Recent Advances in the Development of Anti-tuberculosis Drugs Acting on Multidrug-Resistant Strains: A review

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ABSTRACT

Despite over a century of drug development research, tuberculosis remains a major public health problem with a leading cause of infectious death worldwide. The reality of being deadly is the result of decades of neglect for an important infectious disease, lack of resources for national TB control programs, poor case detection and inadequate/inappropriate therapy in high-burden countries. At present time, the outcome of treatment protocol for MDR-TB is not intrinsically satisfactory, and it is lengthy in duration (18 – 24 months) although the treatment regimen consists of at least four drugs with different mechanism of action. Hence, new drugs are urgently needed to shorten and improve the treatment course in drug resistant TB, and to minimize the occurrence of new infections and death to zero level. Nowadays, various novel investigational drugs, such as bedaquiline (TMC207) [approved for use by FDA and WHO Expert Group], nitroimidazoles (PA-824, OPC-67683), diamines (SQ109), oxazolidinones (Linezolid, PNU-100480 (Sutezolid), ADZ5847), pyrroles (LL3858) and fluoroquinolones (moxifloxacin and gatifloxacin), have entered various clinical trial phases and on progress to be developed for the treatment of MDR-TB. However, new targets should be further identified and discovered that can kill the viable MTB in the latent phase and prevent the occurrence of resistance in bacterial cells. Finally, it is crucial to boost the connection between different parties, like research and development institutes, industries, drug control authorities, and international policy-making bodies to make the future bright and convey the optimum therapy for the patients who are suffering from TB.

Keywords: Tuberculosis, MDR-TB, treatment protocol, new targets, challenges.

INTRODUCTION

Background

Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). According to World Health Organization (WHO) global TB report 2012, there were almost 9 million new cases in 2011 and 1.4 million TB deaths. Besides, the emergence of drug-resistance is becoming a major threat to global TB care and control. WHO estimates that around 310,000 multidrug-resistant tuberculosis (MDR-TB) cases occurred among notified TB patients in 2011. The increasing emergence of drug resistant TB, and HIV infection, which compromises host defense and allows latent infection to reactivate TB, pose further challenges for effective control of the disease. Moreover, TB treatment is remarkably lengthy (takes 6–9 months) with significant toxicity, which creates poor patient compliance resulting in a frequent cause for selection of drug resistant and often deadly MDR-TB bacteria. To tackle this situation, multiple approaches will need to be implemented simultaneously, combining the efforts of government, academic and industrial entities. These approaches include increased funding for research in antibiotic resistance and drug development for TB, development of methods for protecting the efficacy of existing drugs, and prioritization for making use of current non-TB drugs for TB treatment.

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Current treatment regimens for MDR-TB are far from satisfactory: the overall duration is 20 months or more, requiring daily administration of drugs that are more toxic and less effective than those used to treat drug-susceptible TB, and have a high cost. Among MDR-TB patients started on treatment globally in 2009, only 48% were treated successfully, largely as a result of a high frequency of patient deaths (15%) and loss to follow-up (28%), which is commonly associated with adverse drug reactions, among other factors. New drugs that would help build a better, safer, less toxic, shorter and cheaper regimen are therefore urgently needed to reduce patient suffering and mortality. It has been over 40 years since a new drug for tuberculosis has been discovered. Therefore, the development of innovative, effective drug combinations should also be encouraged to diversify therapeutic choices, especially those for drug resistant TB cases. Emphasis, however, should be placed on compounds that attack non-traditional targets, as to lower the risk for acquired drug resistance mechanisms.

**Epidemiology**

Globally in 2011, there were an estimated 630,000 cases of MDR-TB (range, 460 000–790 000) among the world’s 12 million prevalent cases of TB, in which 3.7% of new cases and 20% of previously treated cases were anticipated. Almost 60% of these cases were in India, China and the Russian Federation. While the number of cases of MDR-TB notified in the 27 high MDR-TB burden countries is increasing and reached almost 60,000 worldwide in 2011, this is only one in five (19%) of the notified TB patients estimated to have MDR-TB.

