Old Drugs, New Purpose: Retooling Existing Drugs for Optimized Treatment of Resistant Tuberculosis

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Treatment of drug-resistant tuberculosis is hindered by the high toxicity and poor efficacy of second-line drugs. New compounds must be used together with existing drugs, yet clinical trials to optimize combinations of drugs for drug-resistant tuberculosis are lacking. We conducted an extensive review of existing *in vitro*, animal, and clinical studies involving World Health Organization–defined group 1, 2, and 4 drugs used in drug-resistant tuberculosis regimens to inform clinical trials and identify critical research questions. Results suggest that optimizing the dosing of pyrazinamide, the injectables, and isoniazid for drug-resistant tuberculosis is a high priority. Additional pharmacokinetic, pharmacodynamic, and toxicodynamic studies are needed for pyrazinamide and ethionamide. Clinical trials of the comparative efficacy and appropriate treatment duration of injectables are recommended. For isoniazid, rapid genotypic tests for *Mycobacterium tuberculosis* mutations should be nested in clinical trials. Further research focusing on optimization of dose and duration of drugs with activity against drug-resistant tuberculosis is paramount.

The World Health Organization (WHO) estimates that 650 000 cases of multidrug-resistant (MDR) tuberculosis occurred in 2010. Extensively drug-resistant (XDR) tuberculosis has been found in every country with the means to test for it [1]. To improve treatment of drug-resistant tuberculosis with existing drugs and identify optimized background regimens for trials with new compounds, the Drug Efficacy Subgroup of RESIST-TB (Research Excellence to Stop TB Resistance; www.resisttb.org) reviewed the existing literature on second-line tuberculosis drugs to ascertain the contribution of individual agents to drug-resistant tuberculosis treatment. This review summarizes the preclinical and clinical evidence and gaps in knowledge for antituberculosis agents classified by the WHO as groups 1, 2, and 4. Groups 3 (fluoroquinolones) and 5 (agents of uncertain efficacy) are reviewed separately. Priority ranking of these drugs for future study—and identification of key research questions—has not previously been undertaken.

SEARCH STRATEGY AND SELECTION CRITERIA

In vitro studies were included if they used *Mycobacterium tuberculosis* laboratory or clinical strains and

reported measures of antituberculosis activity. Studies involving animals describing drug efficacy against *M. tuberculosis* infection were included. Clinical studies were included if they had relevant pharmacokinetic (PK), safety, bacteriologic, or clinical end points.

A search strategy using "tuberculosis" and the drug being evaluated as MeSH terms between January 2008 and September 2011 was employed in PubMed and Embase. Articles from 1940–2011 were reviewed if they were in English or French. References at the end of articles and relevant textbook chapters were searched by hand.

GROUP 1: FIRST-LINE ORAL DRUGS WITH POTENTIAL UTILITY AGAINST DRUG-RESISTANT TUBERCULOSIS

Pyrazinamide
Pyrazinamide (PZA) is a prodrug activated by *pncA* whose mechanism of action is still being elucidated (Table 1). PZA requires acidic conditions to exert antituberculosis activity. Resistance to PZA is conferred by mutations in *pncA* or *rpsA* [2]. The absence of dominant mutations in the *pncA* gene represents a substantial limitation for rapid molecular testing.

Preclinical Evaluations
PZA activity is correlated with the ratio of the area under the time-concentration curve (AUC) to the minimum inhibitory concentration (MIC) in preclinical models [3]. Doubling the human-equivalent PZA dose increases bactericidal and sterilizing effects in mice and guinea pigs [3]. Importantly, PZA has synergistic effects when given together with investigational tuberculosis drugs.

Clinical Studies
PZA has treatment-shortening effects in regimens containing isoniazid (INH) with or without rifampin [4]. Early bactericidal activity (EBA) trials with PZA demonstrated minimal EBA from days 0–2 (EBA_{0-2}, marker of early bacterial killing) and modest EBA from days 2–14 (EBA_{2-14} is a proposed surrogate of sterilizing activity), but enhanced EBA_{0-14} of several investigational drugs [5]. Although the contribution of PZA to rifampin-containing regimens is limited to the first 2 months of treatment, its optimal duration in other regimens has not been evaluated. Though PZA is likely to improve drug-resistant tuberculosis treatment outcomes against susceptible strains, use of PZA for drug-resistant tuberculosis is complicated by (1) the high incidence of PZA resistance among drug-resistant tuberculosis strains, (2) challenges in performing drug susceptibility testing, and (3) a poor understanding of clinical implications of PZA resistance [6].

