pre-XDR	0	0	7	84 [84§]
	XDR	0	0	0	96 [96§]

DR^: Mono-resistance to rifampicin or isoniazid, or resistance to both rifampicin and isoniazid (MDR-TB)

MDR*: RR-TB with or without resistance to INH

§: excluding the regimens 4Pa100MZ, 4Pa200MZ and 6Pa200MZ

Standard regimen: 2HRZE/4HR for DS-TB, in the Practecal-TB (MSF) trial the standard regimen was the locally used and approved MDR-TB regimen at the time.

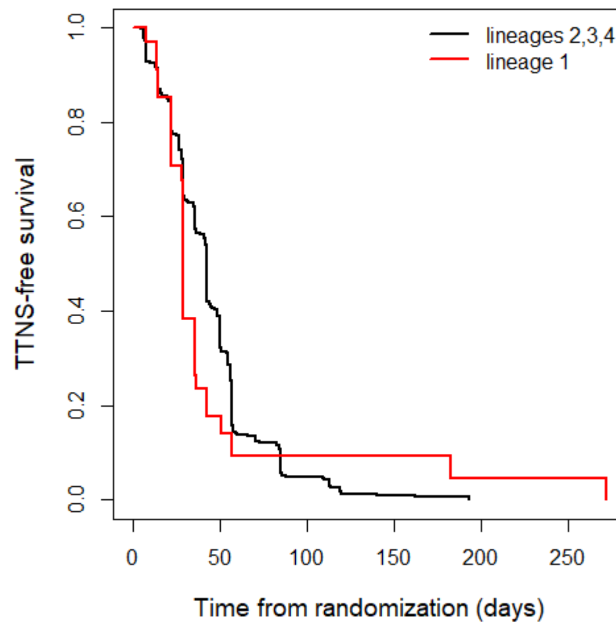
Table 10. Different PA-based regimens by lineage

	Mtb L1 (n=41)	*Mtb L2*, L3 or L4 (n=512)
BPaL	1	71
BPaL1200x26	0	35
BPaL1200x9	0	29
BPaL600x26	0	33
BPaL600x9	1	38
BPaLC	0	14
BPalLM	0	18
4BPaMZ	18	127
6BPaMZ	14	128
4Pa100MZ	2	4
4Pa200MZ	3	12
6Pa200MZ	2	3

4.2.2.2. Time to negative mycobacterial culture

We compared the time to time to culture negative status (TTNS) among participants with L1 TB and L2, L3 or L4 TB. A total of 21 participants (all L2, L3 or L4) were excluded because their sputum cultures were negative between screening for inclusion in the trial and starting treatment and 4 participants of from Ireland were excluded since TTNS information was not provided (all L2, L3 or L4). In addition, 24 participants were unassessable for TTNS: 5 died (L1: n=1, L2, L3 or L4: n=4) and 19 were withdrawn or withdrew (L1: n=1, L2, L3 of L4: n=17); their time was censored at date of death or withdrawal. Overall 34 participants with L1 TB and 468 with L2, L3 or L4 TB were included in the analysis.

Figure 16. Time to sustained negative mycobacterial culture by lineage in participants treated with PA-based regimes



The median TTNS was 43 days (95%CI of the median: 42-43 days, IQR: 27-57 days) for L2, L3 or L4 TB and 29 days (95% CI of the median: 29-36 days, IQR: 22-37 days) for L1 TB (Figure 16). The p-value of the logrank test was 0.1825. Since participants with L1 TB were more likely to have drug susceptible TB, we performed a Cox's proportional hazards regression comparing TTNS between lineages adjusting for the resistance status. The adjusted hazard ratio for L1 vs L2,3,4 was 1.31 (95%CI: 0.90-1.90), with a p-value of 0.164. Therefore, the TTNS seems comparable between the two groups, but it must be considered that the L1 TB group has a very limited sample size.

4.2.2.3 Sensitivity analysis considering only 6BPaMZ, 4BPaMZ, BPaLM and BPaLC regimens

A total of 319 patients were treated with 6BPaMZ, 4BPaMZ, BPaLM or BPaLC regimens (L1: n=32, L2,L3 or L4: n=287, Table 9). Among those, 18/32 (56.3%) *Mtb* L1 and 128/287 (44.6%) *Mtb* L2, L3 or L4 isolates were drug susceptible (Table 9, Table 10).

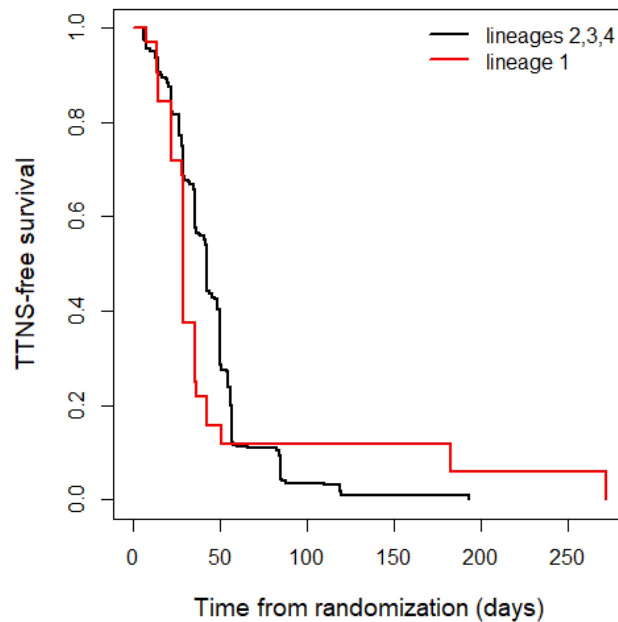
Firstly, we compared favourable vs unfavourable primary outcomes between participants with L1 TB versus L2, L3 or L4 TB. Twentytwo participants with unassessable outcomes were excluded from the analysis (L1: n=3, L2, L3 or L4: n=19). Among participants with L1 TB 23/29 (79.3%, 95% CI: 61.6%-90.2%) had favourable treatment outcomes compared to 226/268 (84.3%, 95% CI: 79.5%-88.2%) with L2, L3 or L4 TB. Although there was no significant difference in unfavourable treatment outcomes across the two groups (p=0.4368), power to detect the observed difference with the available number of participants records was extremely low (11.1%).

The majority of unfavourable outcomes (n=39/48, L1: n=5, L2, L3 or L4: n=34) were due to withdrawal. Excluding unfavourable outcomes due to non-treatment related withdrawal (investigator-initiated or patient-initiated without further information, n=9 all L2,L3 or L4 TB)

from the analysis did not change the results (L1 TB 23/29 (79.3%, 95% CI: 61.6%-90.2%) vs L2,L3 or L4 TB 226/259 (87.3%, 95% CI: 82.6%-90.8%), p= 0.2514, power=22.1%).

We compared the time to TTNS among participants with L1 TB and L2, L3 or L4 TB. Overall 32 participants with L1 TB and 283 with L2, L3 or L4 TB were included in the analysis. The median TTNS was 43 days (95% CI of the median: 41-44 days, IQR: 28-55 days) for L2, L3 or L4 TB and 29 days (95% CI of the median: 29-36 days, IQR: 22-37 days) for L1 TB (Figure 17). The p-value of the logrank test was 0.0919. Since participants with L1 TB were more likely to have drug susceptible TB, we performed a Cox's proportional hazards regression comparing TTNS between lineages adjusting for the resistance status. The adjusted hazard ratio was 1.39 (95% CI: 0.93-2.06), with a p-value of 0.106. Therefore, the TTNS seems comparable between the two groups, but it must be considered that the L1 TB group has a very limited sample size and residual confounding effects could be present.

Figure 17. Time to sustained negative mycobacterial culture by lineage in participants treated with 6BPaMZ and 4BPaMZ regimes



4.2.2. Outcomes in participants L1 TB receiving standard vs PA-based regimens

4.2.2.1. Clinical outcomes

A total of 51 participants were included in this analysis, 41 treated with a PA-based regimen and 10 treated with a standard regimen. Participants with unassessable outcomes were excluded (PA-based regimen: n=6, standard regimen: n=2) leaving a total of 43 participants in the analysis (PA-based regimen: n=35, standard regimen: n=8). Reasons for unassessable outcomes were as followed:

- late exclusion, because of isoniazid resistance (n=5);
- late exclusion, because of randomization failure TB (n=2);
- pregnancy during follow-up (n=1).

Among participants receiving a standard regimen 8/8 (100%, 95% CI: 67.6%-100%) had a favourable outcome compared to 29/35 (82.9%, 95% CI: 67.3%-91.9%) among those receiving a PA-based regimen (p=0.58). With the very small number of participants receiving a standard regimen the power to detect a difference is almost null.

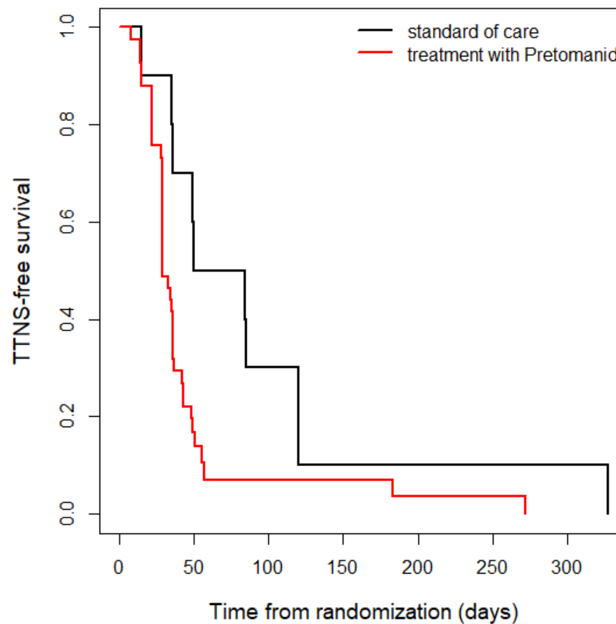
4.2.2.2. Time to negative mycobacterial culture

We compared the time to TTNS between participants receiving a standard regimen versus those receiving a PA-based regimen. Two participants both receiving a PA-based regimen were censored: one died and one was withdrawn, their time was censored at date of death or withdrawal. Thus, the analysis included 10 participants treated with a standard regimen and 41 participants treated with PA-based regimens.

The median TTNS was 67 days (95%CI of the median: 36-NA days, IQR: 36-120 days) for the standard regimen and 29 days (95%CI of the median: 29-36 days, IQR: 28-43 days) for the

PA-based regimen (Figure 18). The p-value of the logrank test was 0.02. Adjustment for confounding was not possible due to the small sample size.

Figure 18. Time to sustained negative mycobacterial culture by treatment in participants with L1 TB



4.3. Sample size calculation for future studies.

4.3.1. Trial comparing standard regimen vs PA-based regimen in participants with L1 TB

The sample size was computed using the following assumptions:

- Primary outcome: death/failure/relapse as primary outcome
- Noninferiority of PA-based regimen compared to standard regimen
- Significant level 0.025
- Power 0.80

We assumed that only participants with L1 drug susceptible TB would be included in the trial. Hence, we assumed 5% of primary outcomes (death/failure/relapse) in the standard regimen arm. Assuming a margin of 3% (thus the proportion of death/failure/relapse in participants receiving PA-based regimens no more than 8%) and actual no difference, a total sample size of 1658 (829 per group) would be required. Assuming a margin of 5% (thus the proportion of death/failure/relapse in participants receiving PA-based regimens is no more than 8%) and actual no difference, a total sample size of 598 (299 per group) would be required.

We assumed a proportion of 15-30% of participants would have a primary outcome (death/failure/relapse) in the standard regimen arm if only participants with L1 MDR TB would be included in the trial. Table 11 summarises the required sample size for different proportions of primary outcomes assuming a margin of 5% and actual no difference.

Table 11. Sample size calculations for a trial among participants with MDR-TB receiving either a standard regimen or a PA-based regimen

Proportion with primary outcomes in the standard regimen arm	Margin	Maximum proportion with primary outcomes in the PA-based regimen arm	Total sample size	Sample size per group
15%	5%	20%	1602	801
20%	5%	25%	2010	1005
30%	5%	35%	2638	1319

4.3.2. Observational study comparing outcomes of PA-based regimens in participants with L1 MDR-TB vs L2, L3 or L4 MDR-TB

Following roll-out of PA-based regimens for people with MDR-TB observational studies could be performed comparing outcomes in those with L1 TB versus those with L2, 3 or L4 TB. If possible, these studies should collect detailed treatment outcome data (not relying on routine treatment outcome data collected as part of routine surveillance), minimise attrition, ensure follow-up beyond treatment completion (for a minimum of one year), perform phenotypic and genotypic drug susceptibility data for all drugs included in the regimen and collect data on time to culture conversion, other biomarkers (e.g. MBLA), clinical and functional data (e.g. anthropometric measurements, muscle strength) and data on quality of life.

We assumed a proportion of primary outcome (death/failure/relapse) of 15-30% among participants with L2, L3 or L4 MDR-TB, a margin of 5% and actual no difference between L1 and L2, L3 or L4 MDR-TB primary outcomes. In addition, we assumed a prevalence of L1 of 10%, 20% and 30% among MDR-TB isolates. Table 12 summarises the sample size required for different assumptions.

Table 12. Sample size calculations for observational studies comparing outcomes of PA-based regimens in participants with L1 MDR-TB and L2, L3 or L4 MDR-TB

Proportion with primary outcomes among participants with L2, L3 or L4 MDR-TB	Margin	Maximum proportion with primary outcomes among participants with L1 MDR-TB	Proportion of L1 among MDR-TB isolates	Total sample size	Sample size L1	Sample size per lineage 2,3,4
15%	5%	20%	10%	4450	445	4005
20%	5%	25%	10%	5590	559	5031
30%	5%	35%	10%	7330	733	6597
15%	5%	20%	20%	2505	501	2004
20%	5%	25%	20%	3140	628	2512
30%	5%	35%	20%	4125	825	3300
15%	5%	20%	30%	1908	573	1335
20%	5%	25%	30%	2391	718	1673
30%	5%	35%	30%	3140	943	2197

5. Conclusions

- None of the PA MIC data summarised in this report were established using the EUCAST reference method for MIC testing of MTBC which is one of the many limitations. Also, MIC data was only available using the MGIT and limited data was available for 7H11.
- PA MIC distributions for H37Rv using MGIT were comparable across laboratories with modes between 0.06 mg/L and 0.25 mg/L. The aggregated distribution spanned four dilutions, demonstrating a good technical reproducibility of MGIT.
- *Mtb* L1 is intrinsically less susceptible to PA compared with L2, L3 and L4. The ECOFF for *Mtb* L2, L3 or L4 isolates was 0.5 mg/L and the tEFOCC for *Mtb* L1 isolates was 2 mg/L. These concentrations merely represent the upper end of these respective distributions and cannot automatically be used to infer susceptibility or resistance, unless sufficient clinical outcome data and, ideally, pharmacokinetics and pharmacodynamics data exist to show that one or both distributions are treatable using a particular exposure of PA as part of a specific regimen.[14]
- The PA MIC distribution of *M. canetti* and non-*Mtb* MTBC members (such as *M. bovis*) were not investigated as part of this review. However, the study by Bateson et al. [4] showed that *M. canetti* isolates have an even high MIC than *Mtb* L1 isolates and may be less likely to respond to PA treatment. Globally *M. canetti* is extremely rare and hence the higher PA MIC of *M. canetti* isolates is of limited concern. Non-*Mtb* MTBC isolates seem to have PA MICs that are either comparable to or lower than those for *Mtb* L2, L3 or L4 isolates [4].

- The 7H11 results were from a single laboratory only and were, consequently, insufficient to even set a tECOFF. *Mtb* L1 isolates were, however, less susceptible, confirming that the L1-effect was not specific to MGIT.
- The analysis of clinical outcomes conducted as part of this report has severe limitations: namely large variety in PA-based regimens, small sample size, a biased sample and no adjustment for confounders (for example not being able to take into account resistance to other drugs in a regimen). Hence no conclusions can be drawn from the analysis.
- Whether or not the intrinsically higher PA MIC of *Mtb* L1 isolates impacts on treatment outcome is currently unknown. Data from trials for L1 TB are very limited and so are observational data. Given the low number of participants with L1 TB included in the trials, more data on treatment outcomes in L1 patients is needed.
- It is important to note that prevalence of L1 TB is highest in South-East Asian countries which also have the highest prevalence of fluroquinolone resistance among MDR-TB isolates. Furthermore, BQD resistance is increasing globally. Hence, understanding whether or not the intrinsically increased PA MIC of *Mtb* L1 isolates is clinically relevant is vital. If PA is less effective in L1 TB, there may be a risk of BDQ resistance evolution in people with L1 TB receiving BPaL regimens, leaving few options to construct alternative regimens. Of note the prevalence of *Mtb* L1 among MDR-TB isolates is not well understood, but data submitted as part of the public WHO call revealed no *Mtb* L1 isolates in Indonesia (1/43). Sample size calculations for trials and well-designed observational studies show that a considerable investment is needed to answer the question of non-inferiority of WHO recommended PA-based regimens for L1 TB.