**TREATMENT AND MANAGEMENT OF MDR-TB**

The current MDR-TB epidemic is the result of decades of neglect for an important infectious disease, lack of resources for national TB control programs, poor case detection and inadequate/appropriate therapy in high-burden countries. Initial outbreaks of MDR-TB in developed countries were associated with very high mortality rates both in HIV-negative and HIV-coinfected patients. Optimization of treatment regimens together with rapid diagnosis and drug susceptibility testing (DST) for first- and second-line drugs, greatly improved the clinical outcome. However, in developing countries, the prognosis is still largely poor due to inadequate laboratory support that is critical for successful management of MDR-TB patients.

Treatment of drug-resistant TB requires a combination various anti-TB drugs with different mechanisms of action. Traditionally, the classes of anti-TB drugs have been divided into first- and second-line drugs as briefly explained in Table 1. Besides, there are some other agents with unclear efficacy (not recommended for routine use) being used for the treatment of MDR-TB and XDR-TB. For MDR treatment, anti-TB drugs are grouped according to efficacy, experience of use and drug class, as shown in Table 2. All the first-line anti-TB drugs are in Group 1, except streptomycin (SM), which is classified with the other injectable agents in Group 2. All the drugs in Groups 2–5 (except SM) are second-line, or reserve drugs. Treatment regimens should consist of at least four drugs with either certain, or almost certain, effectiveness. Each dose in an MDR regimen is given as directly observed therapy (DOT) throughout the treatment.

**Table 1. Some of Anti-TB drugs with their category and mechanism of action**

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Category</th>
<th>Route</th>
<th>Chemical Description</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (INH)</td>
<td>1st line</td>
<td>Oral</td>
<td>Nicotinic acid hydrazide</td>
<td>Inhibits mycolic acid synthesis</td>
</tr>
<tr>
<td>Rifampin/rifampicine (RIF)</td>
<td>1st line</td>
<td>Oral</td>
<td>Rifamycin derivative</td>
<td>Inhibits RNA synthesis by targeting RNA polymerase</td>
</tr>
<tr>
<td>Pyrazinamide (PZA)</td>
<td>1st line</td>
<td>Oral</td>
<td>Nicotinamide derivative</td>
<td>Inhibits cell membrane synthesis</td>
</tr>
<tr>
<td>Ethambutol (EMB)</td>
<td>1st line</td>
<td>Oral</td>
<td>Ethylene di-imine di-1-butanol</td>
<td>Inhibits cell wall synthesis</td>
</tr>
<tr>
<td>Streptomycin (SM)</td>
<td>1st line</td>
<td>Injectable</td>
<td>Aminoglycoside</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Rifabutin /Rifapentine (Rfb)</td>
<td>2nd line</td>
<td>Oral</td>
<td>Rifamycin derivative</td>
<td>Inhibits RNA synthesis by targeting RNA polymerase</td>
</tr>
<tr>
<td>Amikacin (Am)</td>
<td>2nd line</td>
<td>Injectable</td>
<td>Aminoglycoside</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Kanamycin (Km)</td>
<td>2nd line</td>
<td>Injectable</td>
<td>Aminoglycoside</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Capreomycin (Cm)</td>
<td>2nd line</td>
<td>Injectable</td>
<td>Cyclic peptide</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Ofloxacin (Ofx)</td>
<td>2nd line</td>
<td>Oral</td>
<td>Fluoroquinolone</td>
<td>Inhibits DNA synthesis and</td>
</tr>
</tbody>
</table>
Programmatic approaches to MDR-TB treatment depend in part on the type of laboratory method (Conventional and Rapid DST) used to confirm MDR-TB, as shown in Table 3. Once MDR-TB is confirmed, patients can be treated with: a standard MDR-TB regimen (standardized approach); or an individually tailored regimen, based on DST of additional drugs, as shown in Table 4.