Research Priorities
PZA has potent sterilizing activity. Critical areas for future research include determining patterns and frequency of PZA resistance among drug-resistant tuberculosis cases, evaluating the clinical significance of PZA resistance, development of rapid diagnostics to detect resistance, exploration of the risks and benefits of higher doses, including hepatotoxicity with modest dose increases, and establishment of the optimal duration of PZA use for drug-resistant tuberculosis.

Ethambutol
For drug-sensitive tuberculosis, the role of ethambutol (EMB) is to protect companion drugs against resistance. However, EMB resistance among patients with drug-resistant tuberculosis reaches 50%–60%. Mutations in the *embB* gene confer a 2–8-fold increase in MIC [7]. Genotypic testing for EMB resistance is 57% sensitive and 92% specific [8].

Preclinical Evaluations
In mice, the minimal effective dose is 100 mg/kg/d, which produces an AUC equivalent to 15 mg/kg/d in humans. Higher doses are required for bactericidal activity. Although 100 mg/kg prevents emergence of resistance to companion drugs, protection is not complete [9].

Clinical Studies
Doses <15 mg/kg cannot prevent emergence of resistance to companion drugs, so 15 mg/kg/d probably represents the clinical minimal effective dose [10]. In combination with INH, EMB produces superior 6-month culture conversion rates at 25 vs 12.5 mg/kg. EMB at a daily dosage of 25 mg/kg plus a good sterilizing agent is highly active [11]. However, EMB-related optic neuritis is dose and duration dependent and may be irreversible. Incidence is 5% with the 25 mg/kg dose and <1% with the 15 mg/kg/d dose but may be decreased by thrice-weekly dosing [12].

Research Priorities
The principal role of EMB is to prevent resistance to companion drugs. This property should extend to treatment of drug-resistant tuberculosis caused by EMB–susceptible isolates. Higher daily doses (eg, 25 mg/kg) are probably more active but increase the risk of ocular toxicity. Research priorities include determination of frequency of EMB resistance among patients with drug-resistant tuberculosis and the safety and efficacy of intermittent high-dose EMB.

High-Dose Isoniazid
Isoniazid (INH) is a prodrug activated by M. tuberculosis' catalase-peroxidase enzyme KatG. The activated drug binds InhA, an enoyl-acyl carrier protein reductase enzyme, inhibiting fatty acid synthesis. Partial loss of KatG function

Table 1. Summary Information About World Health Organization Class 1, 2, and 4 Drugs for Tuberculosis

