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Rising to the challenge: new therapies for tuberculosis

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Abstract

Standard treatment for tuberculosis (TB) is lengthy, complex and significantly toxic. Drug development for TB has stagnated for decades; but in recent years renewed commitment and coordinated research has generated a modest pipeline of new drugs that hold the potential to make treatment more effective, shorter, less complex and less toxic in the near future. With a particular focus on bedaquiline (TMC207), the first antituberculosis drug of a novel class to be FDA-approved in 40 years, this review summarizes the recent evidence behind new developments in tuberculosis treatment. Novel drug classes, repurposed drugs and host-directed therapies are reviewed. In parallel to these exciting developments in drug-discovery, we propose that it is crucial to develop more rapid and comprehensive diagnostics that will allow for the timely selection of the best regimen for individual patients.

Keywords

Tuberculosis; Bedaquiline; PA-824; Delamanid; Clofazimine; Oxazolidinones

Treatment of tuberculosis: grounded in discoveries of the mid-20th century

Mycobacterium tuberculosis (*Mtb*) infects a third of the world's population, and while many who harbor the bacteria never become ill, in its active state tuberculosis (TB) is a lethal disease. In 2011, TB was responsible for 1.4 million deaths and remains the leading cause of death among people living with HIV [1–4].

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Conflict of Interest

The authors declare no conflict of interest

Ethical Approval

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The current treatment for drug-susceptible TB requires four drugs used in combination for two months, followed by two drugs used in combination for four months (Table 1). If taken correctly, this regimen is often highly effective; but it is also lengthy, complex and significantly toxic. These problems with standard antitubercular therapy have amplified drug resistance, which has emerged in the past 20 years as a deadly problem.[5–8] Aside from the diagnostic challenge of drug-resistant TB, the currently available treatment regimens are more lengthy, more complex, more toxic and less efficacious than standard treatment.

TB drug discovery flourished in the mid-20th century; all of today's first-line drugs were discovered between 1952 (isoniazid) and 1963 (rifampin, Figure 1). On the final day of 2012, however, after over 40 years, the United States Food and Drug Administration (FDA) approved the first new antitubercular drug that represents a novel antibiotic class. In the face of this major development and with additional drugs and regimens moving along the investigative and regulatory pipeline, the time is right to review the recent developments in the field of TB treatment.

In this review we present three of the new drugs that have been recently developed for TB (bedaquiline, PA-824, delamanid), outline new uses for clinically available drugs (linezolid, clofazimine, β -lactams), and discuss the potential utility of host-directed therapies for TB. Finally, we focus on the critical importance of developing novel diagnostics alongside the new drugs to rapidly determine drug-susceptibility patterns of new and old drugs. This approach will allow for the timely selection of the best combination of drugs to maximize treatment successes and minimize the development of drug resistance.

The pipeline begins to deliver: drugs with novel targets

Three exciting new drugs have moved beyond pre-clinical study and have shown promising results in clinical trials in the past year (Figure 2). Please see Box 1 for a description of terms that are commonly used in clinical studies of tuberculosis. The novel drug pipeline has been made possible by over a decade of renewed commitment by stakeholders and by the development of a number of innovative strategies. The 21st century has seen coordinated efforts on TB drug development that has included the cooperation of non-profit organizations, including the Global Alliance for TB Drug Development (TB Alliance), international funders, researchers, clinical trial networks and regulatory agencies. Unprecedented institutional commitments include funding by the Bill and Melinda Gates Foundation and the National Institutes of Health (NIH), coordination of Phase 2 combination trials through the Critical Path to TB Drug Regimens, and accelerated provisional licensing by the US Food and Drug Administration for multidrug-resistant TB (MDR-TB; see Glossary) drugs that fill an unmet need.[9]

Box 1

Definition of commonly used terms in clinical studies of tuberculosis

Definitions for clinical trials

Phase 1: small trials (20–80 healthy volunteers) designed to assess safety of a new drug; additional goals are to determine a safe dosing range and side effects.

Phase 2: medium sized trials (100–300 subjects with disease) designed to assess efficacy of the new drug and to further assess safety.

Phase 3: larger trials (1000–3000 subjects with disease) designed to gather additional information on efficacy and safety in different populations and in combination with different drugs.

Phase 2 and Phase 3 trials may be **controlled** (compared to another treatment), **placebo-controlled** (compared to an inactive substance) or **uncontrolled** (no comparison group). The different interventions in each study arm may be **blinded** (subjects *or* researchers are unaware of the treatment being received by individual subjects), **double-blinded** (subjects **and** researchers are unaware of the treatment being received by individual subjects) or **open-label** (no blinding). Trials may be designed to show **superiority** (better results) or **non-inferiority** (equivalent results) compared to standard treatment.

Common end-points for TB clinical trials

Sputum smear conversion (SSC): proportion of patients who are acid-fast smear negative given time on therapy.

Sputum culture conversion (SCC): proportion of patients whose cultures have converted to negative at a given time on therapy.

Time to positivity (TTP): alternate quantitative method for determining bacillary load in an EBA study by measuring the time until growth detection of *Mtb* in a liquid culture.

Commonly used short-term TB clinical trial design

Early bactericidal activity (EBA): a trial design that aims to assess the bactericidal activity of novel drugs or regimens by providing these agents alone or in combination during the first 14 days of anti-TB therapy. Serial quantitative assessments of the log₁₀ colony forming units (CFU) per milliliter of sputum are performed to determine the decline from baseline CFU counts as an indicator of bactericidal activity or the drug or regimen.

Serial sputum colony counting (SSCC): a trial design that aims to assess the sterilizing activity of regimens over the initial months of therapy (typically eight-week study duration). Quantitative assessments of the log₁₀ colony counts are performed weekly to biweekly.