Table1. MDR-TB Treatment Strategies Depending on Laboratory Method to Confirm MDR-TB

<table>
<thead>
<tr>
<th>Laboratory Method</th>
<th>DST Result</th>
<th>Treatment Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional method to detect MDR-TB</td>
<td>While awaiting DST results for isoniazid, rifampicin; MDR-TB is suspected</td>
<td>Empirical treatment with MDR-TB regimen</td>
</tr>
<tr>
<td></td>
<td>Once MDR-TB is confirmed</td>
<td>Continue standard MDR-TB regimen or Change to individualized MDR-TB regimen (once susceptibility testing for second-line drugs is available).</td>
</tr>
<tr>
<td>Rapid method to detect MDR (takes around 1–2 days to detect MDR-TB)</td>
<td>Once MDR-TB is confirmed</td>
<td>Standard MDR-TB regimen or Individualized MDR-TB regimen (once susceptibility testing for second-line drugs is available).</td>
</tr>
</tbody>
</table>

Table2. Groups of Drugs to Treat MDR-TB

<table>
<thead>
<tr>
<th>Group</th>
<th>Class</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>First-line oral agents</td>
<td>Pyrazinamide (PZA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethambutol (EMB)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rifabutin (RFB)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Injectable Agents</td>
<td>Kanamycin (Km)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amikacin (Am)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capreomycin (Cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptomycin (SM)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Fluoroquinolones</td>
<td>Levofloxacin (Lfx)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moxifloxacin (MFX)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ofloxacin (Ofx)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Oral bacteriostatic second-line agents</td>
<td>Para-aminosalicylic acid (PAS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycloserine (Cs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Terizidone (Trd)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethionamide (Eto)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protionamide (Pto)</td>
</tr>
<tr>
<td>Group 5</td>
<td>Agents with unclear role in treatment of drug resistant-TB</td>
<td>Clofazimine (Cfz)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linezolid (Lzd)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amoxicillin/Clavulanate (Amx/clv)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thioacetazole (Thz)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem/Cilastatin (Ipm/Cln)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-dose isoniazid (high-dose H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clarithromycin (Clr)</td>
</tr>
</tbody>
</table>
Table 2. Potential regimens for the treatment of patients with MDR-TB

<table>
<thead>
<tr>
<th>Pattern of Drug Resistance</th>
<th>Suggested Regimen (Daily Unless Otherwise Stated)</th>
<th>Duration of Treatment (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH (± SM)</td>
<td>RIF, PZA, EMB (a fluoroquinolone may strengthen the regimen for patients with extensive disease)</td>
<td>6</td>
</tr>
<tr>
<td>RIF</td>
<td>INH, EMB, fluoroquinolones, supplemented with PZA for the first 2 months (an Injectable Agents may be included for the first 2-3 months for patients with extensive disease)</td>
<td>12-18</td>
</tr>
<tr>
<td>INH, RIF (± SM)</td>
<td>Fluoroquinolones, PZA, EMB, Injectable Agents (± alternative agent)</td>
<td>18-24</td>
</tr>
<tr>
<td>INH, RIF (± SM) and EMB</td>
<td>Fluoroquinolones, PZA, Injectable Agents, and two alternative agents</td>
<td>24</td>
</tr>
<tr>
<td>INH, RIF (± SM) and PZA</td>
<td>Fluoroquinolones, EMB, Injectable Agents, and two alternative agents</td>
<td>24</td>
</tr>
</tbody>
</table>

Drug Candidates and New Drugs Invented to Treat MDR-TB

New drugs are urgently needed to get to zero deaths, zero new infections, and zero stigma and suffering from TB. While TB has been curable for decades, existing drugs have to be taken for months or even years. New anti-TB drugs are needed for three main reasons: firstly, to shorten or otherwise simplify treatment of TB caused by drug-susceptible organisms; secondly, to improve treatment of drug-resistant TB, and thirdly, to provide more efficient and effective treatment of latent TB infection. Consequently, the landscape of TB drug development has evolved dramatically over the past ten years, and novel drugs are entering Phase III trials for the treatment of MDR-TB. Within a rational and well-funded infrastructure for conducting multinational large clinical trials, the importance of the development of new sterilizing drugs that target persistent bacteria and shorten TB therapy must be intensely promoted by the medical and TB patient advocacy communities.