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PRETOMANID

A SYSTEMATIC REVIEW ON PK AND PK/PD

Report for the World Health Organization, Global TB Program

THE UNIVERSITY OF SYDNEY INFECTIOUS DISEASES INSTITUTE (SYDNEY ID)

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Pretomanid

Introduction

The introduction is intended to provide the readers a quick introduction in basic principles and methods used in the various studies. It is not intended to be a comprehensive overview of the literature. For a more detailed overview we refer to a recent review on this topic [1].

Pharmacokinetics

Pharmacokinetics (PK) describe the behavior of a drug in the patient' s body. Generally, the drug is absorbed (A) into the systemic circulation after oral or parenteral intake, which is then distributed (D) throughout the body including the site of infection. After metabolism (M) primarily by the liver, eventually the drugs are eliminated (E) by the kidneys and released in the urine. Integration of these parameters results in a PK model that describes these processes (ADME). There are many factors that can influence the PK of a drug.

Pharmacodynamics

Pharmacodynamics (PD) describe the biochemical or pharmacological effect of a drug on the *Mycobacterium tuberculosis* (efficacy) and on the patient (toxicity). Anti-TB drugs can be subdivided in bactericidal and bacteriostatic drugs. The maximum achievable response of a drug is described by maximum effect (Emax).

Pharmacokinetics/Pharmacodynamics

Integration of pharmacokinetics and pharmacodynamics (PKPD) will provide information on how the drug concentrations translate into the effect of the drug1.

The correlation between drug concentration and efficacy can be subdivided in the following parameters: 1) area under the concentration time curve in relation to minimal inhibitory concentration (**AUC/MIC**), 2) maximum concentration during the dosing interval in relation to minimal inhibitory concentration (**Cmax/MIC**), and 3) the time the concentration exceeds the minimal inhibitory concentration during the dosing interval (**%T>MIC**).

PKPD is helpful to establish the most appropriate dose. Due to variability in drug concentrations in different patient populations and differences in susceptibility between different bacterial species recommended dosages can be different. PKPD studies can be performed *in vitro*, *in vivo* and in humans.

In vitro studies

In vitro studies are useful in the determination of the efficacy and potency of the extent of the drug or dose by performing time-kill kinetic studies.

In the **static time-kill studies** the drug concentration remains fixed over time and the bacterial response are measured in terms of change on the optical density and/or colony forming units (CFU). The static time-kill studies are commonly performed using the actively replicating logarithmic phase bacteria in cultures and based on the extent of kill drugs are commonly classified as bactericidal or bacteriostatic.

In **dynamic time-kill studies** the drug concentrations to be actively changed over time reflecting more physiological conditions. The most common used dynamic time-kill study is the hollow fibre infection model. The model consists of a cartridge with hollow fibres. Outside the fibres are the bacteria; inside the hollow fibres is a continuous flow of medium. Drugs and nutrients diffuse through the fibre membrane to the bacteria. These systems are of particular interest for studying PKPD because the human PK can be applied in the system. Moreover, the system can be sampled frequently to study bacterial growth and drug pharmacokinetics. This *in vitro* system has been endorsed by European Medical Agency to guide dose finding in TB drug development. Hollow fiber studies can be used to determine whether efficacy is driven by AUC/MIC, Cmax/MIC or T%>MIC by comparing the efficacy of a single dose (Cmax) with the dose divided in two or three dosages (%T>MIC). If the effect of three dosing strategies is the same, the AUC/MIC is the effective PK/PD parameter.

In vivo studies

First of all, it should be clear that mice and man are very different and that PKPD findings in mice should consider the transitional value in the preclinical drug-development. Within murine models of TB, we can study the PKPD relationship and assess the dose- or time dependent nature of the PKPD relationship trough dose fractionation studies. Essential in these studies is that besides dose, the actual drug concentration, preferably at the site of infection, is considered. Measuring the concentration of the (parent drug) compound and the (active) metabolites, via a chromatography based bioanalytical methods, the contribution of PKPD parameters can be made.

Preclinical murine TB models come in many different forms. The route of infection (e.g., intravenous, inhalation, or instillation), the inoculum size, the mycobacterial strain used, the pathology of TB in the specific model, the treatment-free period before starting therapy and the mouse strain used, are all features that can be changed and tweaked to provide different models.

Human studies

The most commonly used study type in humans to evaluate the PKPD effect of an anti-TB drug in TB patients is the early bactericidal activity (EBA) study. EBA is defined as the rate at which a

drug kills actively metabolizing, rapidly multiplying tubercle bacilli in the sputum of patients with TB during the first days of therapy. This methodology has become the first clinical assessment of the efficacy of proposed anti-TB drugs in a relatively small number of sputum smear-positive pulmonary TB patients. Recently, the measurements of killing rate occurring have been divided in an early EBA (between days 0 and 2) and an extended EBA (between days 2 and 7 or between 2 and 14 days). Extended EBA has been advocated as an early measure of sterilizing activity, the ability of a drug to kill slowly replicating, persistent bacilli in tissues.

Pretomanid drug profile

Pretomanid (Pa-824) belongs to the class of nitroimidazoles. It is thought to exert its antimycobacterial effect following the metabolic activation through interruption of mycolic acid synthesis and respiratory poisoning [2-4]. Its activity against both drug-susceptible and resistant TB strains have been reported. In human plasma, 94% of the drug is protein bound [5].

The exact mechanisms of action and factors affecting its pharmacological effect and metabolic pathways are still to be better characterised [6]. Further studies linking specific mutations, in vitro susceptibility, drug exposure and resistance mechanisms to treatment failure with pretomanid should be prioritized [7].

Aim of the report

The intention of the report is to provide insight in PKPD of pretomanid to help make decisions regarding clinical breakpoints and its programmatic use and dosing strategies. Information presented is based on data retrieved from a systematic literature review.

The systematic literature review has the following objectives:

1. Describe the PK of pretomanid, especially the variability and factors relevant for treatment.
2. Identify the PKPD relationship.
3. Describe the target attainment of current dose regimen based on the PKPD relationship.

Review of PK and PD data

The review was conducted in accordance with the principles outlined in the PRISMA statement [8].

Strategy for the systematic review

This systematic review was performed to inform the discussion PKPD of pretomanid.

Search of databases was performed on 14 Aug 2023 without date restriction.

For Pubmed search using "all fields" the search term was:

((Pretomanid) OR (PA-824)) AND (((Tuberculosis) OR (TB)) OR (Mtb)) AND ((pharmacokinetics) OR (concentration) OR (therapeutic drug monitoring) OR (TDM) OR (drug exposure) OR (drug monitoring) OR (pharmacology) OR (pharmacodynamics) OR (pharmacol*) OR (pharmacod*))

For Web of Science core collection search using "all fields" the search term was:

((ALL=(Pretomanid)) OR ALL=(PA-824)) AND

((ALL=(Tuberculosis)) OR ALL=(TB)) OR ALL=(Mtb) AND

((((((((ALL=(pharmacokinetics)) OR ALL=(concentration)) OR ALL=(therapeutic drug monitoring)) OR ALL=(TDM)) OR ALL=(drug exposure)) OR ALL=(drug monitoring)) OR ALL=(pharmacology)) OR ALL=(pharmacodynamics)) OR ALL=(pharmacol*)) OR ALL=(pharmacod*)

Title and abstract screening as well as full text screening was performed by two reviewers independently. In case of differences consensus was reached through discussion. The PRISMA diagram was made to illustrate the study selection and exclusion process.

Studies selection PK

Criteria for selection of pharmacokinetic variability were studies with a prospective, observational or retrospective design. Only studies with actual TB patients were included as PK studies in healthy volunteers are not representative of drug exposure in TB patients. Studies in healthy volunteers were allowed in case a specific effect was studied, e.g. a drug-drug interaction study or food-effect study. We investigated used dosages and judged whether PK sampling was performed in steady state. Assay parameters for analysis were judged and should comply with ICH guideline M10 on bioanalytical method validation and

study sample analysis.

Studies selection PKPD

Criteria for selection of PKPD studies were *in vitro* (hollow fiber infection model), animal and human studies investigating the relationship between drug dose, concentration, and microbiological response. Important was that the study design allowed for the effect of the drug of interest to be assessed. This could be either as monotherapy or as combination therapy where the drug was administered at various dosages/exposures. For better interpretation of the microbiological response the minimal inhibitory concentration had to be assessed.

Exclusion of studies

Excluded were reviews, case reports and studies not providing relevant information to assess the PKPD of the drug of interest. In case of data appearing in different publications and noticed by the reviewers, results were only included once.

Data extraction

Data extraction was performed by one reviewer and verified by a second reviewer. The following data were extracted when available: study design, dose, type of TB, tuberculosis strain, bacterial load, treatment duration and treatment outcome (CFU reduction, sputum culture conversion), minimum inhibitory concentration (MIC) including the method used, AUC and Cmax data, pharmacokinetic sampling scheme, and information on population pharmacokinetic models.

Results

In total, 502 articles were retrieved from PubMed and Web of Science (Figure 1). After the removal of 128 duplicates, 374 articles underwent abstract and title screening resulting in 61 articles for full text screening. After the exclusion of 24 non-relevant articles, 37 articles were included in the final assessment.

A total of 5 *in-vitro* studies, 14 *in-vivo* studies, 9 human studies and 9 modeling studies in were included.

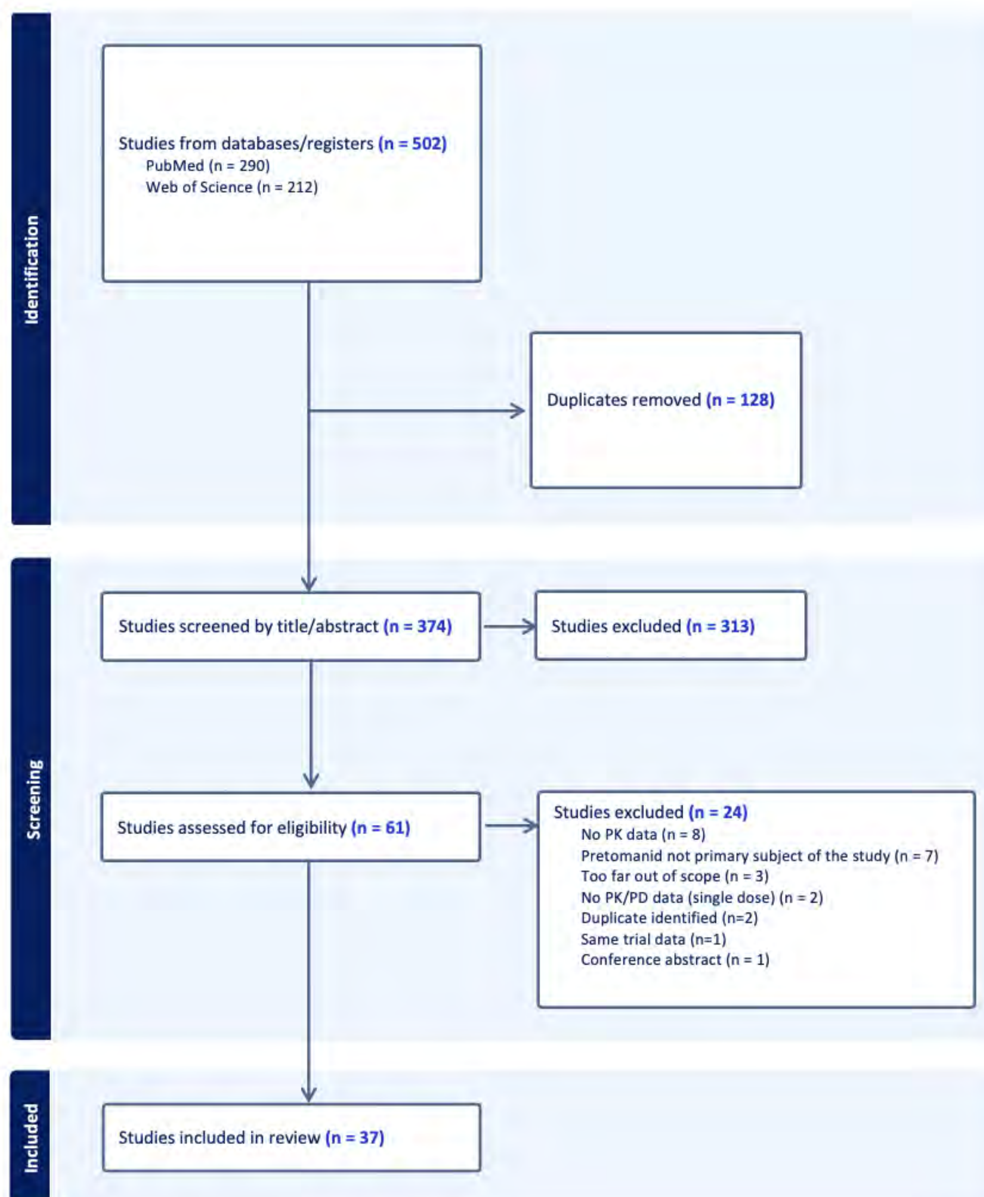

Figure 1: PRISMA diagram

PK variability and factors relevant for treatment

PK *in vivo*

In a murine model of TB, pretomanid exhibited dose-proportional increase in concentrations between 18 to 243mg/kg oral dose. More complex PK with potential saturation of oral absorption was observed at >486mg/kg [9]. Furthermore, late additional peaks at 24 and 48 hours for these higher doses indicated precipitation and redissolution of pretomanid in the gastrointestinal tract. T_{max} was 4 hours and elimination half-life was 4 to 6 hours.

In a guinea pig model of TB [10], repeated dose of 25mg/kg given twice a day gave AUC of mean 42.19 (SD+/-21.04) that is comparable to steady state healthy human exposure reported for 200mg dose (30.2+/-3.7).

Three studies in healthy rats reported distribution of pretomanid into various tissues at multiple timepoints up to 36 hours after a single dose of 20mg/kg, 40mg/kg or 80mg/kg [11-13].

Bratkowska *et al*. observed that after a 20mg/kg dose, pretomanid plasma Cmax was 2.5-fold higher than in lungs and 5-fold higher compared to brain. Similar AUC mean ratios of 0.25 for lung:plasma and 0.27 for brain:plasma was observed during 24 hours post-dose [11].

Distribution of pretomanid into brain during the first 8 hours after dose was further characterised by Shobo *et al* [12]. After a 20mg/kg intraperitoneal dose, pretomanid diffused from cortical region of the brain into the corpus callosum at 60 min, reaching peak at 120min followed by elimination into neighbouring areas of the brain by 480min (8 hours).

In rats, approximately 2-fold greater pretomanid concentration was observed in liver at 6 hours and stomach at 2h and 6 hours after a 40mg/kg oral dose compared with plasma [13]. Distribution into other tissues such as heart, lung, spleen, kidney, intestine and brain was also observed at a lower and variable concentrations.

Two *in vivo* studies explored effect of drug-drug interactions on pretomanid PK [14, 15]. Addition of 40mg/kg moxifloxacin to 20mg/kg pretomanid oral dose in rats caused a significant increase of about 40% in pretomanid T_{max} and $AUC_{(0-t)}$ over 36 hours [14].

In contrast, co-administration of 60mg/kg darunavir and 20mg/kg pretomanid in rats resulted in approximately 50-60% reduction in mean $AUC_{(0-t)}$ and C_{max} of pretomanid [15]. Potential effect of CYP450 enzymes on pretomanid metabolism is a possible mechanism but requires further study.

PK in humans

In total 9 human studies were evaluated, five reported on pharmacokinetics in TB patients [16-20] while 4 other studies presented clinically relevant information on drug-drug/food interaction [21-23] or CNS penetration [24].

In total 5 studies evaluated the pharmacokinetics of pretomanid in clinical trials. Diacon *et al*. and Dawson *et al*. performed phase 2 studies evaluating early bactericidal activity of dosages ranging from 50 – 1200mg once daily [16-19]. Although PK sampling was performed results were not separately reported in these two studies and only partly in two other studies. Solans *et al* reported the C_{min} data from the Nix-TB trial at week 2, 8 and 16 during treatment and showed that the drug accumulated over time [20].

Drug-drug interactions between pretomanid and efavirenz, lopinavir/ritonavir and rifampicin were studied in 52 healthy volunteers. The pretomanid AUC0-24 was reduced by 28% as a result of efavirenz, 13% due to lopinavir/ritonavir and 53% due to rifampicin [21]. Ignatius studied the effect of rifampicin and rifabutin on pretomanid AUC0-24 in patients with TB on day 14 of treatment. The pretomanid AUC0-24 was 30.1 (23.5–35.3) mg*h/L when administered in combination with rifampicin while the pretomanid AUC0-24 was 59.5 (48.0–65.2) mg*h/L in combination with rifabutin, demonstrating the stronger effect on rifampicin on drug clearance [22].

Mota *et al*. performed a dynamic PET/CT imaging study with ^{18}F-Pretomanid after intravenous administration in six healthy volunteers. The drug showed high penetration into the brain parenchyma and the cerebral spinal fluid (CSF), with an AUC (tissue/plasma) ratio >1, however, exposure in CSF was significantly lower than in brain parenchyma [24].

Winter *et al*. studied the effect of food on bioavailability of pretomanid using a cross-over study at various dosages (50, 200 and 1000mg) in 48 healthy volunteers. The $AUC_{0-\infty}$ increased 147.07% (50mg), 187.87%(200mg) and 374.26% (1000mg) as a result of increased solubility in the presence of food [23].