Bedaquiline

Bedaquiline (TMC207, Sirturo) is notable for being the first TB drug of a novel class to be approved by the FDA in over 40 years. It belongs to a new class of drugs, the diarylquinolines, and its mechanism of action is inhibition of mycobacterial ATP synthase resulting in decreased ATP levels and pH imbalance in the organism [10–12]. Although ATP synthase is universally expressed in bacteria and eukaryotes, the mycobacterial enzyme has a C shaped moiety in the c subunit that provides a docking site for bedaquiline. The human structure of the c subunit differs at three amino acids resulting in a different

conformation that is thought to sterically inhibit bedaquiline binding; interestingly mutations at these same sites in *Mtb* result in bedaquiline resistance [11, 13].

Bedaquiline is bactericidal against *Mtb*. The drug has an unusually long half-life (longer than 24 hours in humans) and therefore can be loaded initially with a daily dose for two weeks and subsequently dosed thrice weekly, resulting in steady-state plasma concentrations of 0.5ug/mL, well above the MIC for *Mtb* of 0.06ug/mL. It has a long half-life due to deposition and concentration in tissues with mouse data showing 20 times more drug in the lungs than in the plasma [14, 15].

The FDA approval of bedaquiline for the treatment of MDR-TB in adults was expedited under the accelerated approval program and was based on two Phase 2 trials that showed efficacy on surrogate endpoints (eight-week and 24 week culture conversion). To date, peer-reviewed publications have only reported the details of a small subset of the 440 patients whose results were submitted to the FDA. In a 7-day early bactericidal activity (EBA) study of 75 treatment-naïve, drug-susceptible TB patients were randomized to receive either bedaquiline, isoniazid or rifampin monotherapy; bedaquiline failed to show a significant decrease in colony forming units (CFU) counts during the first four days of therapy [15]. Despite this initial lack of efficacy, the subsequent rate of CFU decline of bedaquiline was essentially equivalent to isoniazid or rifampin, thus further investigations of this agent were pursued. In a second published study, 47 MDR-TB patients were randomized to a background regimen (cycloserine, ethionamide, kanamycin, ofloxacin, and pyrazinamide) with or without the addition of bedaquiline and the regimen containing bedaquiline resulted in a significantly higher rate of eight-week culture conversion than the control (48% vs. 9%, $p = 0.003$) [16]. After 24 months of follow-up, the group receiving bedaquiline also accumulated less resistance to other drugs in the background regimen [17]. In data from the FDA press release (but not yet published in a peer-reviewed journal), 440 MDR-TB patients received bedaquiline either in a randomized Phase 2 trial of bedaquiline and background vs. background alone or in a second one-armed Phase 2 trial. The patients receiving bedaquiline in the randomized trial achieved culture conversion faster (average of 83 days) than those receiving the background regimen alone (125 days, <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm>).

While Phase 3 trials are planned for 2013, a number of concerns about bedaquiline have arisen. The FDA has placed a black box warning on bedaquiline cautioning significantly increased rates of unexplained deaths in the bedaquiline group (11.4%, 9 of 79) compared to the placebo group (2.5%, 2 of 81, $p = 0.03$) in the randomized trial (http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000lbl.pdf). The majority of deaths in the bedaquiline group occurred following completion of the bedaquiline-containing regimen, and the FDA and investigators were unable to determine a pattern to explain the increased deaths in this group. Increased rates of QT prolongation were also noted in the bedaquiline groups, but arrhythmia did not explain the deaths. Drug interactions are also a concern given the drug's metabolism by the cytochrome P450 system, which could cause interactions with rifampin as well as certain antiretroviral medications for treatment of concurrent HIV. In healthy volunteers, co-administration of bedaquiline and rifampin resulted in a 52% decrease in bedaquiline exposure prompting the recommendation

that the combination of rifamycins and bedaquiline be avoided. To date, a Phase 1 pharmacokinetic drug study evaluating co-administration with the antiretroviral agent efavirenz showed that there were unlikely to be significant interactions between these two drugs, but interactions with the protease inhibitors have not been investigated [18].

Table 2 summarizes the Phase 2 clinical studies for bedaquiline. Bedaquiline has been tested in humans primarily in MDR-TB where it has been combined with second-line anti-TB drugs. Hence, it is unclear what role, if any, it may have in drug-susceptible active TB or latent TB. Animal studies have shown significant potency of the combination of bedaquiline and pyrazinamide yet it is difficult to know if this effect will carry over to human since many patients with drug-resistant TB may have pyrazinamide-resistant strains [19–21]. In summary, bedaquiline is the first drug of a new class to be approved for tuberculosis in 40 years; however, it has a narrow indication, namely for drug-resistant TB, and concerns remain regarding its safety profile.

The nitroimidazopyrans

PA-824—PA-824 is a nitroimidazo-oxazine and a metronidazole derivative from the nitroimidazopyran class. PA-824 demonstrates bactericidal activity against both replicating and non-replicating mycobacteria [22]. It has been observed to kill by two distinct mechanisms (i) by interfering with the synthesis of ketomycolate – an essential component of the mycobacterial cell wall and (ii) by acting as a nitric oxide donor and causing respiratory poisoning (in a similar mechanism to cyanide) [23]. It is thought that these two mechanisms account for its aerobic activity against replicating bacteria (cell wall effect) and anaerobic activity against non-replicating bacteria (the nitric oxide effect) [24]. PA-824 has *in vitro* activity against both drug-susceptible and otherwise drug-resistant TB but does not have activity against a range of Gram-positive and Gram-negative bacteria [25].

In mice, PA-824 demonstrates time-dependent killing in both the intensive and continuation phases of treatment, decreasing colony forming units (CFU) counts by 0.1 log₁₀ units/day over 24 days [25]. Human EBA studies have identified the dose of 100 to 200mg daily, each with early bactericidal activity of approximately 0.1 log₁₀ units/day/ml of sputum. [26, 27] In contrast to bedaquiline, PA-824 does not have any cytochrome P450 interactions, so it may cause fewer drug interactions.