Bedaquiline (Diarylquinoline TMC207, R207910)

Bedaquiline [formerly named as TMC207 or R207910] is the first new drug from a new drug class to treat TB to be approved by the United States Food and Drug Administration (FDA) in over 40 years, whose chemical structure is seen in Fig. 1. The drug, to be called Sirturo, was discovered by scientists at Janssen, the pharmaceuticals unit of Johnson and Johnson, and is the first in a new class of drugs that aims to treat the drug-resistant strain of the disease. As a result, the Expert Group suggested that, as an interim recommendation, bedaquiline may be added to a WHO-recommended regimen in MDR-TB adult patients under the following conditions: when an effective treatment regimen containing four second-line drugs in addition to PZA according to WHO recommendations cannot be designed; and when there is documented evidence of resistance to any fluoroquinolone in addition to multidrug resistance.

TMC207 is a first-in-class diarylquinolone compound with a novel mechanism of action and exhibits excellent activity against drug susceptible, MDR and XDR mycobacterium strains, with no cross-resistance to current first-line drugs. It appears that TMC207 has greater potency against mutated drug resistant strains than to fully susceptible isolates, suggesting a unique mechanism of action. Whilst TMC207-resistant strains have appeared, they remain fully susceptible to other anti-TB drugs such as RIF, INH, SM and EMB. Diarylquinolone TMC207 seems to act by inhibiting the ATP synthase, leading to ATP depletion and pH imbalance.

Figure 1. Chemical Structure of bedaquiline (TMC207)
Moreover, diarylquinoline TMC207 has potent bactericidal activity in the established infection in murine TB model. Besides, oral once daily administration of TMC207 has bactericidal activity at a dose of 400 mg when administered as mono-therapy for 7 days in patients with pulmonary TB. Compared to INH and RIF, the bactericidal activity of 400 mg of TMC207 started later but was of similar magnitude on days 4 to 7. Serious adverse effects related to the study drug did not occur. TMC207 has bactericidal and sterilizing activity against Mycobacterium tuberculosis (MTB) and other mycobacterial species but little activity against other bacteria.

Substitution of RIF, INH or PZA with diarylquinoline TMC207 accelerated activity leading to complete culture conversion after 2 months of treatment. In particular, the TMC207-INH-PZA and TMC207-RIF-PZA combinations cleared the lungs of TB in all the mice after two months. Diarylquinoline TMC207 has been also tested in various combination with the second line drugs amikacin, PZA, MFX and ethionamide in mice infected with the drug susceptible virulent MTB strain H37Rv. Diarylquinoline containing regimen were more active than the current recommended regimen for MDR-TB amikacin-PZA-MFX-ethionamide and culture negativity of the both lungs and spleens was reached after 2 months of treatment in almost every case. 

As preliminary stage I data from a double-blind, placebo-controlled, randomized Phase II trial and 2-years followed up results revealed on the randomized placebo-controlled study confirm the significant bactericidal activity of TMC207/bedaquiline in patients treated for MDR-TB. In both studies there is a reduced time to culture conversion and a higher number of culture conversions after 8 weeks of a standard background regimen plus TMC207 in patients with MDR-TB. The compound was safe and well tolerated over the 8-week treatment period. The emergence of drug resistance was substantial and may have been reduced by the concurrent administration of bedaquiline.

**Nitroimidazoles (PA-824 and OPC-67683)**

Another class of compounds that has been the subject of considerable interest because of their potential in TB therapy is the nitroimidazoles. Currently, two nitroimidazoles, the nitroimidazo-oxazine PA824, which is being developed by the TB Alliance, and the dihydroimidazooxazole OPC67683, which is being developed by Otsuka Pharmaceutical, are in clinical development. This class of compounds possessed antimicrobial activity. However, when the lead compound (CGI-17341) was found to be mutagenic in the Ames assay, further development was halted.