Drug	Target/Mechanism of Action	MIC	Pharmacodynamic Parameter Associated with Efficacy	Clinical Dose for Tuberculosis	Equivalent Dose in Animal Model
Pyrazinamide	After activation by PncA, pyrazinoic acid accumulates intracellularly, acidifying *Mycobacterium tuberculosis* and binding to RpsA to inhibit trans-translation, It may also inhibit ATP synthesis	MIC is highly variable, because it is pH dependent	AUC/MIC correlates with bactericidal activity; Time above MIC correlates with suppression of resistance	25–30 mg/kg/d, with adjustments for renal failure	150 mg/kg in mice, 300 mg/kg in guinea pigs [3]
Ethambutol	Targets arabinosyl transferase enzymes involved in biosynthesis of 2 key cell wall components: arabinogalactan and lipoarabinomannan	Wild type: 1–4 µg/mL	AUC/MIC correlates with bactericidal activity; Time above MIC correlates with suppression of resistance	15 mg/kg/d for drug-sensitive tuberculosis; 25 mg/kg/d daily for drug-resistant tuberculosis	100–150 mg/kg in mice
Isoniazid	After activation by KatG, active moiety forms adduct with nicotinamide adenine dinucleotide and InhA, inhibiting mycolic acid synthesis; other potential mechanisms of action have been described	Wild type: 0.03–0.12 µg/mL; Typical *inhA* mutations: 0.25–0.5 µg/mL; Typical *katG* mutations: 2–≥16 µg/mL	AUC/MIC is associated with bactericidal activity	5 mg/kg/d for drug-sensitive tuberculosis; Higher doses (up to 16–18 mg/kg) used experimentally for drug-resistant tuberculosis	10–25 mg/kg in mice
Rifabutin	Inhibits transcription by binding to RpoB, the β subunit of bacterial DNA–dependent RNA polymerase	Wild type: <0.06 µg/mL; Rifampin-resistant: 0.125–≥16 µg/mL	Presumably, AUC/MIC correlates with bactericidal activity, and C_{max}/MIC is associated with resistance suppression, as for rifampin	300 mg/d	5 mg/kg in mice
Kanamycin and amikacin	Inhibits protein synthesis by binding to the 16S ribosomal RNA encoded by *rrs*	Wild type: 0.5–4 and 0.25–1 µg/mL for KM and AMK, respectively	Unknown, but C_{max}/MIC is best predictor of aminoglycoside activity against other bacteria	15 mg/kg daily or thrice-weekly	Although KM and AMK doses ≥100 mg/kg are commonly used in mice, pharmacokinetic studies with AMK in mice suggest that daily doses of 20–45 mg/kg produce more human-equivalent exposures
Capreomycin	Exact mechanism of action unclear, but presumably inhibits protein synthesis by binding to the 16S ribosomal RNA encoded by *rrs*	Wild type: 1–4 µg/mL	Unknown	1 g/d	Not well established
Ethionamide and prothionamide	After activation by EthA, active moiety inhibits InhA and mycolic acid synthesis (like isoniazid)	MIC varies depending on medium used; MICs 0.3–1.2 µg/mL in 7H12 broth, 2.5–10 µg/mL in 7H10 broth	Unknown	15–20 mg/kg/d, usually in divided doses	Unknown
Para-aminosalicylic acid	Not fully elucidated, but PAS may interfere with iron uptake and/or folate biosynthesis [33]	Wild type: 0.3–1.0 µg/mL	Unknown	150 mg/kg/d, in 2–4 divided doses	Unknown
Cycloserine and terizidone	As analogs of D-alanine, CS and TZ inhibit alanine racemase and D-alanine:D-alanine ligase, thus blocking cell wall peptidoglycan synthesis	Wild type: 8–32 µg/mL	Unknown	CS: 500–750 mg/d, in single or divided doses	Unknown

Abbreviations: AMK, amikacin; ATP, adenosine triphosphate; AUC, area under the time-concentration curve; C_{max}, maximum concentration; CS, cycloserine; KM, kanamycin; MIC, minimum inhibitory concentration; PAS, para-aminosalicylic acid; TZ, terizidone.

Table 2. Summary of Research Questions to Inform Optimization of Drug-Resistant Tuberculosis Treatment Regimens