An exciting role for a PA-824 has been identified in a novel combination that appears to outperform standard treatment in both the murine model and in a human EBA trial. The combination of PA-824, moxifloxacin and pyrazinamide (abbreviated PaMZ) was found in the murine model to have both superior bactericidal and sterilizing activity when compared to the standard four-drug combination (isoniazid, rifampin, ethambutol and pyrazinamide). To assess for sterilizing activity, mice were treated with four months of drug therapy and then monitored for relapse. Encouragingly, compared to the mice that received rifampin, isoniazid, and pyrazinamide, 50% of which later relapsed, the mice treated with PaMZ were all cured after only four months of drug treatment [21]. This finding prompted an EBA trial of novel combination therapies in humans which enrolled 85 subjects and randomized them to 6 blinded groups: (i) bedaquiline alone; (ii) bedaquiline and PA-824; (iii) bedaquiline and pyrazinamide; (iv) PA-824 and pyrazinamide; (v) PA-824, moxifloxacin, and pyrazinamide;

and (vi) isoniazid, rifampicin, pyrazinamide, and ethambutol. This study found that in humans, PaMZ had the strongest EBA effect, and was at least as efficacious as the combination of isoniazid, rifampin, pyrazinamide, and streptomycin (Figure 3) [28]. Importantly, in addition to confirming the efficacy of this exciting new combination, this study showed that the murine model can aid in selecting promising combinations for human trials thus significantly accelerating the development of regimens that will have real-world impact [21]. PaMZ is currently being tested in a Phase 2 eight-week trial. In addition to its potent activity, this regimen has a number of other attractive features including its potential for activity against isoniazid- or rifampin-resistant strains and the fact that it is not metabolized by cytochrome P450, thus lessening drug–drug interactions.

Delamanid—Delamanid (OPC-67683), a nitro-dihydro-imidazooxazole derivative, is another metronidazole derivative that inhibits mycolic acid biosynthesis and disrupts the mycobacterial cell wall. It is distinguished from PA-824 by having a MIC for *Mtb* that is 10 times lower but less favorable serum bioavailability requiring twice daily dosing. It does, however, have excellent activity against intracellular *Mtb*; in murine models, when delamanid was combined with rifampin and pyrazinamide, it led to more rapid culture conversion of infected lung tissue than the standard four-drug treatment [29]. In a human 14-day EBA trial, it reduced CFU counts by 0.04 $\pm$ 0.056 log₁₀ units daily [30].

In its largest published trial to date, 481 MDR-TB patients receiving a background drug regimen for MDR-TB were randomized to additionally receive delamanid 100 mg twice daily, delamanid 200 mg twice daily or placebo. The primary endpoint was sputum-culture conversion (SCC) at two months. The two delamanid groups achieved significantly higher rates of two month SCC compared to those receiving the placebo: 45.4% (100 mg delamanid dose) and 41.9% (200 mg delamanid dose) compared to 29.6% (placebo). Delamanid was generally well tolerated at both doses, but asymptomatic QT segment prolongation on monitoring EKGs was more frequent in both delamanid groups (13.1% and 9.9%) than in the placebo group (3.8%) [31]. A Phase 3 randomized, double-blind, placebo-controlled clinical trial is underway to test the safety and efficacy of delamanid 100 mg twice daily and 200 mg once daily for MDR-TB. This trial was designed to include those with HIV-infection on antiretroviral drugs, and the initial results are expected later in 2013 (<http://clinicaltrials.gov/show/NCT01424670>).

In summary, the nitroimidazopyrans have impressive bactericidal activity, may be suitable for once daily dosing, and importantly show potential to improve upon the current standard of care for both drug-susceptible and drug-resistant TB when used in certain combinations. Ongoing clinical testing may reveal strengths and weaknesses of each of these drugs, including toxicities and side-effect profiles.

Old drugs repurposed for modern regimens

While much emphasis has been placed on the discovery of new drugs to treat TB, existing antibiotics may have potential for use in novel combinations. A number of such drugs have been categorized as ‘Group 5’ agents by the World Health Organization (WHO), indicating that they currently have an unclear role in the treatment of drug-resistant TB and are not yet

recommended for routine use. These agents may have unrecognized potential; however, further studies are necessary to elucidate their role in the treatment of TB. The most-promising drugs in this category are discussed here.

Oxazolidinones

Oxazolidinones have shown promise in the treatment of drug-resistant TB [32, 33]. Linezolid, the first commercially available oxazolidinone, was originally developed for resistant Gram-positive organisms and is commonly used to treat methicillin-resistant *Staphylococcus aureus* (MRSA). Linezolid binds the bacterial 23S ribosomal RNA (rRNA) inhibiting protein synthesis. With an MIC against *Mtb* of 0.5 µg/mL [34], linezolid has been used for drug-resistant TB with some success [33, 35–37]. A recent meta-analysis consisting of 11 studies and a total of 148 patients reported a 68% treatment success rate (completion of regimen with either five negative cultures in the last 12-months of therapy or no evidence of treatment failure) among patients receiving linezolid [38]. The long-term use of this drug has been limited by myelosuppression, peripheral neuropathy and optic neuropathy [39, 40], and in the meta-analysis there was a 36% discontinuation rate due to side effects [38]. In a trial of linezolid-containing regimens for XDR-TB in Korea, patients randomized to a decreased dose (300 mg daily rather than 600 mg daily) experienced fewer adverse events [36]. In the face of treatment efficacy and anticipated increases in distribution, there have been early warnings that indiscriminant use could lead to emergence of widespread resistance to linezolid [41].

Sutezolid (PNU-100480) is an oxazolidinone that has shown promising antitubercular activity in mice. With a similar MIC to linezolid, sutezolid appears to have improved *in vivo* activity [42], and may shorten treatment time in drug-susceptible TB in mice [43]. Phase 2 studies of sutezolid are currently underway. Another oxazolidinone, posizolid, (AZD-5847) has also entered Phase 2 clinical investigation.

Clofazimine

Clofazimine is a riminophenazine dye first synthesized in the 1954[44]. While initially developed for TB, clofazimine is mostly known for its use in the treatment of two other mycobacteria, *M. leprae* and *M. lepromatosis*, which are responsible for leprosy. Though the precise mechanism of action of clofazimine has remained elusive, the drug may target NDH-2, the primary NADH dehydrogenase involved in the respiratory chain, resulting in production of bacterial reactive oxygen species [45].