Zou *et al*. [25] evaluated a dispersible tablet formulation useful for future use in children or adults not able to take the current tablet formulation. The exposure was comparable between current and dispersible formulation. A difference was observed in the absorption time between both products, but this did not influence overall absorption.

PKPD relationship

In vitro PKPD

Five *in vitro* studies characterised mycobacterial effect of pretomanid [5, 26-29], including one hollow fibre infection model [28].

In the models of bacterial persistence using 100-day old static cultures, pretomanid showed a dose-related and greater sterilising activity compared to moxifloxacin, especially at concentration of ≥ 10 ug/ml which was sufficient to kill all bacilli [5]. However, this study raised a concern that these concentrations may not be feasible to be the reached at lung cavitary lesions based on 94% protein binding of pretomanid, raising the need to investigate the role of bound and unbound drug.

Subsequently, Drusano *et al*. demonstrated *in vitro that the* addition of moxifloxacin to pretomanid at representative of human of human exposure (C_{max} 1.7mg/L or C_{avg} 1.26mg/L)

resulted in augmented bacterial kill against log-phase *Mtb* and suppressed amplification of less-susceptible pathogens by day 7 which was not possible with pretomanid alone [26].

Similarly, even in acid growth phase of *Mtb* representative of non-replicating state, pretomanid and moxifloxacin combination demonstrated maximal concentration-dependent bactericidal effect at mimicked human C_{max} of 1.7mg/L pretomanid and cleared bacterial load by day 14 [27].

In a hollow fibre infection model study, the combination of pretomanid with pyrazinamide and moxifloxacin (PaMZ) yielded similar kill rates of 0.18 (95% CI 0.13–0.23) log_{10} CFU/ml/day compared with the standard therapy of rifampicin, isoniazid and pyrazinamide, 0.15 (0.08–0.21) log_{10} CFU/mL/day. When investigators simulated the impact of these findings in 1000 patients it was found that only 40.37% (95% CI 39.1–41.34) and 72.30% (71.41–73.17) of patients will achieve sterilisation at 3 months and 4 months respectively compared with 93.67% (93.18–94.13) at 6 months . This indicated that the PaMZ regimen is not able to shorten treatment to less than 6 months [28].

Yamada *et al*. [29] studied combinations of pretomanid, bedaquiline and moxifloxacin against non-replicating bacteria. The combination of pretomanid with bedaquiline was highly active but investigators considered addition of moxifloxacin valuable due distribution to the site of infection and interplay with the immune system.

In vivo PKPD

Eight *in vivo* studies reported on PK and PD outcomes of pretomanid in mice [9, 30-35] or guinea pigs [10].

In a dose-fractionation study in rats receiving 144 to 4,608 mg/kg in divided doses over 24 days, lung CFU counts after treatment showed correlation with the free drug T>MIC (R^2 =0.87) and free drug AUC/MIC (R^2=0.60), but not with the free drug C_{max}/MIC (R^2=0.17) [9]. Free drug $T_{>MIC}$ of 22, 48, and 77% were associated with bacteriostasis, a 1-log kill, and a 1.59-log kill (or 80% of the maximum observed effect, EC_{80}), respectively. In this study, PD simulations based on human phase I data predicted 200 mg/day will result in target attainment including 100% $T_{>MIC}$ for MIC 0.03125-0.0625 ug/ml depending on free drug proportion.

In guinea pigs, free drug T/MIC of 73-100% and variable free drug AUC/MIC of 37-224 was achieved when simulated for low MICs of 0.03-0.06 ug/ml at 25mg/kg every 8 or 16 hours which was the dose yielding exposure comparable to humans [10]. At the higher MICs, variability in the PD parameters depended on the unbound fraction.

Of the eight studies, four studies were related to finding the best combination regimens containing pretomanid [31-34]. One of the earlier studies in mice dose at 100mg/kg/day showed favourable outcome when pretomanid was substituted for rifampicin [32]. Pa-MXF-PZA

as effective as RIF-MXF- PZA in reducing organ CFU counts (> 6log10), but may be less durable culture-negative state after treatment. Sterilizing activity of Pa-MXF-PZA was enough to cure mice more quickly than standard RIF-INH-PZA (lower CFU at 1 and 2 months, $P<0.001$). However, outcomes were not favourable when pretomanid was added to 4-month RIF- MXF-PZA or substituted for MXF or PZA.

In a later study, addition of nitroimidazoles including pretomanid (PMD) or TBA-354 significantly improved sterilizing activities of bedaquiline (BDQ) and sutezolid, with or without pyrazinamide [34].

Addition of LZD significantly increased the activity of BDQ+PMD ($P< 0.01$) [33]. All 2 drug combinations had inferior efficacy compared to 3 drug combinations (BDQ+PMD+ either SZD/LZD) at 2 months ($P<0.01$), indicating each drug contributes to the efficacy.

In the more recent study, all regimens except for the low-dose pretomanid regimen were significantly more active than the RIF+INH+PZA standard regimen resulting in approximately 3.25-log10 reduction of CFU at 1 month ($P < 0.0001$) [31]. By month 3, both PMD+MXF+PZA regimens and the PMD+MXF+BDQ regimen showed significantly greater killing activity ($P <0.0001$).

Mudde *et al.* [36] compared BPaMZ and BPaL in a murine model of TB and found that BPaMZ was more active than BPAL resulting in earlier cure of the animals. Using mathematical modeling the investigators predicted that 95% probability of cure was predicted to occur at 1.6, 4.3, and 7.9 months for BPaMZ, BPaL, and HRZE, respectively.

Human PKPD

The PKPD in humans was studied in 5 clinical trials. When comparable early bactericidal activity was observed at dosages ranging from 200-1200mg [18] the study was repeated at dosages ranging from 50-200mg [17]. In the latter, a more substantial difference was noticed with lowest activity at the lowest dose (50mg). When the pretomanid was combined with various other drugs (bedaquiline, pyrazinamide, clofazimine) it was demonstrated that the combination of bedaquiline, pretomanid and pyrazinamide had a similar activity as the first line regimen over the studied treatment duration of 14 days [19].

Dawson *et al.* studied the combination of moxifloxacin, pretomanid (100 or 200mg) and pyrazinamide in 181 DS-TB and 26 MDR-TB patients [16]. The arms containing pretomanid were more effective and showed a shorted time to sputum culture conversion. However, the difference between the arms containing 100 or 200mg pretomanid was limited. The 200mg arm showed "somewhat improved results" as demonstrated by logCFU count change over 56 days but not in logTTP over the same period. PKPD associations between bactericidal activity and Cmax, AUC, and T>MIC were weak due to confounders and small sample size.

Solans *et al*. analysed the Nix-TB data using bedaquiline (+M2 metabolite), linezolid and pretomanid trough concentrations collected at week 2, 8 and 16 [20]. No relationship between drug exposure and treatment failure, disease relapse and death could be made. Investigators hypothesized that concentrations were at maximum of the concentration-effect Emax curve. Another explanation might be that pathogen susceptibility was not taken into account in the analysis.

Modelling data of pretomanid

A one-compartment PK model with first-order absorption and elimination and a sigmoidal bioavailability dependent on dose, time, and the predose fed state PK model was developed by Lyons [37] based on phase 2 data [17, 18] to be used in further studies to analyse exposure effect relationship of pretomanid.

Lyons [38] developed a model that was able to predict CFU and TTP reflecting mycobacterial load as function of pretomanid plasma concentrations using clinical trial data [17, 18] with pretomanid dosages ranging from 50-1200mg. Interestingly, a dose related increase in EBA0-14 was found with a 27% increase in EBA when increasing the dose from 100-mg/day to 200 mg/day but a further increase to 300 mg/day resulted only in an additional 10% increase in EBA. Subsequent application of mathematical algorithm (multi-objective optimization) to the PKPD model enabled finding the optimal dosing in different regimens based on a combination of variable therapeutic objectives such as CFU counts and adverse effects [39]. The model provided typical population based chacterisation of the current 200mg daily dose, however more importantly provided a computational benefit-risk tool for future regimen designs. Next, Lyons [40] performed PKPD analysis of 2 trials [19, 41] and performed simulations to demonstrate that $B_{200}Pa_{200}Z_{1500}$ once-daily would result in sputum culture conversion in 90% within 3 months.

Mehta *et al*. [42] build a model to predict concentrations of pretomanid at the site of infection and found that a dose of 200mg will be sufficient to kill replicating bacteria may not be sufficient to eradicate non-replicating bacteria as <5% of patients predicted to reach target concentration for non-replicating bacteria at the site of infection.

Nedelman *et al*. [43] pooled data from four clinical trials (NC-002, NC-005, STAND and Nix-TB) and modeled time to sputum culture conversion and side effects. They were particularly interested in the effect of food on drug absorption. They found that pretomanid exposure is associated with efficacy (time to sputum culture conversion) and toxicity (vomiting and gastrointestinal tract symptoms) but that age and baseline time to positivity influenced efficacy and female gender was associated with risk of vomiting. Reducing exposure by switching from 200mg fed dose to 200mg fasted or 100mg fed would reduce exposure and reduce both efficacy and side effect. However, the trade off is not clear as mentioned by the investigators.

Ignatius *et al.* [22] studied the impact of rifamycins on pretomanid exposure and tested if 90% of would achieve a bactericidal effect (T>MIC77%). Their simulation showed that >90% of the population would achieve the target for MIC 0.03125 and 0.0625mg/L.

Salinger *et al.* [44] pooled data from 14 clinical trials (CL-001, CL-002, CL-003, CL-005, CL-007, CL-009, CL-010, DMID 10, NC-001, NC-002, NC-003, NC-005, STAND, and Nix-TB to build a one-compartment model with three transit compartments to represent lagged absorption and an extensive list of covariates with an effect on absorption, clearance and volume of distribution. The final model was considered suitable to be used for exposure/response analyses of pretomanid.

Discussion

Overall, pretomanid has shown generally dose-proportional increase in concentrations in animal models of TB, until potential saturation in oral absorption was observed at higher doses (e.g. >486mg/kg in rats). Phase 2 EBA studies confirmed this observation of linear PK over the lower dose range of 50-200mg daily with accumulation in the first two weeks. However, less than dose-proportional increase in concentrations was reported for higher doses of 200-1000mg daily. Future studies would be most beneficial if they present detailed PK parameters such as sampling timepoints, AUC and Cmax as these were often not presented in some of the included clinical studies, as the focus was on presentation of PD parameters such as CFU counts or TTP for each dose arm rather than per measured drug concentration.

Modelling strategies attempted to better characterise pretomanid PK and identify factors affecting its PK variability such as fasted state resulting in reduced bioavailability.

Both *in vitro* and *in vivo* data inform us about the ability of pretomanid to distribute into other tissues including crossing the blood brain barrier, indicating the potential role in TB meningitis. Of course, there are limitations in the translation of the highly simplified preclinical models [6] and first in human data, and perhaps subsequent clinical studies could explore the role of pretomanid in targeting heterogenous lesions at the infection site [24].

With regards to PK/PD markers, %T/MIC and AUC/MIC based on the free drug were identified as the most significant predictors of pretomanid efficacy. Preclinical models coupled with model simulations for human-equivalent doses (200mg, 400mg daily) were able to show attainment of as high as 100% T/MIC at MIC < 0.1 ug/ml [9, 10]. Similarly, high target attainment of T/MIC

of 92-99% was achieved for pretomanid dose of 100mg-200mg daily in phase 2 studies for the observed MIC of <0.1 ug/ml [17, 19].

Establishment of a critical concentration for pretomanid to guide clinical practice is urgently needed [7]. Based on the limited information available, FDA has supported provisional critical concentration at 1 µg/ml for both REMA and MGIT methods [45]. More than 95% of clinical isolates were reported to have MIC values ≤ 1 µg/ml, although resistant strains exceeded this MIC. Hence, surveillance data from future studies will enable characterisation of MIC distribution in clinical setting for both susceptible strains as well as for those with resistance mechanisms. This will lead to better estimation of attainment of current PKPD targets.

Conclusion

Pretomanid has a clear exposure effect relationship (%T>MIC) and the exposure of the drug is highly dependent on concomitant food intake. Drug-drug interactions with rifamycins can reduce the exposure substantially. Drug exposure in routine care is therefore expected to be variable. The impact of this variable drug exposure on treatment response depends on the MIC for pretomanid but also companion drugs in the regimen. Combination with bedaquiline, moxifloxacin and pyrazinamide seem favorable and may help to compensate for its limited role against non-replicating bacteria in lung lesions.

PK/PD targets established in a mice model show that free drug T>MIC of 22, 48, and 77% were associated with bacteriostasis, a 1-log kill, and EC80, respectively. For programmatic care the exposire of pretomanid should be sufficient to achieve at least kill in 90% of the population (T>MIC48%). Pretomanid $_{Cmax}$ in TB patients on 200mg/daily as part of BPaZC, BPaZ or BPaC was approximately 4mg/L (up to 6mg/L). Based on protein binding and free drug fraction of ~15% (variable 5-15%), the free drug will be ~0.6mg/L (up to 0.9mg/L in some patients). However, as this assumption is based on Cmax timepoint, it is unclear if this concentration >0.5mg/L would be achieved for ~50% of time at the site of infection. Likely, pretomanid MIC tested as part of a combination regimens will show additive/synergistic effect and hence a lower MIC, meaning that the target of T>MIC48% may become more achievable. As long as this result in kill in 90% of the population it should be fine. This means that stasis based on pretomanid alone can be expected in <10% of the population. As long as strong companion drugs are included in the regimen (bedaquiline, linezolid and moxifloxacin) and considering the isolate is susceptible to those drugs. So, if the prevalence of an MIC of 0.5mg/L is <10% of the MIC distribution it should be fine. These are preliminary assessments and the planned comprehensive PKPD analysis of the TB Practical study will provide further insight in relation between PKPD of pretomanid and long term treatment response.

Knowledge gap

This review has shown important *in vitro*, *in vivo* and early clinical trial PKPD data but lacking is a detailed analysis of the relationship between PKPD and long-term treatment outcomes. However, it is likely that the knowledge gap will be resolved soon because of a planned PKPD analysis [46] of the TB-Practical study [47].

The TB-Practical study [47]. evaluated a 24-week regimen of bedaquiline, pretomanid, linezolid, and moxifloxacin for rifampicin-resistant TB in an open label randomised controlled trial vs standard of care and demonstrated to be more effective and safer than standard of careIn addition to the main study investigators planned to perform an extensive PKPD evaluation of the trial [46]. Objectives of this study are to estimate the population exposure metrics of the drugs in the trial using population pharmacokinetic models. More importantly, investigators plan to develop a PKPD model to characterise the relationship between drug exposure, baseline clinical covariates, baseline minimum inhibitory concentrations and early bactericidal effect, long-term treatment outcome and toxicity. The results of this study will address an important knowledge gap regarding PKPD and long-term outcome. Findings can be used optimize treatment and/or provide justification for future studies.

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Tables

Table 1: Summary of studies reporting on pharmacokinetics of pretomanid in TB patients

Author	Study	Country	Subjects	n	Dose	Day	Sampling	AUC (range)	Cmax (range)	PK model
Dawson [16]	Phase 2b EBA	South Africa Tanzania	DS-TB	207	100, 200mg qd	14	N.R.	N.R.	N.R.	N.R.
Diacon [18]	Phase 2 EBA	South Africa	DS-TB	69	200, 600, 1000, 1200mg qd	1,8,14	N.R.	N.R.	N.R.	N.R.
Diacon [17]	Phase 2 EBA	South Africa	DS-TB	69	50, 100, 150, 200mg qd	1,14	0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16h	11.9(1) - - 38.5(1)	456.3 (1)-800 (14)* 625 (1)-1050 (14)* 940 (1)-1500(14)* 1183.0 (1)-2125 (14)*	N.R.
Diacon [19]	Phase 2 EBA	South Africa	DS-TB	105	200mg qd BPaZC BPaZ BPaC	14	0,1,2,3,4,5, 10,16,24	60487(36541-74762) 76292(41080-109,139) 61534(35462-119234)	3600 (2690-4460) 4430 (2880-5500) 3600 (2330-6130)	N.R.
Solans [20]	Phase 3	South Africa	M/XDR-TB	93	200mg qd	14, 56, 112	0	N.R.	2359.3 (218.6–6444.9)# 1922.3 (33.5–5388.7)# 2121.64 (30.5–6831.6)#	N.R.