Research Questions by Theme	Suggested Studies	Relevant Drugs
Translation of Preclinical Findings to Clinical Settings		
What are human equivalent doses for studies in animal models?	PK/PD studies in animals to define the PD parameter most closely correlated with activity	AMK, KM, CM, ETA, PAS
Use of PK/PD Relationships to Optimize Dosing		
Which PK/PD targets correlate best with efficacy and prevention of resistance?	PK/PD studies in *in vitro* and animal models	PZA, EMB, INH, RBT, ETA, PAS, CS/TZ
Can target attainment be further optimized?	Dose-ranging human efficacy trials with PK/PD component	
Can toxicity be reduced through changes in dosing, while preserving efficacy?	Animal and human PK/PD and toxicodynamic studies	PZA, EMB, PAS, CS/TZ, ETA
	Human efficacy and tolerability studies using toxicodynamically optimized doses	
Can toxicity be reduced through changes in formulation and/or delivery?	Animal and human PK/PD and toxicity/tolerability studies of new formulations, studies of novel delivery systems for existing drugs	AMK, KM, CM, ETA, PAS, CS/TZ
Enhanced Use of Genotypic and Phenotypic Testing to Inform Drug Choices		
Can rapid resistance tests identify patients with drug-resistant tuberculosis who are likely to benefit from a drug that ordinarily would not be used?	Correlation of rapid genotypic resistance test results with minimum inhibitory concentrations	INH, RBT, ETA
	Human genotyping to identify mutations likely to affect drug absorption or clearance in human PK and efficacy trials	
What is the correlation between phenotypic resistance and clinical treatment outcomes? Can this be further refined with genotypic testing?	Randomized trial of drug in question in patients whose isolates have documented phenotypic resistance, or collection of phenotypic and genotypic data of the drug in question among patients enrolled in trials of other compounds	PZA, CS/TZ
Designing an Optimized Background Regimen of Existing Drugs		
What is the activity of this drug in humans?	Extended (14-d) EBA study alone and with other drugs	CM
What is the most efficacious injectable?	Randomized trial of an optimized background regimen with substitution of injectable agents	KM, AMK, CM
What is the optimal duration of treatment with a given drug?	Randomized trial comparing different drug durations within a multidrug regimen	PZA, AMK, KM, CM

Abbreviations: AMK, amikacin; CM, capreomycin; CS/TZ, cycloserine and terizidone; EBA, early bactericidal activity; EMB, ethambutol; ETA, ethionamide; INH, isoniazid; KM, kanamycin; PAS, para-aminosalicylic acid; PD, pharmacodynamic; PK, pharmacokinetic; PZA, pyrazinamide; RBT, rifabutin.

typically results in INH MICs of 2–8 µg/mL, while complete loss results in MICs ≥ 16 µg/mL. Mutations in *inhA* and its promoter cause low-level INH resistance and cross-resistance with ethionamide [13]. Data on the distribution of INH MICs of clinical drug-resistant tuberculosis isolates are sparse, but up to 43%–75% of drug-resistant tuberculosis strains may remain susceptible to INH at clinically achievable concentrations.

Preclinical Evaluations

Maximal INH activity in vitro and in mice is achieved at an AUC/MIC ratio value of 100–200. Still, an INH dose of 10 mg/kg/d exhibited bactericidal activity against an *inhA* promoter mutant, and 25 mg/kg/d had bactericidal activity against a *katG* mutant (AUC/MIC of approximately 40 and 15, respectively) [14].

Clinical Studies

In humans, INH exposures are variable and determined by N-acetyltransferase (NAT2) genotype [15]. Against INH-susceptible strains, INH AUC >10.5 µg × h/mL (AUC/MIC > 100) occurs in slow acetylators receiving 3 mg/kg and rapid acetylators receiving 6 mg/kg/d and is associated with near-maximal EBA. However, considerable bactericidal activity is achieved with doses as low as 1.25 mg/kg [16]. To achieve such bactericidal activity against strains with low-level

Table 3. Priority Ranking of Drugs for Additional Research on Optimization for Drug-Resistant Tuberculosis Treatment