**PA-824**

PA-824 is a promising new compound, with the chemical name (S)-2-nitro-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3], as shown on Fig. 2, for the treatment of TB that is currently undergoing human trials. Like its progenitors, metronidazole and CGI-17341, PA-824 is a pro-drug of the nitroimidazole class, requiring bio-reductive activation of an aromatic nitro group to exert an anti-tubercular effect. And it was confirmed that resistance to PA-824 is most commonly mediated by loss of a specific glucose-6-phosphate dehydrogenase (FGD1) or its deazaflavin cofactor F_{220}, which together provide electrons for the reductive activation of this class of molecules. PA-824 has substantial bactericidal activity during both the initial and the continuation phases of treatment in an experimental murine TB model. The minimal effective dose and minimal bactericidal dose were 12.5 mg/kg and 100 mg/kg, respectively. At a dose of 100 mg/kg/day, the bactericidal activity of PA-824 approaches that of INH at the equipotent dosage of 25 mg/kg/day for humans.

![Figure 2. Structural formula of investigational product: PA-824](image)

In the randomized study, PA-824 appeared safe and well tolerated during 14 days of once-daily dosing in drug-sensitive, sputum smear-positive, adult pulmonary tuberculosis (PTB) patients at dosages of 200 to 1,200 mg/day. The number of adverse events was low and the severity of events mostly mild or moderate. PA-824 also showed to have a substantial and linear early bactericidal...
activity (EBA) over 14 days comparable to that of the existing first-line TB treatment agents. The extended EBA of PA-824 suggests that this drug may have sterilizing activity in human PTB and as such could contribute importantly to the sterilizing and treatment shortening ability of a MDR-TB treatment regimen.24 In another study done on 58 healthy subjects, the safety, tolerability, and pharmacokinetics of PA-824 were evaluated in two escalating-dose clinical studies, one a single-dose study and the other a multiple-dose study (up to 7 days of daily dosing). In subjects dosed with PA-824 in these studies, the drug candidate was well tolerated, with no significant or serious adverse events.25

The bicyclic nitroimidazole PA-824 has a very complex mechanism of action active against both replicating and hypoxic, non-replicating MTB. Microarray analysis of the mode of action of PA-824 showed a puzzling mixed effect both on genes responsive to both cell wall inhibition (like INH) and respiratory poisoning (like cyanide). The aerobic killing mechanism of this drug appears to involve inhibition of cell wall mycolic acid biosynthesis through unknown molecular mechanism. Whereas in the case of respiratory poisoning, PA-824 acts directly as nitric oxide (NO) donor, and that NO release from various PA-824 derivatives correlated well with the anaerobic killing of MTB. Thus, PA-824 acts as a “suicide bomb” releasing toxic NO within mycobacterial cells and NO possibly reacts with cytochromes/ cytochrome oxidase to interfere with the electron flow and ATP homeostasis under hypoxic non-replicating conditions.26

Based on the study done in evaluating 3-drug combinations composed of TMC207, PZA, PA-824, MFX; and rifapentine, TMC207 plus PZA plus either rifapentine or MFX was the most effective, curing 100% and 67% of the mice treated, respectively, in 2 months of treatment. Four months of the first-line regimen did not cure any mice, whereas the combination of TMC207, PA-824, and MFX cured 50% of the mice treated. The results reveal new building blocks for novel regimens with the potential to shorten the duration of treatment for both drug-susceptible and drug-resistant TB, including the combination of TMC207, PZA, PA-824, and a potent fluoroquinolone.27

The murine model for TB has been informative in the development of multi component drug regimens. In the studies with aerosol infection of mice with TB, PA-824 showed significant bactericidal activity both alone and when substituted for INH as part of a standard regimen. This finding was supported by pharmacokinetic data that suggested that PA-824 did not significantly affect concentrations of RIF, INH and PZA.28 In other study, testing of the PA-824, MFX and PZA combination revealed that the triple combination cured mice more rapidly than the current first-line regimen components of RIF, INH and PZA. MFX has potent in vitro activity against TB and improves culture conversion in early TB treatment.29