DS-TB drug susceptible TB, N.R. not reported, * interpreted from figure

Table 2: In vitro PKPD data

Author	Model	TB strain	Inoculation	Dose or concentrations	Intervention duration	Outcome	PK
Drusano 2020 [26]	*in vitro* + Monte Carlo simulation	H37Rv	Log-phase growth	C_{max} 1.7 mg/L C_{avg} 1.26 mg/L C_{min} 0.535mg/L	28 days	Pa alone: Amplification of a less-susceptible population by day 7 (complete takeover by day 14 for C_{max} and C_{avg}). Pa + MXF: Excellent bacterial kill and suppressed amplification of less-susceptible pathogens by day 7 1000-iterate Monte Carlo simulations: 3-drug (Pa + MXF + BDQ with its active M2 metabolite) showed faster bacterial load clearance compared with 2-drug (Pa + MXF).	Concentration-dependent killing as a single agent, from 0.85 to 0.36 log_{10} CFU/ml for C_{max} to C_{min}.
Hu 2008 [5]	3 *in vitro* models of bacterial persistence	H37Rv	100-day static culture	0.39-12.5 ug/ml as final concentration in models	-	Little bactericidal activity at low concentrations up to 1.25 ug/ml in each of these models. At ≥ 10ug/ml, activity, sufficient to kill all bacilli (Model 3) and appreciably greater than with MXF.	-
Kim 2021 [27]	Time-kill study + Non-parametric modeling	H37Rv	Acid phase	C_{max} 1.7 mg/L C_{avg} 1.26 mg/L C_{min} 0.535mg/L	-	Pa + MXF at comparable average/peak human concentrations effectively eradicated *Mtb* and prevented emergence of less susceptible isolates. Bacterial clearance were concentration-dependent with C_{max} causing the fastest bacterial clearance by day 14.	-
Srivastava 2020 [28]	HFS-TB	H37Ra and H37Rv	Log phase or semi-dormant	To mimic 200mg daily	56 days	Sterilizing rates of PaMZ vs standard: 0.18 (95% CI 0.13–0.23) vs 0.15 (0.08–0.21) log_{10} CFU/mL/day. Expected % of patients achieving sterilization: 40.37% (39.1–41.34) at 3 months, 72.30% (71.41–73.17) at 4months vs 93.67% (93.18–94.13) at 6 months for standard. PaMZ regimen insufficient to achieve cure in < 6 months.	System was modelled using a one-compartment model with first-order input and elimination

Yamada 2022 [29]	Time-kill study + Non-parametric modeling	NRP state Mtb 18b	mimic human Cmax, Cavg, Cmin (1.7, 1.3, 0.5mg/L)	28 days	Pa+MXF at high and average levels was noticeably more effective than monotherapy. MXF+Pa at low concentrations was no more effective than monotherapy with low-concentration MXF. No sustained emergence of less susceptible isolates for any regimen, unlike other growth states of Mtb.	Nonparametric population algorithms-based model for simulating bacterial population decline for combination regimens.

Pa pretomanid, MXF moxifloxacin, *Mtb Mycobacterium tuberculosis,* PaMZ pretomanid moxifloxacin pyrazinamide, HFS-TB hollow fiber system model of TB

Table 3: In vivo PKPD data

Author	Model	TB strain	Inoculation	Dose or concentrations	Intervention duration	Outcome	PK
Ahmad 2011 [9]	BALB/c mice	H37Rv	Log phase	144 to 4,608 mg/kg in 3 to 48 doses (3 to 1458 mg/kg in single or multiple doses for PK analysis)	Over 24 days	Lung CFU counts correlated with free drug $T_{>MIC}$ (R^2 = 0.87) and free drug AUC/MIC (R^2 =0.60), but not with free drug C_{max}/MIC (R^2=0.17). Free drug $T_{>MIC}$ of 22, 48, and 77% were associated with bacteriostasis, a 1-log kill, and a 1.59-log kill (or 80% of the maximum effect), respectively. PTA (human phase I data) 200 mg/day, 100% $T_{>MIC}$ for MIC 0.03125 100% $T_{>MIC}$ for MIC 0.0625 if free drug >10% 400mg/day, 100% $T_{>MIC}$ for MIC <0.1 ug/ml	Tmax: 4 h. Elimination $t_{1/2}$: 4 to 6h. Dose-proportional increase between 10 to 243 mg/kg. Complex PK (likely saturation of oral absorption) at > 486 mg/kg.
Bigelow 2020 [30]	BALB/c mice	H37Rv and HN878	Log phase	Dose to achieve either a weekly AUC (50 mg/kg) or a TMIC	2 months	Lung CFU counts after 2 months of treatment: Only when pretomanid dose was 100 mg/kg,	_

				(100mg/kg) similar to patients taking 200mg daily		BPaL more active than bedaquiline alone.	
Dutta 2013 [10]	Guinea Pig	H37Rv-JHU	Mid-log phase	12.5 or 50mg/kg (25mg/kg for steady state)	2 months	25mg/kg BD showed exposure comparable to human. PaMZ given at human-equivalent doses was safe and well tolerated and gave culture negative more rapidly than RHZ did. 50% of animals in PaMZ group relapsed at 1month, but no relapse when administered for 2 months. PTA, variable overall. At low MICs of 0.03-0.06, fT/MIC of 73-100% fAUC/MIC of 37-224 At higher MICs, more variable and dependent on unbound fraction.	Doses yielding comparable PK values based on reference human data: Variable for PK parameter. Median AUC after repeated dosing of 25mg/kg every 8/16 h comparable to human exposure at steady state on 200mg dose.
Li 2017 [31]	BALB/c mice	H37Rv	Log phase	50mg/kg or 100mg/kg	3 months	At month 1, all regimens except for the low-dose pretomanid regimen were significantly (P < 0.0001) more active than the RIF+INH+PZA standard (~3.25-log10 reduction of CFU). By month 3, both PMD+MXF+PZA regimens and the BDQ+PMD+MXF regimen showed significantly greater killing activity (P <0.0001). Relapse data shows greater contribution of BDQ, compared to PZA, to the sterilizing activity of the 4-drug regimen.	_
Mudde 2022 [36]	BALB/c mice	Mtb Beijing VN 2002-1585	8log10	100mg/kg/d	upto 13 weeks	6 weeks of BPaMZ achieved cure in all mice. 13 weeks of BPaL did not achieve 100% cure rates. 95% probability of cure was predicted to occur at 1.6, 4.3, and 7.9 months for BPaMZ, BPaL, and HRZE, respectively.	Mathematical model based on mice TB model

Nuernberger 2008 [32]	BALB/c mice	H37Rv	19 days after infection (mean CFU counts in lungs and spleens 7.77+/-0.09 and 5.29+-0.18 log10)	100mg/kg 5 days per week	Up to 3 months	Favourable outcome for: Pa substituted for RIF. Pa-MXF-PZA as effective as RIF-MXF- PZA in reducing organ CFU counts (> 6log10), but may be less durable culture-negative state after treatment. Sterilizing activity of Pa-MXF-PZA was enough to cure mice more quickly than RIF-INH-PZA (lower CFU at 1 and 2 months, P<0.001). No favourable outcome for: Pa added to 4-month RIF- MXF-PZA. Pa substituted for MXF or PZA.	_
Tasneen 2016 [33]	BALB/c mice	H37Rv	Log phase	50 or 100mg/kg 5 days per week	2 months	Addition of LZD significantly increased the activity of BDQ+PMD (P< 0.01). All 2 drug combinations had inferior efficacy compared to 3 drug combinations (BDQ+PMD+ either SZD/LZD) at 2 months (P<0.01), indicating each drug contributes to the efficacy.	_
Tasneen 2015 [34]	BALB/c mice	H37Rv	Log phase	10, 30, 100, 300, or 600 mg/kg or 50mg/kg	Up to 3 months	Addition of either nitroimidazole (PA-824 or TBA-354) significantly improved the sterilizing activities of bedaquiline and sutezolid, with or without pyrazinamide. TBA-354 is 2 to 4 times more potent than PA-824 when combined with bedaquiline.	_
Tyagi 2005 [35]	BALB/c mice	H37Rv	_	50, 100, 200mg/kg 5 days per week	4 months	Dose-dependent activity during the continuation phase. Potent activity during the continuation phase of therapy, targetting bacilli persisting through an initial 2-month intensive phase of treatment with rifampin, isoniazid, and pyrazinamide. At a dose of 100 mg/kg, the activity of PA-824 was significantly greater than that of isoniazid or moxifloxacin and approached that of the combination of rifampin and isoniazid. At 6 month, 100 or 200 mg/kg doses resulted in negative spleen cultures (in all 6 mice).	_

PaMZ pretomanid moxifloxacin pyrazinamide, PMD or Pa or PA-824 pretomanid, BDQ bedaquiline, SZD sutezolid, LZD linezolid, MXF moxifloxacin, PZA pyrazinamide, RIF rifampicin, INH isoniazid

Table 4: Summary of studies reporting on pharmacokinetics pharmacodynamics of Pretomanid

Author	Study	Country	Subjects	n	Dose	Day	CFU	TTP	Conclusion	PKPD
Dawson [16]	Phase 2b EBA	South Africa Tanzania	DS-TB	207	100, 200mg qd	14	$DSTB_{0-56}$, MPa200Z 0·155, (95%CI 0·133–0·178) vs HRZE (0·112, 95%CI 0·093–0·131). $MDRTB_{0-56}$, MPa200Z 0·117 (0·070–0·174).	$DSTB_{0-56}$, MPa200Z 0·020 (95%CI 0·016 - 0·024) vs HRZE 0·017 (95%CI 0·013 - 0·021) $MDRTB_{0-56}$, MPa200Z 0·015 (95%CI –0·001- 0·031)	MPa200Z was more active than HRZE for for CFU (p=0·028) and TTP (p=0·035)	weak associations
Diacon [18]	Phase 2 EBA	South Africa	DS-TB	69	200, 600, 1000, 1200mg qd	1,8,14	Mean $logCFU_{0-14}$ ranged from 0.088 (1200mg)-0.106 (200mg). The log10 CFU time trend was best modeled by bilinear regression.	TTP_{0-14} ranged from 3.818 (200mg) – 4.865 (1000mg). Overall mean increase in TTP_{0-14} 4.106 h/day (SD 4.011).	EBA(0-14), EBA(0-2), and EBA(2-14) were similar at all dosages, at a rate comparable to HREZ.	
Diacon [17]	Phase 2 EBA	South Africa	DS-TB	69	50, 100, 150, 200mg qd	1,14	Mean $logCFU_{0-14}$ ranged from 0.063 (50mg) – 0.112 (200mg) The log10 CFU time trend was best modeled by bilinear regression.	TTP_{0-14} ranged from 2.621(50mg) – 4.640 (200mg)	Substantial EBA (days 0 to 14) at all doses (fall in CFU per ml sputum and the prolongation of TTP). Weak PK-PD correlations. No overall trends within dose groups.	T/MIC(2-14) lowest for 50mg group (91.45%; SD 6.114). For the 100-mg PA-824 group, the T/MIC(2-14) was 93.42% (SD, 5.885); for the 150-mg group, 95.81% (SD, 4.323); and for the 200-mg group, 98.82% (SD, 2.218).
Diacon [19]	Phase 2 EBA	South Africa	DS-TB	105	200mg qd BPaZC BPaZ BPaC	14	Daily rate of change in log10CFU/ml of sputum from D0-14 was mean 0.167 (95% CI 0.075 to 0.257)	TTP_{0-14} was 7.0 (5.1 to 9.4) for BPAZ and 6.3 (4.8 to 7.6) for HRZE	The highest EBA0-14 was found with B-Pa-Z	T/MIC 92% in patients on pretomanid-containing regimen (MICs<0.03 to 0.06 ug/ml for group B-

										Pa-C and < 0.03 to 0.125 for B-Pa-Z-C and B).
Solans [20]	Phase 3	South Africa	M/XDR-TB	93	200mg qd	14, 56, 112		No significant predictors of TTP (Tested dynamic drug concentrations, individual daily exposure and cumulative AUC up to 2 months of treatment).	No relationship between drug exposure & outcome: Plasma concentrations not distinguishable between groups of favourable and unfavourable outcomes.	-

CFU colony forming units, TTP time to culture positivity, MPaZ moxifloxacin pretomanid pyrazinamide, BPaZ bedaquiline pretomanid pyrazinamide, C clofazimine

Table 5: Summary of modelling studies of Pretomanid

Author	Study	Subjects	Dose	Duration	Modelling	Conclusion
Lyons [37]	CL-007, CL-010	DS-TB	50-1200mg	14 days	one-compartment model with first-order absorption and elimination and a sigmoidal bioavailability dependent on dose, time, and the predose fed state	The PK model describes the dose-exposure relationship for pretomanid in adult TB patients and can be used for further studies exploring dose effect relationship
Lyons [38]	CL-007, CL-010	DS-TB	50-1200mg	14 days	PD modelling using a previously developed PK model	Model simulations showed pretomanid at 100, 200, and 300 mg attained 58, 73, and 80%, respectively, of maximum 14-day EBA of 0.136 log10 CFU/ml sputum/day. Model has potential applications to dose optimization of pretomanid-containing regimens
Lyons [39]	CL-007, CL-010	DS-TB	50-1200mg	14 days	Optimal once-daily mean (SD) doses corresponding to the maximum benefit-risk values: 220 mg (10 mg) for population total, 230 mg (10 mg) for male, 200 mg (10mg) for female Dosing interval: twice-daily for 100 mg, once- daily for 200 mg and 300 mg, and once every 36 h for 400 mg. The maximum benefit-risk regimen: 120 mg every 15 h [based on EBA of 0.103 log10 CFU/ml/d and probability of adverse event 0.044], or 100 mg twice daily.	The model provides opportunity to identify optimized individual dosing based on initial PKPD profiles and can help dose selection for clinical trials at individual patient level.

					Loading dose of 350mg for the maximum benefit-risk	
Lyons [40]	NC-001, NC-003	DS-TB	200mg	14 days	The model describes the relationship between drug exposure (B, Pa, Z, M, C) and CFU and TTP for individual drugs as well as combinations.	Importance of pyrazinamide was demonstrated as the synergy between BZ and PaZ compensated the antagonism between B and Pa
Mehta [42]	Various preclinical/clinical studies	TB	200mg	n.a.	A Minimal Physiologically Based Pharmacokinetic (mPBPK) Model Structure was created and validated with pyrazinamide data. Subsequently it was developed for pretomanid including target sites (e.g. lung lesions) to assess target attainment for minimum bactericidal concentration for non-replicating and replicating Mtb.	Pretomanid 200mg may not achieve optimal exposures to eradicate non-replicating bacteria in most patients as <5% of patients predicted to reach target concentration for non-replicating bacteria while >80% of patients predicted to reach target concentrations for replicating bacteria.
Nedelman [43]	NC-002 (PaMZ), NC-005 (BPaZ and BPaMZ), STAND (PaMZ), and Nix-TB (BPaL)		200mg			Significant exposure-response relationships for time to sputum culture conversion (TSCC) and two adverse event classes (vomiting and gastrointestinal symptoms). Pretomanid, 200 mg in the fed state, is appropriate over the range of exposures.
Ignatius 2021 [22]	APT A5306 phase 1	DS-TB HV	200mg	up to 12 weeks	one-compartment disposition model with first- order elimination and dynamic transit compartment absorption. Study effect parameters to account for rifampicin effect on increasing pretomanid CL	Pretomanid coadministered with rifampin or rifabutin under fed conditions showed a favorable probability of target attainment (PTA) at the recommended dose of 200 mg daily. PTA > 90% in both the rifampin and rifabutin arms at a MIC of 0.03125 or 0.0625 mg/liter. When the target corresponding to 1.59-log10 bactericidal activity (77% fT>MIC) was used, the PTA was above 90% at a MIC of 0.03125 or 0.0625 mg/liter.
Mudde 2022 [36]	-	BALB/c mice	100mg/kg/d	upto 13 weeks	Mathematical model based on mice TB model	6 weeks of BPaMZ achieved cure in all mice. 13 weeks of BPaL did not achieve 100% cure rates.

						95% probability of cure was predicted to occur at 1.6, 4.3, and 7.9 months for BPaMZ, BPaL, and HRZE, respectively.
Salinger 2019 [44]	14 studies: Six phase 1 studies, six phase 2 studies, and two phase 3 studies.	HV, DS-, MDR-, XDR-TB	50-1200mg daily	up to 6 months	One-compartment model that at a given dose was linear in its absorption and clearance but where the rate of absorption and extent of bioavailability changed with dose. The median Cavg, Cmax, and C24h (reference subject): 2.4, 3.2, and 1.6 ug/ml, respectively. Factors affecting variability: Relative bioavailability decreased with increasing dose in the fasted condition (not for = or < 200 mg fed state). The median Cavg was 22% higher in females, 13% lower in HS, and 6% lower in HIV+ subjects.	n.a.

N.R. not available, HV healthy volunteers, NRP non-replicating persister

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Technical Report on critical concentrations for drug susceptibility testing of cycloserine and terizidone

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Technical feedback

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Provision of publications

The following individuals helped with obtaining papers for this review: Max Salfinger (University of South Florida, Tampa, Florida, United States of America) and Danila Zimenkov (Russian Academy of Sciences, Moscow, Russian Federation).

Abbreviations

7H10	Middlebrook 7H10
7H11	Middlebrook 7H11
ATCC	American Type Culture Collection
ATU	area of technical uncertainty
BCCM	Belgian Co-ordinated Collections of Micro-organisms
BCG	*Mycobacterium bovis* BCG
CC	critical concentration
CI	exact binomial confidence interval
DCS	D-cycloserine
ECOFF	epidemiological cut-off
gDST	genotypic drug susceptibility testing
gWT	genotypically wild type
INH	isoniazid
ITM	Institute of Tropical Medicine Antwerp
LJ	Löwenstein-Jensen medium
LoF	loss of function
MDR	multidrug-resistant
MGIT	BACTEC™ Mycobacterial Growth Indicator Tube™ 960
MIC	minimum inhibitory concentration
MTBC	Mycobacterium tuberculosis complex
pan-S	pan-susceptible
pDST	phenotypic drug susceptibility testing
pNWT	phenotypically non-wild type
PMID	PubMed ID
pWT	phenotypically wild type
R	resistance/resistant
TB	tuberculosis
TZD	terizidone

1.0 Introduction

A 2018 WHO systematic review of minimum inhibitory concentration (MIC) data identified no studies for terizidone (TZD) and only a limited number of studies for cycloserine (DCS) *(1)*. As a result, the WHO critical concentration (CC) for Löwenstein-Jensen (LJ) at 30 mg/L was withdrawn and no other CCs could be established for Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11) or BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT). As a result, no WHO-endorsed phenotypic drug-susceptibility testing (pDST) method currently exists for either DCS or TZD. There is no commercial genotypic drug-susceptibility testing (gDST) assay either. WHO commissioned an update to the systematic review to evaluate whether sufficient new evidence had been published since 2018 to set a CC for one or more of the above media using the 1% proportion method.