Priority Ranking	Drug	Reasons for Continued Research on Use in Regimens for Drug-Resistant Tuberculosis	Barriers to Optimization for Drug-Resistant Tuberculosis Treatment
High	Pyrazinamide	Sterilizing activity in first-line regimens, so may shorten drug-resistant tuberculosis treatment duration Synergistic effects with new drugs in clinical development	Resistance may be common in multidrug-resistant tuberculosis strains Phenotypic resistance testing problematic Multiple different mutations can confer resistance, impeding development of rapid genotypic resistance test
	Isoniazid	Cheap, well tolerated, and widely available Low-level resistance may be overcome with higher doses Rapid genotypic resistance test may predict which patients with drug-resistant tuberculosis may benefit from higher doses	MIC distribution among resistant strains unknown Correlation between genotypic resistance test result and MIC not established Interpatient variability in PK and acetylation complicates dose selection
	Amikacin, kanamycin, capreomycin	Susceptibility to injectables confers better outcomes in drug-resistant tuberculosis Relative efficacy of injectable drugs is unknown Optimal treatment duration of injectable use is unknown Parenteral use requirement makes this group a target for replacement as new oral drugs are developed	Amikacin not widely available and expensive Poor early bactericidal activity prevents using this method to compare efficacy Large sample sizes needed to study comparative efficacy and treatment duration
Medium	Ethionamide and prothionamide	Only second-line oral drug with potential bactericidal activity Relationship between drug exposure and GI tolerability unknown	Use of isoniazid in initial tuberculosis treatment may select for isoniazid and ethionamide cross-resistance Ability of rapid genotypic tests to predict susceptibility requires further study
	Ethambutol	Better tolerated than many second-line drugs Relationship between drug exposure and ocular toxicity unknown Animal models suggest that neuroprotective agents may prevent optic neuritis, allowing for higher ethambutol dosing	Resistance may be common in drug-resistant tuberculosis strains given the use of ethambutol in first-line tuberculosis treatment Concerns over ocular toxicity may limit use doses that optimize efficacy
Low	Para-aminosalicylic acid	Minimum drug exposure necessary for bacteriostatic effect unknown Lower doses may have similar activity, better tolerability than currently recommended dose	Poor GI tolerability and risk of hypersensitivity
	Cycloserine and terizidone	Minimum drug exposure necessary for bacteriostatic effect unknown Lower doses may have similar activity, better tolerability than currently recommended dose	Not amenable to animal studies given marked interspecies PK differences Serious central nervous system side effects
	Rifabutin	Rifamycins are sterilizing drugs Some drug-resistant tuberculosis strains may retain susceptibility to rifabutin Rapid genotypic resistance tests may predict rifabutin efficacy	Most drug-resistant tuberculosis strains are rifabutin resistant Even drug-resistant tuberculosis strains considered rifabutin susceptible have reduced rifabutin susceptibility compared with wild-type strains Current genotypic resistance tests may not identify discordant susceptibility to rifampin and rifabutin

Abbreviations: GI, gastrointestinal; MIC, minimum inhibitory concentration; PK, pharmacokinetic.

resistance (MIC ≤ 0.5 μg/mL), a dose of ≥ 600 mg is probably needed. Higher INH doses may yield bactericidal activity against strains with MICs of 1 or 2 μg/mL, depending on acetylator status. Addition of high-dose INH (16–18 mg/kg) but not standard-dose INH (5 mg/kg) to drug-resistant tuberculosis treatment increased sputum culture conversion rates in a recent trial [17]. Peripheral neuropathy was more common with high-dose INH, but pyridoxine was not given.

Summary and Areas of Research Interest

High-dose INH may be useful in the treatment of drug-resistant tuberculosis, but efficacy will depend on INH dose, patient acetylator status, and degree of INH resistance. In patients infected with *M. tuberculosis* with isolated *inhA* mutations, high-dose INH should be more effective than ETA. Additional studies are needed to determine the INH MIC distribution among drug-resistant tuberculosis isolates and the ability of rapid genotypic resistance testing to

predict INH MIC. EBA studies can confirm the relationship between INH AUC/MIC and effect and enable optimal dosing recommendations based on genotypic resistance assays. A rapid means of determining acetylator status would be helpful to customize dose selection. The safety of high-dose INH requires investigation.

Rifabutin

Rifabutin (RBT) is a rifamycin antibiotic with more potent *in vitro* activity than rifampin. Although 12%–36% of rifampin-resistant clinical isolates reportedly remain susceptible to RBT, the breakpoint defining susceptibility is not evidence based. In fact, RBT MICs against rifampin-resistant strains are virtually always higher than the wild-type distribution, indicating that RBT is unlikely to be fully active against rifampin-resistant isolates. Although new line probe assays can identify specific *rpoB* mutations that do not shift the RBT MIC as much as the rifampin MIC, it is unclear whether such assays would predict the efficacy of RBT [18].

Preclinical Evaluations

In mice, RBT has dose-dependent bactericidal activity. At 10 mg/kg/d, RBT monotherapy reduces lung and spleen CFU counts by 3–5 log in mice infected with drug-sensitive *M. tuberculosis,* but the exposures observed with this dose may not be tolerable in humans, and efficacy against rifampin-resistant strains has not been assessed [19].