Because clofazimine is a fat-soluble compound with a long half-life (estimated at 72 hours), long-term therapy leads to accumulation in fatty tissues and organs, including the lungs. Tissue accumulation of this phenazine dye leads to a brown/orange pigmentation of the skin due to drug-induced ceroid lipofuscinosis [46]. While this skin discoloration is reversible upon drug discontinuation, this side effect can be off-putting for patients and may limit its use. There have been efforts to improve on this class of antibiotics and to eliminate the skin discoloration adverse effect.[47] Clofazimine has a low MIC to *Mtb* ranging from 0.06 to 2.0 µg/mL [48]. A recent meta-analysis examined treatment outcomes among drug-resistant TB patients treated with clofazimine [49]. Compiling data from 12 studies with a total of

3500 patients, this pooled analysis demonstrated a 62% treatment success (defined as number of patients cured or completing therapy) among patients receiving clofazimine (95% CI, range of 52.79%–71.12%), which is comparable to previously reported MDR-TB outcomes.[50] Additionally, there are some data that suggest that clofazimine-containing regimens may allow for regimen shortening of MDR-TB [51]. Additional investigation, including dose-ranging studies will be necessary to optimize clofazimine use in TB.

β-lactams

Initially developed in the 1940s, β-lactam antibiotics bind transpeptidases that cross-link peptidoglycans and thus inhibit cell wall synthesis. As safe and well-tolerated antibiotics, multiple β-lactams have been interrogated for antitubercular activity, including amoxicillin and the carbapenems (imipenem, ertapenem and meropenem). *Mtb* possesses a highly active β-lactamase, BlaC, which hydrolyzes β-lactams rendering them ineffective against *Mtb* except in the presence of a β-lactamase inhibitor [52]. Clavulanate, an oral β-lactamase inhibitor, irreversibly inhibits BlaC, and thus enhances β-lactam activity [53]. The carbapenems are relatively resistant to hydrolysis by β-lactamases, nonetheless their MIC can be decreased significantly by the addition of clavulanate [54].

Of the β-lactam–β-lactamase inhibitor combinations, meropenem–clavulanate appears to have the highest *in vitro* activity against XDR-TB strains [54, 55], and it has been associated with encouraging clinical outcomes in a limited number of patients [56]. However, intravenous dosing makes meropenem an expensive and impractical selection in many settings. WHO guidelines recommend empiric dosing for amoxicillin–clavulanate and imipenem, however, the recommended schedule and dosing has not been formally studied. In summary, the repurposed drugs discussed here (especially the oxazolidinones and clofazimine) have the potential to contribute to novel treatment combinations for drug-sensitive and drug-resistant TB and should be looked for in upcoming clinical trials.

Host-directed therapies for TB: potential role of immunomodulatory therapies

Host-directed immunotherapies hold the potential to augment antibiotic based treatment regimens. It is hoped that manipulation of the immune response could potentially (i) shorten regimens for drug-susceptible TB, (ii) provide additional therapeutic options for XDR-TB, (iii) prevent certain immunopathologies such as the development of pulmonary fibrosis and (iv) strengthen the memory immune response thereby decreasing the chance of relapse or reinfections.

Potential immunotherapies fall into diverse categories: (i) therapeutic exposures to fragments of *Mtb* intended to illicit an effective immune response (DNA vaccines and *Mtb* liposomal preparation), (ii) therapeutic infections with non-TB mycobacteria (*M. vaccae* and *M. indicus pranii*), (iii) recombinant cytokines or monoclonal cytokine antagonists which aim to boost the bactericidal cellular immune responses [interleukin-2 (IL-2) and interferon-γ (IFN-γ)], and (iv) immunomodulatory drugs for inflammatory diseases [anti-tumor necrosis factor (TNF) agents and phosphodiesterase inhibitors]. These have been

comprehensively reviewed.[57–59] Here we will focus on drugs from the latter two categories (recombinant cytokines or cytokine antagonists and immunomodulatory agents) and highlight recent data from human and animal studies.

The balance between Th1 and Th2 immune responses is critical to the killing of intracellular TB, and boosting the Th1 response is the therapeutic rationale for trials of cytokines including IL-2 and interferon gamma (IFN- γ). The addition of daily low-dose recombinant IL-2 to a failing antibiotic regimen appeared to improve symptoms and sputum conversion in MDR-TB in an uncontrolled study in 1997 [60]; however, in a 2003 randomized placebo-controlled, double-blind study of 110 subjects with drug-susceptible TB, the group receiving IL-2 in addition to standard drugs had delayed culture conversion compared to the group receiving standard therapy alone [61]. In contrast, although initial small trials on the use of recombinant IFN- γ (rIFN- γ) showed unclear microbiologic benefit [62–64], a recent larger clinical trial demonstrated efficacy [65]. IFN- γ is hypothesized to enhance the cytotoxic activity of infected macrophages to improve bacterial killing and prime a more robust T cell response, while reducing pulmonary inflammation. In a study of adjuvant IFN- γ , patients with cavitary pulmonary TB were randomized to receive 16 weeks of standard therapy alone, standard therapy plus nebulized recombinant IFN- γ 1b (rIFN- γ 1b) or standard therapy plus subcutaneous rIFN- γ 1b. There was a trend towards improved bacterial clearance with the nebulized administration of rIFN- γ 1b (60% four-week smear conversion compared to 36% in the control group, $p = 0.03$; four-week culture conversion 32% vs. 18%, $p = 0.15$). Inhaled rIFN- γ 1b also decreased constitutional symptoms and inflammatory cytokines in the lung [65]. It is hypothesized that the delivery of rIFN- γ 1b directly to the alveolar rather than intravenous compartment may account for the improved efficacy compared to earlier trials.