1.1 Known resistance mechanisms

DCS is a cyclic analogue of D-alanine, which consequently acts as an antagonist of DCS *(2)*. Consequently, the DCS MIC increases with higher D-alanine concentrations that depend on the composition of the medium as well as its precise preparation (e.g. autoclaving often releases D-alanine) *(3, 4)*. Other factors, such as the ion content or pH, also affect the MIC *(3)*. There is likely cross-resistance between DCS and TZD in vitro, as the latter compound combines two DCS molecules (whether TZD offers pharmacokinetic/pharmacodynamic or clinical advantages is a separate question) *(3)*.

It is unclear if alanine racemase (Alr (Rv3423c)) or D-alanine–D-alanine ligase (DdlA (Rv2981c)) is the primary target of DCS, but only Alr mutations have been shown to correlate with acquired resistance *(5-9)*. Moreover, mutations in *ald* (*Rv2780*), which encodes L-alanine dehydrogenase, have been implicated in both acquired and intrinsic DCS resistance *(10)*. The mechanism for this phenotype is believed to be the inability of *ald* mutants to convert L-alanine to pyruvate, which would increase the pool of L-alanine and therefore counteract competitive inhibition by DCS *(10)*.

The *Mycobacterium bovis* BCG (BCG) vaccine strain is intrinsically resistant to DCS *(11, 12)*. Chen et al. have demonstrated that the G122S mutation in *cycA* (*Rv1704c*) only partially explains this phenotype *(2)*. Desjardins et al. have proposed that an *ald* frameshift could contribute to the intrinsic resistance of BCG *(10)*. However, the complementation of BCG with the wild type *ald* gene did not result in a change in the DCS MIC in this study, using the 10% LJ proportion method. Nevertheless, the complemented strain had a significant growth disadvantage compared to the unmodified, parental BCG strain in the presence of DCS, suggesting that the frameshift likely plays a role in the intrinsic DCS resistance of BCG. Notably, the *ald* frameshift in BCG is shared by the entire RD9 branch of *Mycobacterium tuberculosis* complex (MTBC), raising the possibility that lineage 5 and lineage 6 (previously known as *M. africanum*) as well as all animal-adapted strains might have elevated MICs compared to *M. tuberculosis (10, 13)*. However, more data are required to confirm this hypothesis, as *M. bovis* was the only RD9 strain tested by Desjardins et al. *(10)*.

Additional mechanisms have been implicated in resistance to DCS, but more data are needed to confirm these *(13-15)*.

1.2 Methods

1.2.1 Search methodology

A PubMed search without date restrictions was conducted on the 6th June 2023 using "(cycloserine OR terizidone) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)". The search terms were intentionally broad since the titles or abstracts of papers do not necessarily mention MIC data. Moreover, MIC data were also solicited from the WHO Supranational Reference Laboratory Network and directly from key researchers, as identified through the literature search and a public call for data by WHO. Only studies that were not already considered for the 2018 review were considered further.

Studies in the following languages were reviewed independently by one or more people:

1. English: Francisco Olivença and Claudio Köser
2. French: Margo Diricks
3. Japanese: Satoshi Mitarai
4. Korean: Soyoun Shin
5. Portuguese: Francisco Olivença
6. Russian: Danila Zimenkov
7. Turkish: Ferda Yılmaz

1.2.2 Inclusion criteria

Studies identified as containing any MIC data through the full-text screening were further reviewed in detail by Claudio Köser. Studies that met the following criteria were included in the review:

1. The MICs for at least one of the anti-TB compounds of interest (with at least three concentrations tested per drug) were determined using the proportion method with a critical proportion of 1%, using LJ, 7H10, 7H11 or MGIT.
2. The drug concentrations tested were clearly defined (i.e. to assess potential truncations of the MIC results).
3. The number of isolates tested at each concentration was given (i.e. to evaluate the shape of the MIC distributions and determine the mode of the distributions).
4. The MIC data were available for at least 10 isolates per drug.

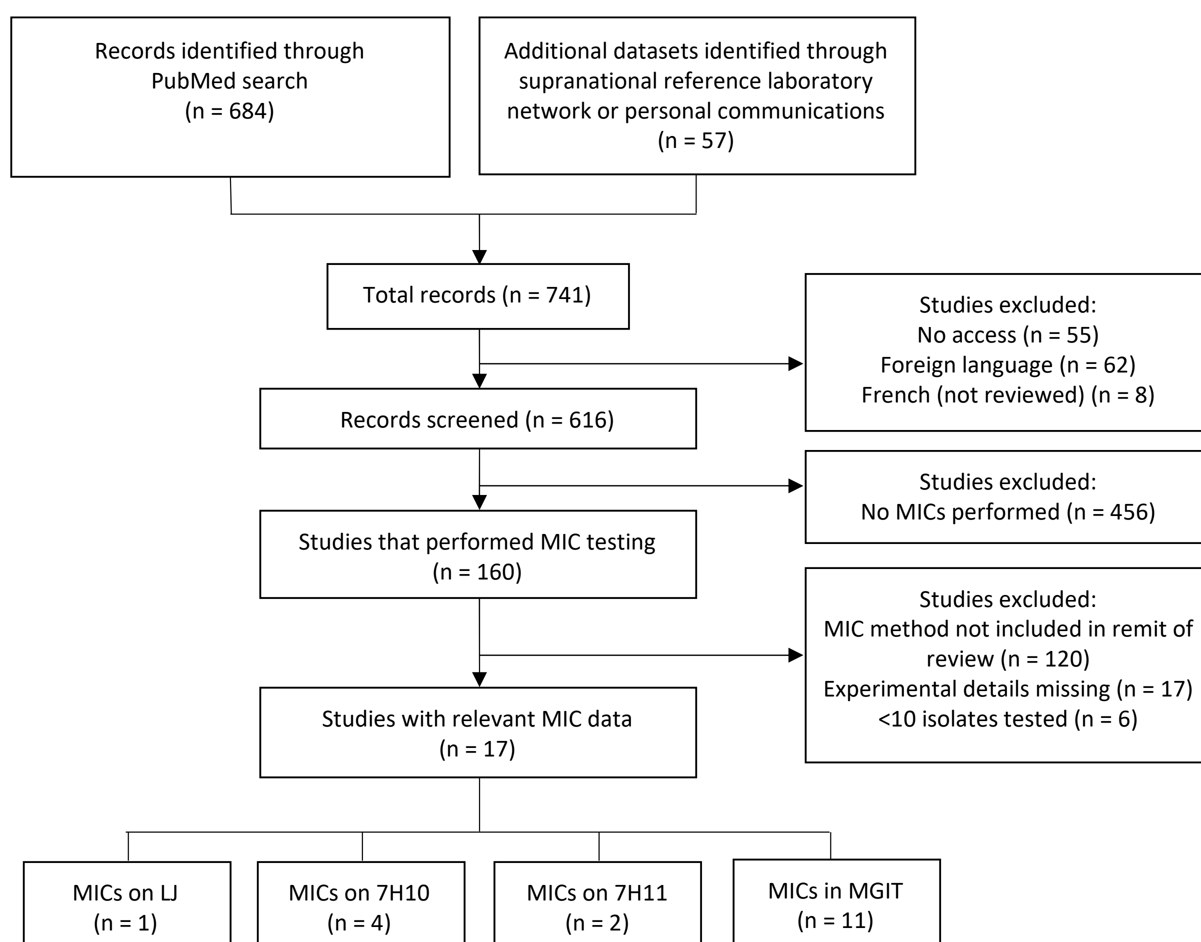
For studies that reported only MIC ranges (i.e. did not meet the third criterion), raw study data were solicited directly from the corresponding authors and/or their co-authors. These

studies were excluded if detailed MIC data could not be obtained. In exceptional circumstances, studies that did not meet all these criteria were still included if they presented data that were particularly valuable.

1.2.3 Studies identified through the systematic review

741 studies were identified, of which 162 had not been considered in 2018 and were reviewed for this report (Figure 1). 17 studies met all inclusion criteria for DCS, compared with just six in 2018, and were further stratified by medium (NB: the sum of the studies for individual media does not correspond to 70 as some studies featured MICs for multiple media). The corresponding studies can be found in the "PRISMA" worksheet in the Supplementary File. No studies met all inclusion criteria for TZD.

Figure 1. PRISMA diagram for DCS and TZD search results and exclusion criteria



1.2.4 MIC data stratification

MIC data from different media were analysed separately as systematic differences between media may exist *(16, 17)*. All mutations in the coding or upstream regions of *ald*, *alr* and *cycA* were included, where known. Strains without mutations or only synonymous mutations were reported as genotypically wild type (gWT). Frameshifts in *ald* were assumed to confer a loss of function (LoF) phenotype as WHO does for other non-essential resistance genes *(18, 19)*.

Three different annotations currently exist for *alr* (

Figure 2). The start codon in the current annotation of H37Rv (GenBank accession CCP46245.1 in AL123456.3) that most bioinformatic pipelines use is 24 amino acids longer than the experimentally confirmed start by Strych et al. *(20, 21)*. In addition to the evidence from Strych et al., in vitro selection experiments using DCS underline that the H37Rv annotation is incorrect. Specifically, a guanine to thymidine change 57 base pairs upstream of the Strych et al. start codon was selected in the H37Rv laboratory strain in three independent laboratories (Web Annex *3*.

Table). This would correspond to a premature stop codon at codon 6 using the H37Rv annotation, as has been reported in the literature, but is impossible as *alr* is an essential gene (Web Annex *3*.

Table) *(22, 23)*. In 2014, a UniProt curator manually extended the Strych et al. start codon by two amino acids (UniProt P9WQA9;

Figure 2), which was adopted by at least three studies from The Francis Crick Institute *(7, 8, 24, 25)*. Although such a short extension is unlikely to affect the function of the protein, Cesira de Chiara, Dimitrios Evangelopoulos and Luiz Pedro S. de Carvalho, the authors of the aforementioned studies from The Francis Crick Institute, and Kurt Krause, one of the authors of Strych et al. and the first crystal structure of Alr from *M. tuberculosis (20, 26)*, reached the consensus that the Strych et al. start codon should be used for two reasons. First, UniProt provided no evidence for its extension of the protein and did not respond to a query to clarify this point.[8] In fact, even the crystal structure from The Francis Crick Institute used the Strych et al. start and only extended the sequence *in silico* because of the Uniprot annotation. Second, if a nucleotide change occurred in the first two codons of the UniProt protein that did not abolish the start codon or that resulted in a synonymous mutation in the second amino acid, most analysis approaches would assume that these cannot cause DCS resistance. This includes the methods employed by WHO to expand its mutation catalogue for gDST *(18)*. Therefore, the Strych et al. was used as the primary annotation for *alr* mutations in this report and the H37Rv annotation was included in parentheses purely to make it easier for readers to compare the mutations with their historical results.

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[8] Evangelopoulos D, personal communication, 2023.

Figure 2. Overview of three different Alr annotations

```
                                                         -20        -10
                                                      MKRF WENVGKPNDT TDGRGTTSLA

         10         20         30         40         50         60         70
MTPISQTPGL LAEAMVDLGA IEHNVRVLRE HAGHAQLMAV VKADGYGHGA TRVAQTALGA GAAELGVATV

         80         90        100        110        120        130        140
DEALALRADG ITAPVLAWLH PPGIDFGPAL LADVQVAVSS LRQLDELLHA VRRTGRTATV TVKVDTGLNR

        150        160        170        180        190        200        210
NGVGPAQFPA MLTALRQAMA EDAVRLRGLM SHMVYADKPD DSINDVQAQR FTAFLAQARE QGVRFEVAHL

        220        230        240        250        260        270        280
SNSSATMARP DLTFDLVRPG IAVYGLSPVP ALGDMGLVPA MTVKCAVALV KSIRAGEGVS YGHTWIAPRD

        290        300        310        320        330        340        350
TNLALLPIGY ADGVFRSLGG RLEVLINGRR CPGVGRICMD QFMVDLGPGP LDVAEGDEAI LFGPGIRGEP

        360        370        380
TAQDWADLVG TIHYEVVTSP RGRITRTYRE AENR
```

M: protein start in H37Rv according to GenBank CCP46245.1 in AL123456.3

L: protein start according to UniProt P9WQA9

M: protein start according to Strych et al. that is considered correct

1.2.5 Format of MIC tables

This report contains abridged versions of the complete data that can be found in the Supplementary File, which also provides a “filter key” to allow the reader to recreate the abridged tables in this report. Details for the information provided in each column of these files can be found below. However, only essential columns were included in this report. For example, the column with the “total [number of] MICs” performed was included only if these numbers differed from the numbers of unique isolates tested (i.e. when isolates were tested repeatedly, as was the often the case for H37Rv).

The following points are relevant for the interpretation of the data:

If a cell is empty, no information regarding the particular category were available (i.e. in the case of the “genotypic results” column, blank cells are not equivalent to gWT (where sequencing or another genotypic method was carried out but no relevant genetic changes were found)).

MICs from different studies cannot be compared unless the concentrations and ranges of concentrations tested are considered. Shaded cells therefore designate the concentrations tested for each group of isolates (NB: some studies tested a wide range of concentrations.

Table 1 provides an overview of how MIC data are displayed.

Table 1. Overview of MIC data presentation.

Studies	RIF MIC [mg/L]					
	0.25	**0.5**	**1**	**2**	**4**	**8**
study A			**15**	2	2	
study B	20		15			2

Shaded cells correspond to the concentrations tested in a particular study (e.g. concentrations of 0.5, 1 and 2 mg/L were tested for study A, whereas 0.5 and 2 mg/L were not tested in study B, which means that MICs of 1 mg/L in both studies are not equivalent). Truncated MIC values were highlighted in red. If red was used in a shaded cell, the MIC was either ≤ or ≥ the concentration in question. For example, the lowest MIC value for study B was ≤0.25 mg/L, whereas the highest MICs were 8 mg/L. If red was used in an unshaded cell, the MIC was > the last concentration tested (for study A, the highest MICs were >2 mg/L, as opposed to 4 mg/L). The mode of the putative phenotypically wild type (pWT) MIC distribution was indicated by highlighting the corresponding number of MICs in **bolded** text (e.g. 1 mg/L for study A). In the case of study B, the truncation of the MIC values meant that a mode could not be identified (e.g. it was possible that the MICs of all 20 isolates with MICs ≤0.25 mg/L were actually 0.25 mg/L, in which case 0.25 mg/L would be the mode of the MIC distribution).

The following information are provided in each data column.

"Studies" column:

The names of the studies with notable limitations were highlighted in red (e.g. if the same laboratory participated in multiple studies that used the same medium or a method other than sequencing was used for gDST). The corresponding limitations were detailed below the tables in the footnotes in this report and in the 'comment' column in red in the supplementary MIC file.

"Lab" column:

The laboratories that participated in multiple studies using the same medium were highlighted in red.

"Unique isolates" & "total MICs" columns:

Red entries correspond to isolates that were tested multiple times.

"Comment" column:

Additional remarks regarding the study in question were included in this column. Important limitations were highlighted in red.

1.3 DCS MIC data on LJ

1.3.1 DCS MICs for pWT isolates on LJ

Nakatani et al. only reported MICs for H37Rv tested in two laboratories and thus provided little insight into the pWT MIC distribution (Table 2) *(5)*.

Table 2. DCS MICs for pWT and mutated isolates on LJ.

Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic results	DCS MIC (mg/L) 1.87	3.75	7.5	10	15	20	30	40	60
1) Nakatani 2017	1		1	H37Rv ATCC 27294				**1**						
	1	clinical	1	MDR	gWT parent					**1**				
	1	clinical	1	MDR	*alr* c-8t (S22L)									1
	2		1	H37Rv ATCC 27294					**1**					
	2	clinical	1	pre-XDR	*alr* M319T (M343T)								1	
	2	clinical	1	pre-XDR	*alr* Y364D (388D)								1	
	2	clinical	1	pre-XDR	*ald* LoF & *alr* R373L (R397L)								1	

The orange line denotes former WHO CC.

1.3.2 DCS MICs for mutated isolates on LJ

Clinical isolates

Nakatani et al. demonstrated that the acquisition of a c–8t mutation upstream of *alr* during multidrug-resistant (MDR) TB treatment correlated with a DCS MIC increase from 15 to 60 mg/L (Table 2) *(5)*. Three additional *alr* mutations, one of which coincided with an *ald* mutation, also correlated with MICs above the former WHO CC. Nakatani et al. provided additional evidence by molecular modelling and direct measurements of enzymatic activity that these three *alr* coding mutations are likely responsible for DCS resistance.