Clinical Studies

RBT is widely distributed in tissues, has poor bioavailability, and autoinduces its metabolism. At 600 mg/d (twice the usual clinical dose), RBT has a much lower EBA_{0-2} than rifampin [20]. Trials comparing rifampin 600 and RBT 300 mg/d (together with standard tuberculosis drugs) against drug-sensitive tuberculosis, found similar efficacy and tolerability [21]. Owing to cross-resistance, RBT is rarely used for drug-resistant tuberculosis, but RBT has been part of successful regimens to treat XDR tuberculosis.

Research Priorities

RBT may retain some activity against a small proportion of rifampin-resistant drug-resistant tuberculosis strains. However, more rigorous study of the impact of specific *rpoB* mutations on RBT susceptibility in light of achievable RBT exposures is needed to gauge the accuracy of rapid *rpoB* genotyping and identify the minority of patients who may benefit from RBT.

GROUP 2: INJECTABLE SECOND-LINE AGENTS

Aminoglycosides (Kanamycin and Amikacin)

Kanamycin (KM), its semisynthetic derivative amikacin (AMK), or capreomycin (see below) is given by injection for ≥6 months of drug-resistant tuberculosis treatment. KM and AMK inhibit ribosomal protein synthesis. Both have poor activity against slowly multiplying mycobacteria. Mutations in *rrs* confer high-level resistance to both agents, although some KM-resistant strains retain susceptibility to AMK. The Hain® GenoType MTBDR*sl* assay is sensitive and specific for detecting KM/AMK resistance.

Preclinical Evaluations

AMK is more potent than KM *in vitro* and in mice. Whereas weak bactericidal activity is observed with human-equivalent doses of AMK, similar KM doses are bacteriostatic [22].

Clinical Studies

As monotherapy, AMK at doses of 5–15 mg/kg/d has minimal EBA and no dose-response effect [23]. No clinical trial has evaluated the contribution of KM or AMK to drug-resistant tuberculosis regimens. However, findings in cohort studies suggest that patients with multidrug-resistant tuberculosis and resistance to injectables have lower treatment success than patients with preserved susceptibility to injectables [24, 25]. KM and AMK cause nephrotoxicity, vestibulotoxicity, and ototoxicity; the latter 2 are related to cumulative dose and commonly irreversible [26].

Research Priorities

KM is the most often-used injectable agent for drug-resistant tuberculosis. AMK is more potent but more expensive, and the mouse-to-human PK/PD correlates are unknown. Both drugs have significant, potentially irreversible, toxicities related to cumulative dose. Despite its modest bactericidal activity in mice, AMK exhibits little to no EBA in patients. However, improved outcomes in patients with drug-resistant tuberculosis with preserved susceptibility to injectables compel their use, despite medical and logistical disadvantages. Understanding the comparative efficacy of KM and AMK, their specific contribution to multidrug therapy, and the optimal duration of treatment will require preclinical and clinical trials.

Capreomycin

Capreomycin (CM) is a polypeptide antibiotic that inhibits protein synthesis. Mutations in the mycobacterial *tlyA* gene confer CM resistance, and mutations in its *rrs* gene may confer cross-resistance to aminoglycosides and CM.

Preclinical Evaluations

In one *in vitro* experiment, CM was the only drug tested with significant activity against hypoxic, nonreplicating *M. tuberculosis.* However, its activity against persistent *M. tuberculosis* in animals has not been evaluated [27]. In mice, CM has bacteriostatic activity and a narrow therapeutic margin. Daily doses of 120–150 mg/kg have weak growth inhibitory effects [28].

CM prevents emergence of resistance to INH at 300 mg/kg/d, but such doses produce renal tubular necrosis in mice.