**OPC-67683**

![Figure 3. Chemical Structure of OPC-67683](image)

A more recently discovered nitroimidazole, OPC67683, is a dihydroimidazo-oxazole under development by Otsuka Pharmaceutical specifically for the treatment of TB. After undergoing single- and multiple-dose trials in normal volunteers, the compound is presently being tested in patients in an EBA trial. OPC67683 has extremely potent in vitro and in vivo activity against MTB.30 OPC-67683, as its structure shown on Fig. 3,31 is a pro-drug and requires activation by MTB for activity; experimentally isolated OPC-67683-resistant mycobacteria did not metabolize the compound and a mutation in the MTB Rv3547 gene (responsible for activating PA-824) among the resistant organisms suggests that this enzyme is involved in activating OPC-67683. The compound has been shown to inhibit mycolic acid biosynthesis and kill MTB in vitro.32

In a mouse model of chronic infection, the efficacy of OPC67683 was superior to that of currently used TB drugs. The effective plasma concentration was 0.1mg/mL, which was achieved with an oral dose of 0.625 mg/kg, confirming the remarkable in vivo potency of the compound. Besides, OPC-67683 showed no cross-resistance with any of the currently used anti-TB drugs.33 OPC-67683 showed
dose-dependent killing of drug-tolerant clinical strains of MTB that was superior to INH and equivalent to RIF, the most strongly sterilizing TB drug. To the extent that findings in the mouse model indicate potential sterilizing activity in human TB, the eradication rate of a new regimen containing OPC-67683 was compared with that of the standard regimen. The OPC-67683-containing regimen exerted a rapid and consistent reduction during the first 3 months. At 3 months after the start of treatment, only one colony was detected in one of the six animals; at 4 months, no colonies were detected in any of the six animals. In contrast, at 6 months for the standard regimen, colonies were detected in four out of five mice. These results suggest that a new regimen containing OPC-67683 could dramatically reduce the treatment duration by at least 2 months.

Diamine(SQ-109)

Sixty-nine 1,2-ethylenediamines were re-tested for in vitro activity against MTB H37Rv in a micro-broth dilution assay. For compound SQ109, its structure shown on Fig. 4, the best minimum inhibitory concentration (MIC) of 1.56µM was determined, selectivity index of 16.7 and 99% inhibition activity against intracellular bacteria, demonstrated potency in vivo and limited toxicity in vitro and in vivo. SQ109 was also tested against Erdman and single drug resistant strains of MTB and demonstrated significant activity: Erdman (0.7µM), ethambutol-resistant (1.4µM in Alamar Blue, 0.99µM in the BACTEC), INH-resistant (1.4µM), RIF-resistant (≤0.7µM). The fact that SQ109 works against an EMB-resistant strain suggests different specific target, mechanism of action and/or different activation pathways for SQ109 and EMB.

Figure 4. Chemical Structure of SQ109

The efficacy of SQ109 against MTB was demonstrated through in vitro macrophage model followed by further testing in an in vivo animal model. Both models were infected with the same MTB strain H37Rv. SQ109 exhibited both in vitro antimicrobial activity against MTB strain H37Rv grown inside the host murine macrophage cells (i.e. its ability to penetrate into macrophage phagosome, where MTB replicates) and in vivo antimicrobial activity on the mouse model inoculated with the H37Rv. The activity of SQ109 in this regard was comparable to that of INH in the macrophage test system, but superior over that of EMB.

SQ109 interacts synergistically with INH and RIF, two of the most important front-line TB drugs. SQ109 at 0.5 of its MIC demonstrated strong synergistic activity with 0.5 fraction of MIC INH and as low as 0.1 fraction of MIC RIF in inhibition of MTB growth. Additive effects were observed between SQ109 and SM, but neither synergy nor additive effects were observed with the combination of SQ109 with EMB or PZA. The synergy between SQ109 and RIF was also demonstrated using RIF-resistant strains; SQ109 lowered the MIC of RIF for these drug-resistant strains. Substitution of the new diamine antibiotic SQ109 for EMB in a mouse model of chronic TB improved efficacy of combination drug therapy with first-line TB drugs RIF and INH, with or without PZA: at 8 weeks, lung bacteria were 1.5 log lower in SQ109-containing regimens.