1.3.3 Conclusion for DCS CC for LJ

Given that no new data were identified compared with the 2018 review, no CC could be set.

1.4 DCS MIC data on 7H10

1.4.1 DCS MICs for pWT isolates on 7H10

Two studies were identified that reported DCS MIC data for the pWT population on 7H10 (Table 3). Schön et al. tested 110 clinical isolates that had a pWT MIC distribution of 8–32 mg/L (with a mode at 32 mg/L) *(27)*. Pholwat et al. reported a pWT MIC distribution of 3.75–15 mg/L (with a mode at 15 mg/L) for 21 clinical isolates *(28-30)*.

Table 3. DCS MICs for pWT and mutated isolates on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	DCS MIC (mg/L) 3.75	4	7.5	8	15	16	30	32
2) Schön 2011	3		1	4	H37Rv ATCC 27294						**4**		
	3	clinical	110	110	different levels of R				10		41		**59**
3) Pholwat 2011, 2012 & 2015	4		1	1	H37Rv ATCC 27294					**1**			
	4	clinical	21	21	different levels of R	1		9		**11**			

1.4.2 DCS MICs for mutated isolates on 7H10

No studies presenting MICs for mutated isolates were identified.

1.4.3 Conclusion for DCS CC for 7H10

Given that no new data were identified compared with the 2018 review, no CC could be set.

1.5 DCS MIC data on 7H11

1.5.1 DCS MICs for pWT isolates on 7H11

One new study was identified for 7H11 compared with the 2018 review (Table 4). However, Meacci et al. only tested 10 serial isolates from the same patient *(31)*. Moreover, it was conducted in the same laboratory as Fattorini et al. that had tested 46 clinical isolates, which were enriched for resistance to other drugs, and found an MIC distribution of 7.5–60 mg/L (with a mode at 15 mg/L) *(32)*.

Table 4. DCS MICs for pWT and mutated isolates on 7H11.

					DCS MIC (mg/L)			
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**7.5**	**15**	**30**	**60**
4) Fattorini 1999	5		1	H37Rv ATCC 27294	**1**			
	5	clinical	46	R to at least 2 first-line drugs	2	**22**	18	4
5) Meacci 2005	5	clinical	10	serial isolates from one patient			**10**	

The novel study identified compared with the 2018 review is shown in **bold**.

1.5.2 DCS MICs for mutated isolates on 7H11

No studies presenting MIC distributions for mutated isolates were identified.

1.5.3 Conclusion for DCS CC for 7H11

Given that all data were from a single laboratory, the evidence was insufficient to set a CC.

1.6 DCS MIC data in MGIT

1.6.1 DCS MICs for pWT isolates in MGIT

In 2018, Naktani et al. was the only study with results for MGIT *(5)*. Data from nine additional laboratories were identified in this review (Table) *(24, 33-37)*.[9,10] All nine sites tested a total

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[9] Augustynowicz-Kopeć E, personal communication, 2023; Jou R, Liu K-H, Wu S-H and Chan H-H, personal communication, 2023; Robledo J, Hurtado U A, Alvarez N, Realpe T and Osorio A, personal communication, 2023; Werngren J, Mansjö M, Mengshoel AH, Groenheit R and Schön T, personal communication, 2023.

[10] The results published as Dyuzhik et al. 2016 represent a subset of results included in the thesis by Dyuzhik 2017. The latter results were consequently included in this report.

of 72 replicates of the *M. tuberculosis* laboratory strain H37Rv, of which two variants are considered equivalent:

27294 from the American Type Culture Collection [ATCC];

500735 from the Belgian Co-ordinated Collections of Micro-organisms/Institute of Tropical Medicine Antwerp (BCCM/ITM), which is considered equivalent to ATCC 27294 by BCCM/ITM;

ATCC 25618, which differs slightly from ATCC 27294 *(38)*;

A variant directly obtained from the Pasteur Institute that was also the original source of the ATCC and BCCM/ITM variants. It is not known whether this variant is more closely related to ATCC 25618 or ATCC 27294.

Excluding six replicates that had truncated MICs, the above H37Rv MIC variants were found to have an MIC distribution of 4–8 mg/L.

Augustynowicz-Kopeć, Gonçalves et al. and Robledo et al. were the only studies to feature 43 untruncated MICs for pan-susceptible (pan-S) strains (7% of isolates from Dyuzhik were also pan-S but their MICs were not presented separately from the remaining isolates that were at least resistant [R] to isoniazid [INH]). The corresponding MICs spanned 4–16 mg/L with consistent modes at 8 mg/L. Setting aside the results from Dyuzhik et al., the remaining 435 MICs from eight laboratories were for strains that were predominantly MDR or at least resistant to one drug, excluding DCS. The modes of the MIC distributions from the studies that tested at least 10 isolates were either 4 mg/L or 8 mg/L. The highest MIC for pWT isolates in all laboratories was 16 mg/L.

The results from Dyuzhik differed from the remaining studies because a bimodal MIC distribution was reported. The mode of the primary distribution was 10 mg/L (or 15 mg/L if 10 mg/L had not been tested in favour of a doubling dilution scheme in accordance with the International Organization for Standardization *(39)*). The secondary mode was 30 mg/L. Notably, all isolates with MICs ⩽15 mg/L tested susceptible using the absolute concentration method on LJ using a CC of 30 mg/L, which is not WHO-endorsed *(40)*. Conversely, all isolates with MICs >15 mg/L were resistant according to the absolute concentration method, which corresponds to a resistance rate of 25% (95% exact binomial confidence interval [CI]: 19–31).[11] H37Rv was not included in every batch of this study, which means that shifts towards higher concentrations due to factors, such as the instability of DCS, cannot be excluded *(3, 16, 41, 42)*. However, it is plausible that isolates with high MICs are genuinely phenotypically non-wild type (pNWT) because Dyuzhik not only included predominantly MDR-TB isolates but focused on isolates from patients with suspected relapses or chronic TB to enrich for pNWT isolates. Moreover, the secondary mode at 30 mg/L is consistent with the MICs observed for some *alr* mutants from other studies (Web Annex *3*.

[11] This compares with a resistance rate of 14% (95% CI: 14–15%) using the absolute concentration method with a CC of 30 mg/L for 14 022 isolates that were at least MDR from 5 677 patients from the Republic of Moldova between 2009–2022. During the same period, only 2% (95% CI: 1–3) of isolates that were susceptible to rifampicin and INH from 980 patients were resistant using the same method (Crudu V, personal communication, 2023).

Table). However, given that some resistance mechanisms exist that confer borderline MIC increases in MGIT (Web Annex *3*.

Table), it was notable that no discordances with the absolute concentration method were reported. In fact, this agreement was only achieved after repeating discordant MGIT results at least twice.[12]

Table 5. DCS MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	DCS MIC (mg/L) 0.5	1	2	4	8	10	15	16	20	30	40
6) Zhu 2023	6		1	9	H37Rv ATCC 27294					**9**							
	6	clinical	159	159	MDR-TB			2	35	**71**	50			1			
7) Wu 2022	7		1	3	H37Rv ATCC 27294					**3**							
	7	clinical	117	117	at least MDR	gWT		11	35	**44**	24			3			
8) Robledo	8		1	30	H37Rv BCCM 500735					**16**	14						
	8	clinical	30	30	pan-S					2	**16**			12			
	8		29	29	at least MDR		1				**12**			16			
	8	in vitro	1	5	H37Rv BCCM 500735	gWT parent				2	**3**						
9) Gonçalves 2014	9		1	1	H37Rv ATCC 27294					**1**							
	9	clinical	10	10	pan-S						**10**						
	9		32	32	different levels of R					2	**29**			1			
10) Dyuzhik 2016 & 2017	10		1	2	H37R ATCC 25618					2							
	10	clinical	226	226	mostly at least R to INH					7	37	**70**	56		14	31	11
11) Jou	11		1	4	H37Rv ATCC 27294					3	1						
	11	clinical	1	1	pan-S	gWT				1							
	11		78	78	different levels of R	gWT				25	**46**			7			
12) Augustynowicz-Kopeć	12		1	1	H37Rv ATCC 27294						**1**						
	12	clinical	4	4	pan-S					1	**3**						
	12		11	11	different levels of R						**9**			2			
13) Werngren	13		1	15	H37Rv ATCC 27294					2	**13**						
	13		1	1										1			
	13	clinical	1	1	pan-S									1			
	13		5	5	different levels of R						4			1			
14) Nakatani 2017	14		1	1	H37Rv ATCC 27294					**1**							
	14	clinical	4	4	different levels of R	gWT								**4**			
15) Evangelopoulos 2019	15		1	1	H37Rv Pasteur	gWT parent					**1**						

The new studies identified compared with the 2018 review are shown in **bold**.

1.6.2 DCS MICs for mutated isolates in MGIT

alr mutants

In vitro isolates

Three laboratories reported the MICs for *alr* in vitro mutants (Web Annex *3*.

Table). Jou et al. found a g–57t upstream mutation in ATCC 35826, which was derived from H37Rv ATCC 27294, to correlate with an MIC of 64 mg/L.[13] The same MIC was obtained for two independent mutants with the same mutation selected from H37Rv Pasteur by Evangelopoulos et al. (one of these mutants was deposited as BCCM 501137). Western blot analysis demonstrated that this mutation resulted in the overexpression of *alr (24)*. The same mutation was selected by Robledo et al. from H37Rv BCCM 500735, yielding an MIC of only 16 mg/L.[14] *alr* D320N was independently selected by Evangelopoulos et al. and Robledo et al. with corresponding MICs of 32–64 mg/L (one of these mutants is now available as BCCM 501136). Moreover, *alr* D320N was selected in vitro in a third study that did not meet the

[12] Smirnova T, personal communication, 2023.

[13] Jou R, Liu K-H, Wu S-H and Chan H-H, personal communication, 2023.

[14] Robledo J, Hurtado U A, Alvarez N, Realpe T and Osorio A, personal communication, 2023.

inclusion criteria for this review *(14)*. The structural basis of this resistance mechanism, which has also been reported in clinical isolates, is well understood *(8, 23, 24, 43)*. The last mutant available from BCCM/ITM (BCCM 501135) had an MIC of 64 mg/L, was originally isolated by Evangelopoulos et al. and harbours a large inter-genic deletion causing *alr* to be over-expressed *(24)*.

<u>Clinical isolates</u>

10 unique *alr* mutations were reported in clinical isolates from three laboratories (Web Annex *3.*

Table) *(5, 36)*.[13] Some of these mutations were likely not related to DCS resistance (e.g. Q6R), whereas others consistently yielded high MICs at multiple sites (e.g. 32–64 mg/L for L89R, which is discussed in more detail in Section 1.7, and M319T) and are likely resistance mutations (5, 10). Notably, two mutations that were selected in vitro by Robledo et al. and were also observed in clinical isolates from China by Jou et al. and Wu et al. correlated with more modest MIC increases (8–32 mg/L for c–14t and 16–32 mg/L for c–8t) *(36)*.[13,14] In contrast, c–8t correlated with more marked MIC increases on LJ (Table 2) *(5, 10, 23)*.

Table 6. DCS MICs for mutated isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Genotypic results	DCS MIC (mg/L) 4	8	16	32	64	Comment
15) Evangelopoulos 2019	15	in vitro mutants	1	1	*alr* large upstream deletion					1	One mutant deposited as BCCM 501135.
15) Evangelopoulos 2019	15		2	2	alr g-57t (E6*)					2	One mutant deposited as BCCM 501137.
11) Jou	11		1	1	alr g-57t (E6*)					1	Deposited as ATCC 35826.
8) Robledo	8		1	1	alr g-57t (E6*)			1			
8) Robledo	8		3	4	*alr* c-14t (T20M)		1	2	1		
8) Robledo	8		1	1	*alr* c-8t (S22L)			1			
8) Robledo	8		1	1	alr R296W (R320W)			1			
8) Robledo	8		12	12	*alr* D320N (D344N)				12		
15) Evangelopoulos 2019	15		8	8	*alr* D320N (D344N)					8	On mutant deposited as BCCM 501136. Proposed resistant control strain.
7) Wu 2022	7	clinical	1	1	*alr* a-188c			1			
11) Jou	11		1	1	*alr* t-71g (M1G)	1					
11) Jou	11		1	1	*alr* g-24a (G17R)		1				
11) Jou	11		1	1	*alr* c-14t (T20M)				1		
7) Wu 2022	7		1	1	*alr* c-8t (S22L)				1		
11) Jou	11		7	7	*alr* Q6R (Q30R)	6	1				
7) Wu 2022	7		2	2	*alr* L89R (L113R)				1	1	
11) Jou	11		1	1	*alr* L89R (L113R)				1		
11) Jou	11		1	1	*alr* R219S (R243S)				1		
11) Jou	11		1	1	*alr* S237N (S261N)			1			
14) Nakatani 2017	14		3	3	*alr* M319T (M343T)					3	
11) Jou	11		2	2	*alr* M319T (M343T)				1	1	
7) Wu 2022	7		1	1	*alr* M319T (M343T)					1	
7) Wu 2022	7		1	1	*ald* LoF				1		
7) Wu 2022	7		1	1	*ald* E32G				1		
11) Jou	11		1	1	*ald* E118K			1			
11) Jou	11		1	1	*ald* A184T		1				
11) Jou	11		2	2	*ald* LoF & *alr* R379C (R403C)	1		1			

The new studies identified compared with the 2018 review are shown in **bold**.

ald mutants

Only four isolates from two laboratories with an *ald* mutation were identified with MICs of 8–32 mg/L (Web Annex *3.*

Table) *(36)*.**Error! Bookmark not defined.**

ald/alr double mutant

Jou et al. reported two double mutants with the same *ald* frameshift and *alr* R379C with MICs of ≤4 mg/L and 16 mg/L (Web Annex *3*.

Table).[15]

1.6.3 Conclusion for DCS CC for MGIT

In this review, MGIT had a good technical reproducibility with a tentative quality control range of 4–8 mg/L. In contrast, BACTEC™ 460 is known to yield much higher and potentially inconsistent DCS MICs, rendering it unsuitable for pDST of DCS *(44, 45)*. The higher D-alanine concentration of Middlebrook 7H12 used by BACTEC™ 460, which contains casein hydrolysate unlike the Middlebrook 7H9 used by MGIT, likely accounts for this difference *(44)*.

Although the quantity of the available evidence about the shape of the pWT distribution for MGIT improved significantly compared with the 2018 review, there were limitations. First, there were an order of magnitude more MICs for strains that were predominantly MDR than pan-S strains, based on which epidemiological cut-off (ECOFF) value should ideally be set *(46, 47)*. Second, truncations precluded the use of ECOFFinder to model the pWT distribution *(46)*. Third, sequencing information was available for only some strains, which meant that pNWT strains with MICs overlapping with the pWT distribution could not be considered systematically *(48)*. Moreover, it was not clear how representative the tested strains were of the global MTBC diversity as typing information was not available for most isolates. However, given that most strains were from Brazil, China, Colombia and Russia, they likely were predominantly lineage 2 and 4, the most relevant lineages in high-burden countries for rifampicin-resistant TB *(49, 50)*.

Despite these limitations, the available evidence suggested that 16 mg/L corresponds to the tentative ECOFF and could be endorsed as the interim CC for DCS, which should be used as a surrogate for TZD resistance *(51)*. This CC is valid for MGIT only and should not be used for other methods, even those using Middlebrook 7H9 (e.g. it has been noted that Sensititre MYCOTB MICs may be higher than MGIT MICs, although this remains to be evaluated more systematically *(51, 52)*). The BCCM 501136 *alr* D320N in vitro mutant appears to have MICs of 32–64 mg/L and could serve as a resistant control strain.

Given the known heat instability of DCS, DCS powder should be stored as instructed by the manufacturer and stocks solutions should be stored at –70° C ± 10° C for no longer than one year (i.e. –20° C should not be used and vials should never be re-frozen) *(42)*.

1.7 Future research priorities

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[15] Jou R, Liu K-H, Wu S-H and Chan H-H, personal communication, 2023.

The following priorities were identified to facilitate both pDST and gDST of DCS:

A well-characterised collection of pan-S MTBC strains should be tested to re-evaluate the tentative CC and to determine whether lineage 5, lineage 6 and animal-adapted MTBC strains have intrinsically elevated MICs to DCS *(13, 47)*.

The *alr* D320N mutant BCCM 501136 should be tested in additional laboratories to establish whether it tests reliably resistant at 16 mg/L (BCCM 501135, BCCM 501137 and BCG could be included as comparators) and external quality control assessment schemes for pDST should be established *(53-56)*.

Strains with genomes and MGIT MICs should be included in the next update of the WHO mutation catalogue *(18)*.

Based on the evidence from this report or the literature, *ald* mutations and some *alr* mutations appear to confer borderline phenotypes, resulting in an overlap between pWT and pNWT MICs at 16 mg/L *(10)*. False-susceptible results could be minimised by setting an area of technical uncertainty, as defined by the European Committee on Antimicrobial Susceptibility Testing *(17)*. Additional MIC testing of *alr* and *ald* mutations would allow WHO to evaluate whether routinely testing 8 mg/L in addition to 16 mg/L to accommodate an area of technical uncertainty is warranted for routine pDST and how discordant DST results are best resolved *(17, 57, 58)*. Moreover, it would facilitate classifying these mutations during the next update of the WHO mutation catalogue, which would allow countries to screen their existing genomes to identify potential hotspots of DCS resistance *(59, 60)*. Ideally, a variety of mutations should be tested, but the following criteria could be used to prioritise mutations for this purpose:

Frequency of mutations in MDR-TB strains. For example, *alr* L89R, which appears to be a good candidate for a resistance mutation based on the results from this report and the literature, was found to be the most frequent *alr* mutation in over 32 000 isolates and was particularly frequent amongst extensively drug-resistant isolates (according to the old WHO definition that includes aminoglycosides) from Belarus and South Africa *(61-68)*.