Clinical Studies
Current dose and treatment duration for CM are largely based on case series from the 1960s [29]. Among previously treated patients, CM at a dosage of 1 g/d plus 2 additional drugs was successful in 50%–85%. CM for 120 days was better than treatment for 60 days when CM was given daily with PAS; [29] treatment beyond 6 months, though, provided no further benefit. CM use is limited by reversible, dose-dependent renal toxicity (1%–2% of patients) and potentially irreversible ototoxicity, which is related to cumulative exposure (2%–3%).

Research Priorities
In mice, CM is bacteriostatic and less effective than aminoglycosides. Despite the intriguing observation of bactericidal activity against nonreplicating *M. tuberculosis in vitro*, this finding has not been demonstrated in vivo. The most common mycobacterial resistance mutations occurring with CM use do not confer cross-resistance to KM or AMK, but the converse may not be true. Whether use of CM as the initial injectable agent affords a treatment advantage by keeping other options open is unclear, because CM is less potent than KM or AMK in preclinical studies, and there are no comparative clinical trials. The optimal dose and duration of treatment are unknown.

GROUP 4: ORAL ANTITUBERCULOSIS DRUGS USED IN SECOND-LINE REGIMENS

Ethionamide and Prothionamide
Ethionamide (ETA) and prothionamide are prodrugs requiring enzymatic activation by *M. tuberculosis* EthA to inhibit InhA, a target shared with INH. Mutations in mycobacterial *inhA* or its promoter confer reduced susceptibility to INH and ETA; mutations in *ethA* cause ETA and prothionamide monoresistance [13, 30].

Preclinical Evaluations
In mice, doses ≥25 mg/kg are bactericidal. Doses as low as 12.5 mg/kg prevent selection of drug-resistant mutants by INH [30]. There are no data confirming pharmacodynamic (PD) targets or human-equivalent mouse doses.

Clinical Studies
ETA was originally evaluated as an agent to prevent resistance to INH or treat INH-resistant disease [31]. Interest in ETA waned when better-tolerated alternatives became available. Gastrointestinal intolerance is the Achilles' heel of ETA and mandates gradual dose increases to the highest tolerable dose.

Strategies to improve tolerability by dividing doses may negatively affect efficacy by preventing serum concentrations from exceeding MIC [32]. Hypothyroidism is a significant clinical concern.

Summary and Areas of Research Interest
Among group 4 agents, only ETA has bactericidal activity against *M. tuberculosis*. However, attainment of bactericidal drug exposures in patients is limited by poor tolerability. Information regarding the drug exposures needed to produce bactericidal effects and the potential for achieving such exposures in patients with drug-resistant tuberculosis remains scarce. PK/PD studies in *in vitro* and animal models could help determine PD targets and establish the human-equivalent dose in mice. In addition, better understanding of human population PK and toxicodynamics could identify the minimal effective dose of ETA. The utility of rapid genotypic resistance testing to identify patients who are unlikely to benefit from ETA should be studied.

Para-aminosalicylic Acid
Highly specific for *M. tuberculosis*, para-aminosalicylic acid (PAS) is a bacteriostatic agent. Resistance to PAS is associated with mutations of mycobacterial *thyA*, but this mechanism accounts for only 6% of phenotypic resistance [33].

Preclinical Evaluations
Early guinea pig studies demonstrated that daily treatment was more effective than intermittent therapy, but the human-equivalent dose in animals and the PK/PD correlates of PAS activity are unknown [34].

Clinical Studies
New delayed-release formulations produce higher PAS exposures, overcoming the rapid metabolism and clearance of early formulations [35]. Although PAS has not been evaluated in EBA studies, clinical trials showed that monotherapy at a dosage of 10 g/d for 3 months produced clinical improvement and had efficacy similar to that of streptomycin [36]. Case reports describe effective drug-resistant tuberculosis treatment with PAS-containing regimens. The use of PAS is limited by its toxicities—gastrointestinal irritation, myxedema, hypokalemia, life-threatening hypersensitivity reactions, and B12 deficiency.

Research Priorities
PAS is a poorly tolerated, bacteriostatic agent most useful for preventing emergence of resistance to companion drugs. As with other oral second-line agents, little is known about PK/PD correlates of PAS activity. In vitro and animal model studies to define the lowest exposure necessary for maximal (presumably bacteriostatic) effect, in concert with human PK

studies, could identify lower, more tolerable PAS doses that retain similar efficacy.