Anti-TNF- α monoclonal antibodies are useful in the treatment of rheumatologic disease but were found to carry a risk of reactivation of latent TB [66]. This phenomenon led to the hypothesis that the disruption of the TNF- α -dependent homeostasis between host and pathogen could possibly represent a therapeutic opportunity. Unbalancing host containment of non-replicating bacteria and coaxing the organism into a replicative (and thus more chemotherapeutically vulnerable) state could enhance the organism's susceptibility to drugs and potentially shorten treatment. Thalidomide is a potent inhibitor of TNF- α and when used adjunctively with standard drug therapy in a rabbit model of TB meningitis was shown to reduce TNF- α levels, cerebral spinal fluid leukocytosis, brain pathology and mortality [67]. Due to its teratogenicity, thalidomide is difficult to use in humans, but etanercept, one of the anti-TNF monoclonal antibodies, was tested in a Phase 1 trial during the initial treatment of HIV-associated TB. Treatment arm subjects received eight doses of etanercept over the first four weeks of standard TB treatment while controls received standard treatment alone. Those who received etanercept showed a trend towards improved time to sputum culture conversion and cavity closure [68].

Animal models of TB have provided recent evidence of other potential immunomodulators that may have an adjunctive effect on host clearance of TB. A phosphodiesterase-4 (PDE-4) inhibitor CC-3052 increases intracellular cAMP in macrophages and reduces TNF- α production. Compared to isoniazid alone, CC-3052 caused improved bacillary clearance in a rabbit model of TB [69]. Tofacitinib, a new anti-inflammatory Janus kinase (JAK) inhibitor

used for rheumatoid arthritis, has also recently been shown to reduce host containment of *Mtb* indicating that it might also have a role as an adjunctive therapy for *Mtb* when used alongside effective antibiotics [70].

Though some of the host-directed therapies currently show experimental promise, none has sufficient evidence to support inclusion in first-line regimens for either drug-susceptible or drug-resistant TB. Significantly more work is needed in understanding the balance between protective and detrimental immune responses to TB. Before immunotherapies take their place in the armamentarium of TB chemotherapeutics, it will be necessary to tease out the individual host factors (timing of disease, site and severity of disease, and genetic factors) that will allow for the successful application of host-directed therapies for TB.

Next steps: preserving new drugs will depend on improving diagnostics for drug resistance

Diagnostic limitations are a major barrier to the early recognition and subsequent management of drug-resistant TB. Currently, while awaiting conventional (phenotypic) drug susceptibility testing results, patients may be treated for months with ineffective antibiotics resulting in the amplification of drug resistance. Development and implementation of rapid diagnostics to identify drug resistance early in therapy is essential to preserving the continued susceptibility to and future use of TB drugs.

Diagnostic methods that define resistance genotypically rather than phenotypically have proven useful in accelerating the detection of drug-resistant TB. Currently, these molecular technologies, which include the GeneXpert [71] and the Hain Line Probe Assay [72], detect a limited number of genetic markers associated with resistance to a few key anti-TB drugs. However, there is a significant portion of drug resistance that does not appear to be associated with known genetic mutations, and there are many first, second and third line drugs for TB for which no rapid molecular tests are available. Creating genotypic tests for these other drugs will be more challenging and less rewarding, both financially and academically.

A critical barrier to the development of rapid molecular diagnostics is a limited understanding of the genomic basis of drug-resistance. For each anti-TB drug, there are several known *Mtb* genetic mutations commonly associated with bacterial drug resistance (Table 3). To date, single gene models of resistance have been insufficient to explain a large proportion of drug-resistance, and there is much drug resistance that remains unaccounted for by these mutations [71, 73, 74]. It is likely that there are yet unidentified mutations in known genes or novel genes in *Mtb* that confer drug resistance. Alternatively, drug-resistance may not be a monogenic phenomenon, but rather a multigenic process that requires concurrent mutations in multiple genes. Answering these questions will require a complex analysis of the entire *Mtb* genome.

To address this need, various international consortia are currently being assembled to provide coordinated efforts at whole genome sequencing of drug-resistant clinical isolates. These research efforts aim to apply deep sequencing technology to derive the DNA

sequences of clinical *Mtb* isolates from various parts of the globe. Comparing these sequences to the known drug-resistance phenotypes will hopefully allow for the identification of genes that play interdependent roles in the establishment of a drug-resistant phenotype. The ultimate goal of these research efforts is to improve molecular diagnostics so as to shorten the time to diagnosis, drug-susceptibility profiling and initiation of targeted treatment of drug-resistant TB.

At the threshold of a new era in tuberculosis treatment

With the FDA approval of bedaquiline, the first TB drug with a novel target in 40 years, and with additional other drugs poised in clinical development, we finally have the prospect of new tools to tackle the epidemic of TB. Before these tools can be effectively used in public health programs, there are many remaining questions that must be answered. What will be the most effective combinations of antimicrobials and host-directed therapies?

Further incorporating murine EBA experiments, which may be useful for pre-clinical assessment of novel drugs and regimens prior to clinical trials. Other challenging questions can only be answered in Phase 2 and 3 human clinical trials: how effective is each drug in HIV-associated TB? What are the interactions of the new drugs with various combinations of antiretroviral therapies? Finally, there is considerable work to be done on diagnostics that will allow for the efficient choice of appropriate therapy for each patient diagnosed with TB. Going forward we need to develop improved diagnostics for the organism (to determine rapidly and comprehensively the drug-susceptibilities so as to use the best combination of antimicrobials) and the host (to determine the genetic and disease specific factors that may be improved by immunomodulatory agents). Given the global realities of the epidemic, developing cost-effective diagnostics for drug-resistance will be as crucial to conquering the epidemic as the furthering of the drug development pipeline.