Homoplasic mutations *(5, 68)*.

Mutations that arose during treatment where closely related wild-type strains are available as controls *(5, 63, 69)*.

The *alr* annotation in the H37Rv genome and at UniProt should be corrected according to Strych et al. *(20)*.

Alternative resistance mechanisms should be studied *(13-15)*.

MICs must be integrated with pharmacokinetic/pharmacodynamic and clinical outcome data to optimise DCS and TZD treatment *(17, 37)*.

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Cycloserine

A systematic review on PK and PK/PD

Report for the World Health Organization, Global TB Program

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Introduction

The introduction is intended to provide the readers a quick introduction in basic pharmacokinetic (PK) and pharmacodynamic (PD) principles and methods used in the various studies. It is not intended to be a comprehensive overview of the literature. For a more detailed overview we refer to a recent review on this topic.[1]

Pharmacokinetics

PK describes the behavior of a drug in the patient' s body. Generally, the drug is absorbed (A) into the systemic circulation after oral or parenteral intake, which is then distributed (D) throughout the body including the site of infection. After metabolism (M) primarily by the liver, eventually the drugs are eliminated (E) by the kidneys and released in the urine. Integration of these parameters results in a PK model that describes these processes (ADME). There are many factors that can influence the PK of a drug e.g. renal function, body weight, and drug-drug interactions.

Pharmacodynamics

PD describes the biochemical or pharmacological effect of a drug on the *Mycobacterium tuberculosis* (efficacy) and on the patient (toxicity). Anti-TB drugs can be subdivided in bactericidal and bacteriostatic drugs. The maximum achievable response of a drug is described by maximum effect (Emax). In addition, prevention of acquired drug resistance is another aspect that is relevant for the evaluation of a drug. Most drug can display different effects at different concentrations, e.g. a drug can be bacteriostatic at a low concentration while it can be bactericidal at higher concentrations.

Pharmacokinetics/Pharmacodynamics

Integration of PK and PD (PKPD) will provide information on how the drug concentrations translate into the effect of the drug. The correlation between drug concentration and efficacy can be subdivided in the following parameters:

1) area under the concentration time curve in relation to minimal inhibitory concentration (**AUC/MIC**),

2) maximum concentration during the dosing interval in relation to minimal inhibitory concentration (**Cmax/MIC**), and

3) the time the concentration exceeds the minimal inhibitory concentration during the dosing interval (**%T>MIC**).

PKPD is helpful to establish the most appropriate dose. Due to variability in drug concentrations in different patient populations and differences in susceptibility between different bacterial species recommended dosages can be different. PKPD studies can be performed *in vitro*, *in vivo* and in humans. [1]

In vitro studies

In vitro studies are useful in the determination of the efficacy and potency of the extent of the drug or dose by performing time-kill kinetic studies.

In the **static time-kill studies** the drug concentration remains fixed over time and the bacterial response are measured in terms change on the optical density and/or colony forming units (CFU). The static time-kill studies are commonly performed using the actively replicating logarithmic

phase bacteria in cultures and based on the extent of kill drugs are commonly classified as bactericidal or bacteriostatic.

In **dynamic time-kill studies** the drug concentrations are actively changed over time reflecting more physiological conditions. The most common used dynamic time-kill study is the hollow fibre infection model. The model consists of a cartridge with hollow fibres. Outside the fibres are the bacteria; inside the hollow fibres is a continuous flow of medium. Drugs and nutrients diffuse through the fiber membrane to the bacteria. These systems are of particular interest for studying PKPD because the human PK can be applied in the system. Moreover, the system can be sampled frequently to study bacterial growth and PK of the drug. This *in vitro* system has been endorsed by European Medical Agency to guide dose finding in TB drug development.[2] Hollow fiber studies can be used to determine whether efficacy is driven by AUC/MIC, Cmax/MIC or T%>MIC by comparing the efficacy of a single dose (Cmax) with the dose divided in two or three dosages (%T>MIC).[3] If the effect of three dosing strategies is the same, the AUC/MIC is the effective PK/PD parameter.

In vivo studies

PK in mice and man are very different and PKPD findings in mice should consider the transitional value in the preclinical drug-development. Within murine models of TB, we can study the PKPD relationship and assess the dose- or time dependent nature of the PKPD relationship trough dose fractionation studies. Essential in these studies is that besides dose, the actual drug concentration, preferably at the site of infection, is considered. Measuring the concentration of the (parent drug) compound and the (active) metabolites, via a chromatography based bioanalytical methods, the contribution of PKPD parameters can be made.

Preclinical murine TB models come in many different forms. The route of infection (e.g., intravenous, inhalation, or instillation), the inoculum size, the mycobacterial strain used, the pathology of TB in the specific model, the treatment-free period before starting therapy and the mouse strain used, are all features that can be changed and tweaked to provide different models.

Human studies

The most used study type in humans to evaluate the PKPD effect of an anti-TB drug in TB patients is the early bactericidal activity (EBA) study. EBA is defined as the rate at which a drug kills actively metabolizing, rapidly multiplying tubercle bacilli in the sputum of patients with TB during the first days of therapy. This methodology has become the first clinical assessment of the efficacy of proposed anti-TB drugs in a relatively small number of sputum smear-positive pulmonary TB patients. Recently, the measurements of killing rate occurring have been divided in an early EBA (between days 0 and 2) and an extended EBA (between days 2 and 7 or between 2 and 14 days). Extended EBA has been advocated as an early measure of sterilizing activity, the ability of a drug to kill slowly replicating, persistent bacilli in tissues. In addition to EBA studies PKPD can also be assessed in relation to parameters like time to sputum culture conversion, sputum culture conversion and a specific month and treatment outcome.

Cycloserine/terizidone drug profile

Cycloserine is an isoxazoline derivate and a bacteriostatic drug that inhibits cell wall synthesis. Cycloserine is well absorbed after oral administration and widely distributed throughout the body. The drug is partially metabolized and approximately 70% is renally excreted. Terizidone consists of two molecules of cycloserine linked by terephthalaldehyde and is hydrolyzed in the gastrointestinal tract to form cycloserine. Terizidone is considered to be interchangeable with cycloserine. The currently recommended dose is 10-15mg/kg.

Cycloserine is well-known for its side effect profile behavioral changes including depression, psychosis, and suicidal thoughts[4].

Aim of the report

The intention of the report is to provide insight in PKPD of cycloserine to help make decisions regarding clinical breakpoints and its programmatic use and dosing strategies. Information presented is based on data retrieved from a systematic literature review.

The systematic literature review has the following objectives:

Describe the PK of cycloserine, especially the variability and factors relevant for treatment.

Identify the PKPD relationship.

Describe the target attainment of current dose regimen based on the PKPD relationship.

Review of PK and PD data

The review was conducted in accordance with the principles outlined in the PRISMA statement.[5]

Strategy for the systematic review

This systematic review is an update of an earlier report which was written to inform the "Technical report on the pharmacokinetics and pharmacodynamics (PK/PD) of medicines used in the treatment of drug-resistant tuberculosis" (Geneva: World Health Organization; 2018 (WHO/CDS/TB/2018.6, licence: CC BY-NC-SA 3.0 IGO)[6].

Search of databases was performed on 14 Aug 2023 with date restriction.

Pubmed Search (used All fields):

((Cycloserine) OR (D-Cycloserine)) OR (Terizidone)) AND (((Tuberculosis) OR (TB)) OR (Mtb)) AND ((pharmacokinetics) OR (concentration) OR (therapeutic drug monitoring) OR (TDM) OR (drug exposure) OR (drug monitoring) OR (pharmacology) OR (pharmacodynamics) OR (pharmacol*) OR (pharmacod*))

Filters: from 2017 - 2023

Web of Science core collection search (used All fields):

((ALL=(Cycloserine)) OR ALL=(D-Cycloserine)) OR ALL=(Terizidone) AND

((ALL=(Tuberculosis)) OR ALL=(TB)) OR ALL=(Mtb) AND

((((((((ALL=(pharmacokinetics)) OR ALL=(concentration)) OR ALL=(therapeutic drug monitoring)) OR ALL=(TDM)) OR ALL=(drug exposure)) OR ALL=(drug monitoring)) OR ALL=(pharmacology)) OR ALL=(pharmacodynamics)) OR ALL=(pharmacol*)) OR ALL=(pharmacod*)

Filters from 2017 Jan 05 to 2023 Aug 14

Title and abstract screening as well as full text screening was performed by two reviewers independently. In case of differences consensus was reached through discussion. The PRISMA diagram was made to illustrate the study selection and exclusion process.

Studie selection PK

Criteria for selection of pharmacokinetic variability were studies with a prospective, observational or retrospective design. Only studies with actual TB patients were included as PK studies in healthy volunteers are not representative of drug exposure in TB patients. Studies in healthy volunteers were allowed in case a specific effect was studied, e.g. a drug-drug interaction study or food-effect study. We investigated used dosages and judged whether PK sampling was performed in steady state. Assay parameters for analysis were judged and should comply with ICH guideline M10 on bioanalytical method validation and

study sample analysis.

Studie selection PKPD

Criteria for selection of PKPD studies were *in vitro* (hollow fiber infection model), animal and human studies investigating the relationship between drug dose, concentration, and microbiological response. Important was that the study design allowed for the effect of the drug of interest to be assessed. This could be either as monotherapy or as combination therapy where the drug was administered at various dosages/exposures. For better interpretation of the microbiological response the minimal inhibitory concentration had to be assessed.

Exclusion of studies

Excluded were reviews, case reports and studies not providing relevant information to assess the PKPD of the drug of interest. In case of data appearing in different publications and noticed by the reviewers, results were only included once.

Data extraction

Data extraction was performed by one reviewer and verified by a second reviewer. The following data were extracted when available and relevant: study design, dose, type of TB, tuberculosis strain, bacterial load, treatment duration and treatment outcome (CFU reduction, sputum culture conversion), minimum inhibitory concentration (MIC) including the method used, AUC and Cmax data, pharmacokinetic sampling scheme and information on population pharmacokinetic models.

Results

In total, 192 articles were retrieved from Pubmed and Web of Science (Figure 1) on the 14th of August 2023 covering the period since the previous report[7]. After the removal of 62 duplicates, 130 articles underwent abstract and title screening resulting in 24 articles for full text screening. After the exclusion of 5 non-relevant articles, 18 articles were included in the final assessment. Review of the references of the included articles resulted in 1 additional article to be included for final analysis.

A total of 1 *in-vitro* studies, 1 *in-vivo* studies and 17 human studies in were included.

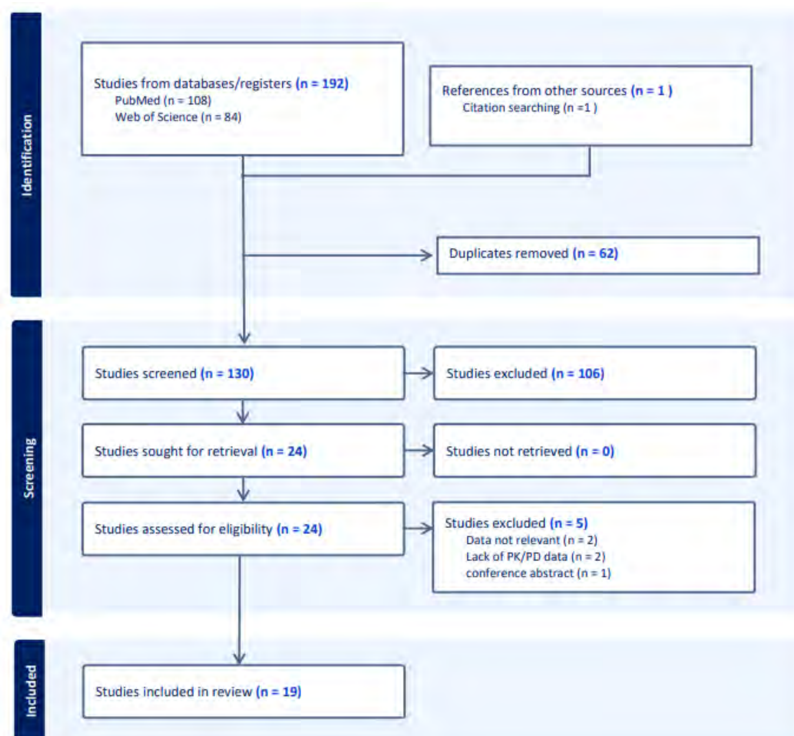

Figure 1: PRISMA diagram

PK variability and factors relevant for treatment

Ten studies evaluated the PK of cycloserine (table 1) and four studies presented other relevant information, including penetration in tissue (cerebrospinal fluid, bone, lung).

Alghamdi *et al* used 5 data sets consisting of 247 patients on various cycloserine dosages to develop a one-compartment population PK model, with a first-order absorption and lag time[8]. The creatinine clearance had a significant effect on the drug clearance and body weight had a significant effect on the drug distribution.

Chang *et al* developed a one-compartment population PK model, with a first-order absorption and lag time using data from 14 patients[9]. No influencing factors on drug distribution or clearance of cycloserine were detected due to small sample size and limited variation in renal function in study participants.

Chirehwa *et al* used data on 132 patients to develop one-compartment population PK model with first-order absorption and lag time[10]. Renal clearance accounted for 55% of the clearance of the drug. The drug distribution was associated with the fat free mass of the participants. Smoking was found to be an important factor as it increased non-renal clearance with 41%.

Court *et al* studied the pharmacokinetics of 35 patients receiving terizidone but did not develop a population pharmacokinetic model [11]. None of the tested factors were associated with the drug exposure of cycloserine. This was likely due to small sample.

Kumar *et al* used multiple linear regression analyses to identify factors influencing drug exposure using the data of 25 children and found that age, gender, weight for age, height for age were not significantly associated[12]. This was likely due to small sample.

Based on data from 39 patients Mulubwa *et al* developed a one - compartment pharmacokinetic model with first - order absorption and linear elimination for terizidone which was modified to include the biotransformation of terizidone to cycloserine[13].

Galien *et al* created a one-compartment population PK model, with a first-order absorption without lag time based on 15 patients[14]. The absence of a lag time in the model was explained by the lack of blood samples collected during the absorption phase of the drug. Authors were not able to correlate drug exposure expressed as AUC0–24 h with sex, age, weight, body mass index, length, diagnosis, body surface area, creatinine clearance, absorption time, use of alcohol, or smoking due to the small sample size of the study. Authors did perform an additional analysis to evaluate if a single sample could predict drug exposure and they found that a sample collected 4h after drug intake correlated well with the AUC0-24 (r2 0.99).

In 80 patients, Yu *et al* measured cycloserine concentrations 2h after administration of cycloserine showing a mean concentration of 22.06 (range 11.21-36.90) after intake of 500mg daily and 36.03 (range 28.57-46.51) after intake of 750mg daily[15]. In 7 patients dose adjustments were effectively performed, i.e. a dose reduction in 5 patients and a dose increase in 2 patients.

Zhu *et al* performed a retrospective study of routinely collected data of 390 patients receiving cycloserine (500mg<50kg, 750mg>50kg) as part of their treatment[16]. Samples were collected 2h after drug intake and concentrations were <20mg/L (14.9 ± 3.72mg/L) in 214/390 patients, between 20-34 mg/L (25.57 ± 4.04 mg/L) in 156/390 patients and > 35mg/L (39.66 ± 5.98 mg/L) in 20 patients. Investigators performed a univariate analysis to explore differences in drug concentration using data from patients 200 patients with a complete data set. Age, gender, chronic liver disease, obesity, diabetes and eGFR were not associated with drug exposure. A limitation is that only a single sample 2h after drug intake was available for analysis.

Zhu *et al* developed a two-compartment model with first-order absorption and a lag time using a cohort of 62 patients with intensive PK sampling (before and at 1, 2, 4, 6, and 8 h after observed intake)[17]. Renal function and body size were significantly associated with drug exposure.

Court *et al* found in a prospective observational study (n=144) that incident or worsening peripheral neuropathy was associated with a Cmax>35mg/L (1.89 (1.04–3.44), *P*=0.035)[18].

When crushing of terizidone tablets was evaluated in a prospective sequential PK Study (n=15) by Court *et al* no difference in drug exposure was observed based on AUC0-10 evaluation (*P*=0.49).[19]

Mulubwa et al assessed the amount of cycloserine after intake of terizidone (n=39) and came to the conclusion that the amount of cycloserine is lower than expected based on 2:1 ratio.[20]

The findings may have implications for dosing but no recommendations were provided besides using therapeutic drug monitoring of terizidone.

Drug concentrations in tissue

Kempker *et al* analyzed, as part of a larger study, 5 patients who received cycloserine for TB meningitis and collected paired samples at 2 and 6h after drug intake[21]. The median CSF concentrations were comparable at 2h (15.90mg/L) and 6h (15.10 mg/L) resulting in a median CSF/serum ratio of 0.52 at 2 h and 0.66 at 6 h.