Cycloserine and Terizidone

Cycloserine (CS) and terizidone have broad-spectrum antimicrobial activity. As an N-methyl-D-aspartate receptor partial agonist, CS competes with γ-aminobutyric acid in the brain, causing central nervous system (CNS) side effects. Overexpression of mycobacterial *alr*, which encodes the primary target, confers CS resistance. Phenotypic susceptibility testing is notoriously difficult to perform, and little correlation exists between treatment outcomes and *in vitro* findings [37].

Preclinical Evaluations

It is difficult to demonstrate the efficacy of CS in animal models. This is partly due to PK differences. The half-life of CS is 12 hours in humans and 23 minutes in mice [38]. Furthermore, greater circulating concentrations of D-alanine in mice may antagonize CS activity. Therefore, the human-equivalent dose of CS remains a mystery. In mice and guinea pigs, daily doses of 150–300 mg/kg have little or no therapeutic effect [39].

Clinical Studies

CS was studied in the 1950s in complicated or resistant tuberculosis cases. CS monotherapy (1–1.5 g in 4 divided doses) produced rapid clinical improvement, and in one study cultures were converted to negative in one-third of patients with new or chronic tuberculosis. When CS was combined with INH, clinical improvements were seen, but INH resistance emerged [40]. CS and ETA promoted culture conversion in a majority of patients in whom other regimens failed [31]. To date, the individual contribution of CS in drug-resistant tuberculosis regimens has not been evaluated. CNS side effects are common, serious, and dose related, severely limiting the use of CS.

Research Priorities

CS is a bacteriostatic agent with clinically proven antituberculosis efficacy. Its use is hindered by serious CNS toxicity. Although animal models are not informative, *in vitro* models could be used to define the PK/PD correlates of activity and optimize drug exposures necessary for bacteriostatic effects.

CONCLUSIONS

Programmatic management of drug-resistant tuberculosis is complex. Increased toxicity and reduced potency or accessibility of second-line drugs result in poor outcomes. The evidence base to guide doses and combinations of these drugs for treatment is limited and of low quality [41]. Heeding a call for research into "the most effective use of existing second-line antituberculosis therapies and other antimicrobials available to treat drug-resistant TB" [42–44], this review identifies and prioritizes research opportunities to optimize the use of WHO-defined group 1, 2, and 4 drugs in the treatment of drug-resistant tuberculosis (Table 2). Important gaps in understanding the pharmacokinetics and pharmacodynamics of these agents prevent confident recommendations regarding dosing and duration needed to maximize efficacy with acceptable safety and tolerability. Focused research using preclinical models and small proof-of-concept trials may be sufficient to guide dose optimization. Questions regarding appropriate duration require longer studies with efficient adaptive designs. Better understanding of the clinical implications of resistance testing (phenotypic and genotypic) is also critical.

Such research efforts are essential to complement observational studies, eg, of the "Bangladesh" regimen, [45] which are not designed to evaluate individual contributions of drugs. The 3 high-priority drug groups reviewed were used in the Bangladesh regimen and are part of short-course regimens being explored in clinical trials.

New drug candidates in clinical development call into question the need for research to optimize use of existing second-line drugs. Existing drugs, however, will be used in combination with new agents for the foreseeable future, making it imperative to optimize their use to protect new drugs (Table 3). In addition, evolution of resistance will inevitably follow the introduction of new drugs, making it unlikely that existing agents will be removed from clinical use. Finally, studies that result in regulatory approval of new agents may not inform their best use. They may not be studied in combination with other new agents, with truly optimized background regimens, or for adequate durations. Additional trials will be necessary to define their optimal use. The same lessons highlighted in this review need to be applied to new drugs; such trials will provide opportunities to address key research priorities established here. Improved quality of evidence, through dedicated preclinical and clinical research, is essential to achieve the goal of shorter, more effective, and less toxic regimens for drug-resistant tuberculosis.

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*For a full list of relevant references for the drugs reviewed in this manuscript, the reader is referred to the RESIST-TB website, www.resisttb.org.