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Glossary

Multidrug-resistant TB (MDR-TB)

Mtb with *in vitro* resistance to the two cornerstone medications of the first-line regimen: isoniazid and rifampin

Extensively drug-resistant TB (XDR-TB)

Mtb resistant to isoniazid, rifampin, a quinolone (ofloxacin, moxifloxacin or levofloxacin) and one of the injectable drugs (kanamycin, amikacin or capreomycin)

Sources

1. Lawn SD, et al. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS*. 2008; 22(15):1897–908. [PubMed: 18784453]
2. Wong EB, et al. Causes of death on antiretroviral therapy: a post-mortem study from South Africa. *PLoS One*. 2012; 7(10):e47542. [PubMed: 23094059]
3. Rana FS, et al. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. *J Acquir Immune Defic Syndr*. 2000; 24(1):23–9. [PubMed: 10877491]
4. Global Tuberculosis Report 2012. World Health Organization; Geneva, Switzerland: 2012.
5. Andrews JR, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *J Infect Dis*. 2007; 196(Suppl 3):S482–90. [PubMed: 18181698]
6. Shah NS, et al. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis*. 2007; 13(3):380–7. [PubMed: 17552090]
7. Gandhi NR, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet*. 2006; 368(9547):1575–80. [PubMed: 17084757]
8. Cox HS, et al. Emergence of extensive drug resistance during treatment for multidrug-resistant tuberculosis. *N Engl J Med*. 2008; 359(22):2398–400. [PubMed: 19038891]
9. Lienhardt C, et al. New drugs for the treatment of tuberculosis: needs, challenges, promise, and prospects for the future. *J Infect Dis*. 2012; 205(Suppl 2):S241–9. [PubMed: 22448022]
10. Andries K, et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science*. 2005; 307(5707):223–7. [PubMed: 15591164]
11. Koul A, et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. *Nat Chem Biol*. 2007; 3(6):323–4. [PubMed: 17496888]
12. Koul A, et al. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. *J Biol Chem*. 2008; 283(37):25273–80. [PubMed: 18625705]
13. Haagsma AC, et al. Selectivity of TMC207 towards mycobacterial ATP synthase compared with that towards the eukaryotic homologue. *Antimicrob Agents Chemother*. 2009; 53(3):1290–2. [PubMed: 19075053]
14. Rouan MC, et al. Pharmacokinetics and pharmacodynamics of TMC207 and its N-desmethyl metabolite in a murine model of tuberculosis. *Antimicrob Agents Chemother*. 2012; 56(3):1444–51. [PubMed: 22155815]
15. Rustonjee R, et al. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. *Antimicrob Agents Chemother*. 2008; 52(8):2831–5. [PubMed: 18505852]
16. Diacon AH, et al. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med*. 2009; 360(23):2397–405. [PubMed: 19494215]
17. Diacon AH, et al. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob Agents Chemother*. 2012; 56(6):3271–6. [PubMed: 22391540]
18. Dooley KE, et al. Safety, tolerability, and pharmacokinetic interactions of the antituberculous agent TMC207 (bedaquiline) with efavirenz in healthy volunteers: AIDS Clinical Trials Group Study A5267. *J Acquir Immune Defic Syndr*. 2012; 59(5):455–62. [PubMed: 22126739]
19. Andries K, Gevers T, Lounis N. Bactericidal potencies of new regimens are not predictive of their sterilizing potencies in a murine model of tuberculosis. *Antimicrob Agents Chemother*. 2010; 54(11):4540–4. [PubMed: 20713662]
20. Veziris N, et al. Sterilizing activity of second-line regimens containing TMC207 in a murine model of tuberculosis. *PLoS One*. 2011; 6(3):e17556. [PubMed: 21408613]
21. Nuernberger E, et al. Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin, and pyrazinamide in a murine model of tuberculosis. *Antimicrob Agents Chemother*. 2008; 52(4):1522–4. [PubMed: 18285479]

22. Stover CK, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature*. 2000; 405(6789):962–6. [PubMed: 10879539]
23. Singh R, et al. PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO release. *Science*. 2008; 322(5906):1392–5. [PubMed: 19039139]
24. Manjunatha U, Boshoff HI, Barry CE. The mechanism of action of PA-824: Novel insights from transcriptional profiling. *Commun Integr Biol*. 2009; 2(3):215–8. [PubMed: 19641733]
25. Tyagi S, et al. Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. *Antimicrob Agents Chemother*. 2005; 49(6):2289–93. [PubMed: 15917523]
26. Diacon AH, et al. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrob Agents Chemother*. 2012; 56(6):3027–31. [PubMed: 22430968]
27. Diacon AH, et al. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrob Agents Chemother*. 2010; 54(8):3402–7. [PubMed: 20498324]
28. Diacon AH, et al. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet*. 2012; 380(9846):986–93. [PubMed: 22828481]
29. Matsumoto M, et al. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. *PLoS Med*. 2006; 3(11):e466. [PubMed: 17132069]
30. Diacon AH, et al. Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. *Int J Tuberc Lung Dis*. 2011; 15(7):949–54. [PubMed: 21682970]
31. Gler MT, et al. Delamanid for multidrug-resistant pulmonary tuberculosis. *N Engl J Med*. 2012; 366(23):2151–60. [PubMed: 22670901]
32. Schecter GF, et al. Linezolid in the treatment of multidrug-resistant tuberculosis. *Clin Infect Dis*. 2010; 50(1):49–55. [PubMed: 19947856]
33. Migliori GB, et al. A retrospective TBNET assessment of linezolid safety, tolerability and efficacy in multidrug-resistant tuberculosis. *Eur Respir J*. 2009; 34(2):387–93. [PubMed: 19282348]
34. Schon T, et al. Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis*. 2011; 15(4):502–9. [PubMed: 21396210]
35. Koh WJ, et al. Daily 300 mg dose of linezolid for multidrug-resistant and extensively drug-resistant tuberculosis: updated analysis of 51 patients. *J Antimicrob Chemother*. 2012; 67(6):1503–7. [PubMed: 22403262]
36. Lee M, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. *N Engl J Med*. 2012; 367(16):1508–18. [PubMed: 23075177]
37. Sotgiu G, et al. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. *Eur Respir J*. 2012; 40(6):1430–42. [PubMed: 22496332]
38. Cox H, Ford N. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis. *Int J Tuberc Lung Dis*. 2012; 16(4):447–54. [PubMed: 22325685]
39. Gerson SL, et al. Hematologic effects of linezolid: summary of clinical experience. *Antimicrob Agents Chemother*. 2002; 46(8):2723–6. [PubMed: 12121967]
40. Bressler AM, et al. Peripheral neuropathy associated with prolonged use of linezolid. *Lancet Infect Dis*. 2004; 4(8):528–31. [PubMed: 15288827]
41. Chang KC, Leung CC, Daley CL. Linezolid for multidrug-resistant tuberculosis. *Lancet Infect Dis*. 2012; 12(7):502–3. [PubMed: 22742623]
42. Williams KN, et al. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. *Antimicrob Agents Chemother*. 2009; 53(4):1314–9. [PubMed: 19075058]
43. Williams KN, et al. Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. *Am J Respir Crit Care Med*. 2009; 180(4):371–6. [PubMed: 19520903]
44. Barry VC, et al. A new series of phenazines (rimino-compounds) with high antituberculosis activity. *Nature*. 1957; 179(4568):1013–5. [PubMed: 13430770]
45. Yano T, et al. Reduction of clofazimine by mycobacterial type 2 NADH:quinone oxidoreductase: a pathway for the generation of bactericidal levels of reactive oxygen species. *J Biol Chem*. 2011; 286(12):10276–87. [PubMed: 21193400]