Liang *et al* performed a case study of a patient with pre-extensive drug resistant TB and based on paired sample collection over a period 12h the CSF/plasma ratio was determined to be 0.59[22].

Zhang *et al* studied the cycloserine concentration in 28 patients with osteoarticular TB receiving 500-mg daily[23]. The median concentrations in plasma and bone were 16.29 (IQR, 6.47-22.76) mg/mL and 24.33 (IQR, 14.68-39.01) mg/g respectively. The median bone/plasma penetration ratio (v/v) was 0.76 (range, 0.33 to 1.98).

Zhang *et al* performed microdialysis in adult male Sprague–Dawley rats to measure the free drug concentration of cycloserine in the lung[24]. A dose of 25 mg/kg was considered to equivalent to a human dose of 250 mg and was orally administered. The free drug concentration ranged from 3.51 to 10.61 mg/L in the blood and from 0.57 to 5.05 mg/L in the lung resulting in a lung/blood ratio of 0.41 (range 0.07-1.29).

PKPD relationship

Three studies presented information on PKPD properties of cycloserine; one in vitro study and two observational cohort studies.

The PKPD properties of cycloserine as monotherapy were studied in hollow fiber system model of tuberculosis by Deshpande *et al* mimicking human exposure from earlier studies[25]. The experiment lasted for 28 days, and the system was sampled for PK (0, 1, 6, 11, 21, 23.5, 48, 72, 96, 120, 144, and 168 h) and PD (0, 3, 5, 7, 10, 14, 21, and 28 days). To assess the bacterial burden they used Mycobacterium Growth Indicator Tube time to positivity and CFU count on Middlebrook 7H10 agar. Deshpande *et al* found that cycloserine maximal kill of extracellular Mtb was >1000-fold higher than for intracellular Mtb. Interestingly, the AUC/MIC best described the response to treatment while this converted to T>MIC after 7 days. The study resulted in the following targets:

T>MIC 20% resulted in stasis.

T>MIC 30% was associated with bactericidal activity (1.0 log10 CFU/mL kill below day 0)

T>MIC 64% represented the 80% of the maximal kill (EC80)

T>MIC 100% was associated with complete suppression of acquired drug resistance.

Zheng et al collected in an observational cohort study data on cycloserine exposure was collected from 186 patients with MDR-TB[26]. Treatment response was evaluated by sputum spear positivity at 2 months and 6 months and treatment outcome. Drug exposure/susceptibility was divided into quartiles and patients in the quartile four were more likely to return a negative sputum culture at 2 and 6 months compared to patients in quartile one (M2, aOR 3.45 (95%CI 1.38–8.61), M6 5.26 (95%CI 1.88–14.7)) as well as treatment outcome (4.87 (95%CI 1.28–18.5).

Zhu *et al* performed a prospective study in MDR-TB patients (n=159) and collected intensive PK data at week 2, MIC and treatment response[17]. Treatment response, as sputum culture conversion was assessed after 2 months and 6 months of treatment, in addition to final treatment outcome. The authors used classification and regression tree analysis and found that T>MIC of 33.2% predicted 6-month sputum culture conversion. Authors also found that the ratio of area under drug concentration-time curve (AUC0-24h) over MIC of 36 predicted final treatment outcome well.

Target attainment of current dose regimen

In total nine studies evaluated whether specific dosages were adequate to attain the target exposure (table 2). Studies either evaluated the traditional Cmax target[27] or evaluated T>MIC targets[25].

Deshpande *et al* performed Monte Carlo simulations for both pulmonary TB and TB meningitis[25]. They found that a dose of 750 mg twice daily was able to achieve the exposure target of T>MIC 20%, T>MIC 30%, and T>MIC 64%, in 93%, 92%, and 81% of the patients. For the target of T>MIC 64%, a dose if 750 mg twice a day would achieve 90% at around 16 mg/L. For TB meningitis: 500 mg twice daily or 750mg once daily achieved exposure target T>MIC 30% up to an MIC of 32 mg/L. T>MIC 64%was only achieved at 750 twice daily for MIC 16 mg/L.

Alghamdi *et al*[8] used Monte Carlo simulation together with T>MIC ≥30% and T>MIC ≥64% as targets to assess for which MIC values the target could be attained in ≥90% of the population. They assumed that Cmax was associated with neurotoxicity and therefore simulated once, two-, three- and four-times daily dosing. Their data showed that current dosing strategies are sufficient for MICs up to 16mg/L. Higher MICs required dosages exceeding 1000mg which may result in toxicity.

Chang *et al* [9] evaluated target attainment using Cmax of 20–35 mg/L. Target attainment was simulated for the following oral dosages: 250 mg BID, 35.8%; 500 mg QD, 49.5%; 750 mg QD, 37.1%; 500 mg BID, 30.9%; 1000 mg QD, 18.3%.

In the study by Chirehwa et al [10] The proportion of virtual patients attaining a T>MIC of ≥30% was at least 90% for MIC values of ≤16 mg/liter. However, the proportion of patients achieving T>MIC values of ≥64% and 100% was more than 90% only with MICs of ≤8 mg/liter. Doses of 500 mg (for those weighing ≤45 kg) and 750 mg (for those weighing >45 kg) were effective at suppressing the emergence of resistance only in isolates for which MIC values were ≤4 mg/liter.

Kumar et al[12] evaluated the target attainment using traditional Cmax evaluation (20-35mg/L), and found that 11 patients (44%) had a level below target, 4 patients (16%) had a level within the target range, while 10 patients (40%) had a level above the target range.

Galien *et al* used the developed model to simulate target attainment for MIC values up to 32.5 mg/L[14]. Target attainment dropped rapidly when MICs exceed 10mg/L to 48% at MIC of 20mg/L and 0% at 32.5mg/L.

Yu *et al* found that a Cmax/MIC≥1 was associated with a favorable outcome (OR 8.000 (95%CI 1.399-45.756) in 8/15 patients while only 3/12 had a favorable response with a Cmax/MIC<1. T>MIC could not be calculated as investigators collected only a single sample for concentration measurement in each patient[15].

Zhang *et al* concentrations of cycloserine that exceeded 16 mg/mL were observed in 53.6% (15/28) plasma samples and 28.6% (8/28) of bone samples[23]. %T.MIC of 30% with the 500-mg/day dosage, whereas the target exposure of a T>MIC64% remained unattainable.

Zhu *et al* used an independently developed population PK model to identify PKPD thresholds for treatment response in a cohort of 159 patients[17]. For target attainment they used T>MIC30% and T>MIC64%. Simulations showed that 500 mg (<45kg) and 750 mg (>45 kg) resulted in a probability of target attainment of >90% at MIC of 16 mg/L in MGIT. Patients with demonstrating a T>MIC>30% were more likely to have a favorable response to treatment (aHR 2.6 (95%CI 1.7, 3.9)).

Discussion

Since the release of the the report “Technical report on the pharmacokinetics and pharmacodynamics (PK/PD) of medicines used in the treatment of drug-resistant tuberculosis” a range of studies have been conducted[6]. This was helpful to overcome the evidence gap shown in the previous systematic review on cycloserine no preclinical studies and very few human studies

with sparse PK sampling[25]. The current review provides a more detailed understanding of the PK and PKPD of cycloserine and its potential implications for drug dosing as part of programmatic care.

In the six studies developing a population PK model all except 1 study found that a one compartment model with first-order absorption described the data well. A lag time or transition compartment was used to account for any delay in absorption of bioconversion from terizidone to cycloserine. Important factors associated with drug exposure were renal function and body size. These factors were found in larger sized studies. Smoking could be an additional factor influencing drug exposure as it increased the non-renal clearance route of cycloserine. As non-renal clearance accounts for 30% of the total clearance it is not clear if dose adjustments need to be made for smoking status. Overall, PK of cycloserine is consistent between studies as demonstrated by comparable structures for the population pharmacokinetics models but its variability in exposure between patients is significant, prompting TDM in some studies.

As cycloserine in an old drug very few preclinical studies investigated the PKPD relationship of the drug. The *in vitro* study by Deshpande et al is the only preclinical study that investigated the relationship between drug exposure and microbiological response. Overall T>MIC was able to predict microbiological response and with an increasing percentage T>MIC the effect of cycloserine increased from stasis (20%), bactericidal (30%), 80% of maximum kill (64%) to prevention of acquired resistance (100%). There were two human studies that investigated the PKPD relationship. The study by Zheng et al ranked drug exposures in patients and showed with higher exposure responded better to treatment[26]. The study by Zhu *et al* collected information on drug exposure, treatment response and pathogen susceptibility in a large prospective cohort[17]. Using classification and regression tree analysis they found that treatment response was determined by T>MIC 33.2%. This study can be considered a clinical validation of the preclinical PKPD study performed by Deshpande *et al*[25] and demonstrated that like for other TB drug the PKPD parameters are comparable between *in vitro* – *in vivo* and human studies.

The relationship with T>MIC established by Deshpande *et al*[25] encouraged many investigators to assess target attainment. For such assessment the ratio between drug exposure and pathogen susceptibility (MIC) is important and therefore most clinical studies used local PK data and MIC distributions. Investigators used T>MIC 30% and T>MIC 64% to determine of >90% of the population would achieve either one of both to these PKPD targets using simulated dosages of 250-1000mg ranging from once to four times daily in some studies (table 2). A few studies used more traditional targets to assess target attainment using a Cmax of 20-35mg/L or included the MIC value in the assessment by stating Cmax>MIC ≥1. Overall, there is a clear trend that higher dosages (500mg bid) are required to attain the therapeutic target of T>MIC 30% for MIC16mg/L. The target for maximum kill can only be attained at lower MIC values (≤8). As a substantial number of patients displays Cmax concentration >35mg/L at higher dosages side effects will increase[18]. Hence, based on the current information cycloserine can be considered a drug with a narrow therapeutic window[28].

Although various PKPD targets have been developed, ranging from statis to prevention of acquired resistance, the question remains which one will be used to select the dose for programmatic treatment. Aiming for stasis (T>MIC20%) does not seem to make sense from efficacy point of view while aiming for prevention of acquired resistance (T>MIC100%) would result in too many side effects. When setting a breakpoint based on maximum kill (T>MIC64%) the MIC would likely be much lower than the ECOFF hence setting a breakpoint based on T>MIC30% makes more sense from ECOFF point of view as well as from treatment tolerability point of view[28].

Conclusion

Cycloserine is a drug with substantial PK variability and a narrow therapeutic window. With a concentration effect relationship (T>MIC) supported by preclinical and human data, PKPD considerations can help to decide on the dose most likely to be beneficial for the treatment of patients with MDR-TB. Considering a target of T>MIC30% makes most sense as aiming for higher targets (T>MIC64-100%) will likely require too high dosages to be well tolerated. A daily dose of 750mg (250+500) or 500mg bid will highly likely achieve T>MIC30% in case of MIC ≤16mg/L.

Knowledge gap

As cycloserine is a drug with a narrow therapeutic window, meaning that the concentration to achieve maximum therapeutic effect is close to the concentration associated with toxicity a personalized treatment approach to manage treatment in patients experiencing toxicity can be considered. As cycloserine is not one of the key drugs to treat MDR-TB very few alternatives are left to replace the drug in case of toxicity. Hence therapeutic drug monitoring may therefore be helpful to guide dosage adjustment in case patients experience toxicity[29]. One of the studies already used TDM to adjust the dose to achieve therapeutic targets[15] and another study presented a limited sampling approach to facilitate drug exposure evaluation with a single blood sample[14]. As no commercial of-the-shelf tests are available to measure cycloserine in house tests need to be developed. Various assays have been published including assays on more basis HPLC-UV equipment[30] as was demonstrated in one of the included studies[12]. If TDM were to be recommended for managing toxicity practical guidance regarding its application would be helpful[29].

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Tables

Table 1: Summary of studies reporting on pharmacokinetics of cycloserine/terizidone

Author	Study	Country	Subjects	n	Dose	Sampling	Concentration	PK model
Alghamdi[8]	PK	USA	HV	12	500mg single dose	0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 24, 36, 48	Cmax median 26.5 (range 7.5 to 97.9) mg/L	one-compartment model, with a first-order absorption and lag phase
	PK	Georgia	MDR-TB	69	500-1000mg	0, 2, 6 to 8, 10 to 12, 24		
	PK	Bangladesh	MDR-TB	42	500-1000mg	1, 2, 6, 12 2, 6		
	Clin	USA	MDR-TB/NTM	54	250-750mg	2, 10		
	Clin	USA	N.S.	70	250-750mg	2, 6		
Chang[9]	PK	South Korea	MDR-TB	14	500-1000mg	0, 0.5, 1, 2, 4, 6, 12	Cmax mean 21 (range 7 to 64) mg/L*	one-compartment model, with a first-order absorption
Chirehwa[10]	PK	South Africa	RR-TB/MDR-TB	132	<33kg 15-20mg/kg 33-50kg 500mg >50kg 750mg	0, 2, 4, 6, 8, 10, 12♦, 24♦, 26♦	Cmax median 37 (range 12 to 98) mg/L* (fig 1A)	one-compartment model, with a first-order absorption and transit compartment

Court[11]	PK	South Africa	MDR-TB	35	250-750mg	0, 2, 4, 6, 8, 10	Cmax median 38.1 (IQR 32.6-47.2)	N.D.
Kumar[12]	PK	India		25	16-25kg 250mg 26-45kg 500mg 46-70kg 750mg	0, 2, 4, 6, 8	Cmax median 31.8 (range 10.6–63.0)	N.D.
Mulubwa[13]	PK	South Africa	MDR-TB	39	750mg	0, 0.5, 1, 2, 3, 3.5, 4, 8, 16, 24	Cmax median 24.1 (range 0.54–63.5)	one-compartment model, with a first-order absorption and transit compartment
van der Galien[14]	PK	Belarus	MDR-TB	15	<50kg 500mg >50kg 750mg	0, 1, 2, 3, 4, 7, 12	Cmax median 23.31 (IQR 20.14–33.30)	one-compartment model, with a first-order absorption
Yu[15]	PK	China	MDR-TB	73	500mg 750mg	2	Cmax mean 22.06 (range 11.21-36.90) Cmax mean 36.03 (range 28.57-46.51)	N.D.
Zhu[16]	Clin	China	N.S.	200	<33kg 500mg 33-50kg 500mg >50kg 750mg	2	14. ± 3.72mg/L n=214 25.57 ± 4.04mg/L n=156 39.66 ±5.98mg/L n=20	N.D.
Zhu[17]	PK	China	MDR-TB	159	500 to 750mg twice daily	0,2,6	Cmax median 22.5 mg/L (IQR 19.6-39.3 mg/L)	one-compartment model, with a first-order absorption with lag time

PK pharmacokinetic study, Clin clinical data, N.S. not specified, * extrapolated from graph, ♦ subset of patients. N.D. not done,

Table 2: Summary of studies reporting on target attainment in ≥ 90% of the population at MICs at various dosages

Author	n	Targets	MIC*	250mg qd	250mg bid	500mg qd	500mg bid	750mg qd	750mg bid	1000mg qd
Deshpande[25]	10000#	Pulm T>MIC 20%	8^	0	0	0	2	16	32	-
		TBM T>MIC 20%		8	8	16	16	16	32	
		Pulm T>MIC 30%		0	0	0	2	8	32	
		TBM T>MIC 30%		4	8	8	16	16	32	
		Pulm T>MIC 64%		0	0	0	0	2	8	
		TBM T>MIC 64%		0	4	2	8	2	8	
Alghamdi[8]	1000#	T>MIC 30%	[15,25]	4	8	8	16	16	32	-
		T>MIC 64%		4	8	8	16	8	32	-
Chang[9]	1000#	Cmax 20-35mg/L	N.A.	-	35.8%	49.5%	30.9%	37.1%	-	18.3%
Chirehwa[10]	1000#	T>MIC 30%	2 – 1%^	-	-	16	-	16	-	16
		T>MIC 64%	4 – 8%^	-	-	8	-	8	-	8
		T>MIC 100%	16 – 54%^	-	-	2	-	2	-	2
			32 – 17%^							
Kumar[12]	25	Cmax 20-35mg/L	N.A.		44% at 14.3	(10.0–18.0)	mg/kg			
van der Galien[14]	1000#	T>MIC 30%	up to 32	-	-	-	-	8	-	-

Yu[15]	80	Cmax 20-35mg/L Cmax≥1	<32 or ≥32~	-	-	66% N.R.	-	43% N.R.	-	-
Zhang[23]	28	Cmax 20-35mg/L		-	-	25%	-	-	-	-
Zhu[17]	1000#	T>MIC 30% AUC/MIC>36	Median 4 MIC99 8&	-	-	-	32	16 250mg+500mg	32	-

simulated patients, * MIC if measured in the study, - test not performed, N.A. not applicable, ^ Sensititre MycoTB plate test range 2-32mg/L; sensitive ≤16mgL, ~ microplate alamarBlue assay, N.R. not reported, & Bactec MGIT 960 system

About the authors

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Dr Hannah Yejin Kim is a postdoctoral researcher and a hospital pharmacist. Her research focuses on optimising dosing and drug monitoring strategies for antimicrobial drugs.

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