46. Job CK, et al. Skin pigmentation from clofazimine therapy in leprosy patients: a reappraisal. *J Am Acad Dermatol.* 1990; 23(2 Pt 1):236–41. [PubMed: 2145325]
47. Zhang D, et al. Identification of less lipophilic riminophenazine derivatives for the treatment of drug-resistant tuberculosis. *J Med Chem.* 2012; 55(19):8409–17. [PubMed: 22931472]
48. Jagannath C, et al. Chemotherapeutic activity of clofazimine and its analogues against *Mycobacterium tuberculosis*. In vitro, intracellular, and in vivo studies. *Am J Respir Crit Care Med.* 1995; 151(4):1083–6. [PubMed: 7697235]
49. Dey T, et al. Outcomes of clofazimine for the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. *J Antimicrob Chemother.* 2013; 68(2):284–93. [PubMed: 23054996]
50. Orenstein EW, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *Lancet Infect Dis.* 2009; 9(3):153–61. [PubMed: 19246019]
51. Van Deun A, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. *Am J Respir Crit Care Med.* 2010; 182(5):684–92. [PubMed: 20442432]
52. Wang F, Cassidy C, Sacchetti JC. Crystal structure and activity studies of the *Mycobacterium tuberculosis* beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. *Antimicrob Agents Chemother.* 2006; 50(8):2762–71. [PubMed: 16870770]
53. Hugonnet JE, Blanchard JS. Irreversible inhibition of the *Mycobacterium tuberculosis* beta-lactamase by clavulanate. *Biochemistry.* 2007; 46(43):11998–2004. [PubMed: 17915954]
54. Hugonnet JE, et al. Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. *Science.* 2009; 323(5918):1215–8. [PubMed: 19251630]
55. Gonzalo X, Drobniewski F. Is there a place for beta-lactams in the treatment of multidrug-resistant/extensively drug-resistant tuberculosis? Synergy between meropenem and amoxicillin/clavulanate. *J Antimicrob Chemother.* 2013; 68(2):366–9. [PubMed: 23070734]
56. Payen MC, et al. Clinical use of the meropenem-clavulanate combination for extensively drug-resistant tuberculosis. *Int J Tuberc Lung Dis.* 2012; 16(4):558–60. [PubMed: 22325421]
57. Paige C, Bishai WR. Penitentiary or penthouse condo: the tuberculous granuloma from the microbe's point of view. *Cell Microbiol.* 2010; 12(3):301–9. [PubMed: 20039878]
58. Uhlin M, et al. Adjunct immunotherapies for tuberculosis. *J Infect Dis.* 2012; 205(Suppl 2):S325–34. [PubMed: 22457298]
59. Report of the expert consultation on immunotherapeutic interventions for tuberculosis, 2007, World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases: Geneva.
60. Condos R, Rom WN, Schluger NW. Treatment of multidrug-resistant pulmonary tuberculosis with interferon-gamma via aerosol. *Lancet.* 1997; 349(9064):1513–5. [PubMed: 9167461]
61. Johnson JL, et al. Randomized trial of adjunctive interleukin-2 in adults with pulmonary tuberculosis. *Am J Respir Crit Care Med.* 2003; 168(2):185–91. [PubMed: 12702550]
62. Suarez-Mendez R, et al. Adjuvant interferon gamma in patients with drug - resistant pulmonary tuberculosis: a pilot study. *BMC Infect Dis.* 2004; 4:44. [PubMed: 15500691]
63. Giosue S, et al. Aerosolized interferon-alpha treatment in patients with multi-drug-resistant pulmonary tuberculosis. *Eur Cytokine Netw.* 2000; 11(1):99–104. [PubMed: 10705306]
64. Koh WJ, et al. Six-month therapy with aerosolized interferon-gamma for refractory multidrug-resistant pulmonary tuberculosis. *J Korean Med Sci.* 2004; 19(2):167–71. [PubMed: 15082886]
65. Dawson R, et al. Immunomodulation with recombinant interferon-gamma1b in pulmonary tuberculosis. *PLoS One.* 2009; 4(9):e6984. [PubMed: 19753300]
66. Keane J, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med.* 2001; 345(15):1098–104. [PubMed: 11596589]
67. Tsenova L, et al. A combination of thalidomide plus antibiotics protects rabbits from mycobacterial meningitis-associated death. *J Infect Dis.* 1998; 177(6):1563–72. [PubMed: 9607834]
68. Wallis RS, et al. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. *AIDS.* 2004; 18(2):257–64. [PubMed: 15075543]

69. Subbian S, et al. Phosphodiesterase-4 inhibition alters gene expression and improves isoniazid-mediated clearance of *Mycobacterium tuberculosis* in rabbit lungs. PLoS Pathog. 2011; 7(9):e1002262. [PubMed: 21949656]
70. Maiga M, et al. Risk of tuberculosis reactivation with tofacitinib (CP-690550). J Infect Dis. 2012; 205(11):1705–8. [PubMed: 22474037]
71. Helb D, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010; 48(1):229–37. [PubMed: 19864480]
72. Miotto P, et al. Genotype MTBDRplus: a further step toward rapid identification of drug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol. 2008; 46(1):393–4. [PubMed: 17977988]
73. Vilcheze C, Jacobs WR Jr. The mechanism of isoniazid killing: clarity through the scope of genetics. Annu Rev Microbiol. 2007; 61:35–50. [PubMed: 18035606]
74. Simons SO, et al. Validation of pncA gene sequencing in combination with the mycobacterial growth indicator tube method to test susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. J Clin Microbiol. 2012; 50(2):428–34. [PubMed: 22090409]
75. Almeida Da Silva PE, Palomino JC. Molecular basis and mechanisms of drug resistance in *Mycobacterium tuberculosis*: classical and new drugs. J Antimicrob Chemother. 2011; 66(7): 1417–30. [PubMed: 21558086]
76. Ma Z, et al. Global tuberculosis drug development pipeline: the need and the reality. Lancet. 2010; 375(9731):2100–9. [PubMed: 20488518]

Figure 1. Chronology of TB drug discovery

TB drug discovery flourished in the mid 20th Century. Drugs appear alongside their date of discovery. Red: drugs that are FDA- approved for use in TB. Black: drugs that are FDA- approved for other uses. Blue: drugs not yet FDA-approved. Timeline adapted from [76].

Figure 2. Multiple agents are currently being investigated for use in TB

While many compounds are in the initial discovery phase, other drugs have begun pre-clinical or clinical development in the form of Phase 1, 2 or 3 clinical trials. Reprinted from Stop TB Partnership, working group for new TB drugs (www.newtbdrugs.org).

Figure 3. PA-824-pyrazinamide-moxifloxacin (PaMZ) outperforms standard treatment in a human 14-day early bactericidal activity (EBA) study. The combination of PaMZ appears to have a synergy that produces superior reduction of viable bacterial count in sputum compared to standard therapy four-drug therapy (rifampin-isoniazid-pyrazinamide-ethambutol, RHZE) and other bedaquiline-containing combinations. Figure from [28].

Table 1

WHO recommended regimen for drug-susceptible TB^a

Drug	Daily dose	Adverse effects
Isoniazid (INH)	5mg/kg (maximum daily dose 300mg)	Hepatitis, peripheral neuropathy, lupus-like syndrome
Rifampin (RIF)	10mg/kg (maximum daily dose 600mg)	Orange discoloration of secretions, hepatitis, gastrointestinal upset, fever
Pyrazinamide (PZA)	25mg/kg	Hepatitis, arthritis, hyperuricemia
Ethambutol (EMB)	15mg/kg	Optic neuritis

For patients newly diagnosed with TB who are presumed or known to have drug-susceptible disease, the standard regimen recommended by the WHO includes an 'intensive phase' of two months of a standard four drug combination (INH, RIF, PZA and EMB), followed by a 'continuation phase' of four months of INH and RIF. Drugs are administered by mouth and are listed above with their recommended daily dose and notable adverse effects.

Table 2

Data on the efficacy of bedaquiline (TMC207)^a

Clinical trial phase	Trial name	Trial design	Type of TB	Number of subjects	Control arm(s)	Interventional arm	Key results	Refs
Phase 2		Randomized controlled EBA	Drug susceptible	75	(A) Rifampin alone, (B) isoniazid alone	Bedaquiline alone at varying doses	Decrease in log10 CFU counts from baseline to day 7: rifampin (1.88 +/- 0.74), isoniazid (1.7 +/- 0.71), bedaquiline 400 mg daily (0.26 +/- 0.64); bedaquiline well tolerated and without adverse events.	[15]
Phase 2	TMC207-C208, Stage 1	Randomized controlled trial	MDR-TB	47	Standard background	8 weeks of bedaquiline plus standard background	8-week culture conversion: bedaquiline (48%), control (9%)	[16]
Phase 2	TMC207-C208, Stage 2	Randomized controlled superiority trial	MDR-TB	161	Standard background	24 weeks of bedaquiline plus standard background	2-year development of other drug resistance: bedaquiline (4.8%), control (21.7%) Median time to sputum culture conversion: bedaquiline (83 days), control (125 days)	[17] http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm
Phase 2	C209 TMC207-	Open-label uncontrolled trial	MDR-TB, XDR-TB	233		24 weeks of bedaquiline + individualized background regimen	Unexplained deaths: bedaquiline (11.4%), control (2.5%) Median time to sputum culture conversion: 57 days	http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000bl.pdf http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm

^aPhase 2 clinical trials of bedaquiline have shown decreased time to sputum conversion in MDR-TB patients treated with bedaquiline. Phase 3 trials are currently underway.

Table 3

Mtb genes associated with drug resistance^a

Drug	<i>Mtb</i> gene	Role of gene product
Isoniazid	<i>katG</i>	Catalase/oxidase
	<i>inhA</i>	Enoyl reductase
	<i>aphC</i>	Alkyl hydroperoxide reductase
Rifampin	<i>rpoB</i>	β-subunit of RNA polymerase
Pyrazinamide	<i>pncA</i>	Pyrazinamidase/nicotinamidase
Streptomycin	<i>rpsL</i>	S12 ribosomal protein
	<i>rrs</i>	16S rRNA
	<i>gidB</i>	7-methylguanosine methyltransferase
Ethambutol	<i>embB</i>	Arabinosyl transferase
Fluoroquinolones	<i>gyrA/gyrB</i>	DNA gyrase
Kanamycin/Amikacin	<i>rrs</i>	16S rRNA
Capreomycin/Viomycin	<i>tlyA</i>	rRNA methyltransferase
Ethionamide	<i>inhA</i>	Enoyl reductase
<i>p</i> -amino salicylic acid	<i>thyA</i>	Thymidylate synthase A
PA-824 and Delamanid	<i>Rv3547</i>	Hypothetical 16.4 kDa protein
TMC-207	<i>atpE</i>	ATP synthase

^a Mutations in these genes are commonly associated with resistance to the antibiotics listed in the first column. Table adapted from [75].