The combination of SQ109 with TMC207 improved an already excellent TMC207 MIC for MTB H37Rv by 4- to 8-fold, improved the rate of killing of bacteria over the rate of killing by each single drug, and enhanced the drug post antibiotic effect by 4 h. In no instance, antagonistic activities with the combination of SQ109 and TMC207 were observed. In another study, the two investigational drugs being developed for the treatment of TB, SQ109 and PNU-100480, combinations were additive and improved the rate of MTB killing over individual drugs. It showed an overall lack of antagonism between SQ109 and PNU-100480 in the range of expected therapeutic drug concentrations.

Oxazolidinone[Linezolid, PNU-100480(Sutezolid) and AZD5847]

The oxazolidinones represent a novel class of antibacterial agents whose mechanism of action appears to be inhibition of protein synthesis by binding to the 50S ribosomal subunit at a site close to the
Amanuel Godebo et al. “Recent Advances in the Development of Anti-tuberculosis Drugs Acting on Multidrug-Resistant Strains: A Review”

site(s) to which chloramphenicol and lincomycin bind but that the oxazolidinones are mechanistically distinct from these two antimicrobial agents. Two oxazolidinones, PNU-100480 and linezolid, have promising anti-MTB activities in the murine test system. AZD5847 also showed anti-mycobacterial activity.

**Linezolid**

Linezolid, its structure shown on Fig. 4, is the first oxazolidinone to be developed and introduced in clinical use. In vitro studies have shown good activity against different species of mycobacteria, including resistant strains. The linezolid MIC to inhibit the growth of 90% of organisms for MTB is in the range of 1–2 µg.L⁻¹. Linezolid has modest EBA against rapidly dividing tubercle bacilli in patients with cavitary PTB during the first 2 days of administration, but little extended EBA.

![Structure of Linezolid](image)

**Figure 5. Structural formula of Linezolid**

Linezolid is a valid alternative in patients with MDR-TB. Its anti-mycobacterial activity sharply increases the efficacy of other second-line therapies. However, the most limiting problem related to the prolonged use of linezolid in MDR-TB is toxicity, mainly anemia and peripheral neuropathy. As a result, it is recommended to use it with caution, in a dose never exceeding 600 mg, once a day, in patients with MDR-TB and XDR-TB, who have few other drug options. Overall, the available data suggest that linezolid is a potentially useful drug in treating the significant proportion of MDR-TB patients in whom second-line regimens fail or who are infected with TB strains with such significant resistance as not to allow the formulation of an appropriate second-line regimen using recommended drugs.

In the study done from 2003-2007, 30 patients received linezolid therapy at a dosage of 600 mg once daily (2 received intermittent or lower-dose therapy because of low body weight). Of the 30 patients, 22 successfully completed treatment. Among patients who completed treatment, there was no relapse during a mean follow-up of 1.5 years. All 29 patients with PTB achieved culture conversion, at a median time of 7 weeks. Five additional patients continued to receive treatment and were tolerating linezolid well at data censure. Two patients defaulted, and one experienced treatment failure, with an isolate with the same susceptibility pattern as the initial episode of MDR-TB.

![Structure of PNU-100480 (Sutezolid)](image)

**Figure 6. Structure of PNU-100480 (Sutezolid)**

In another study involving 39 patients with XDR pulmonary tuberculosis who had not had a response to any standard treatment regimen for 6 months or more, the immediate addition of linezolid at a dose of 600 mg per day to the ongoing background treatment regimen had a significant beneficial effect on the time to sputum-culture conversion on solid medium, as compared with the delayed addition of linezolid at the same dose. During the first 6 months, 34 of the 39 patients (87%) had confirmed culture conversion, at a median of 76 days. Even though, the role of linezolid in the treatment of MDR-TB and XDR-TB has been considered controversial, an aggressive, comprehensive management programme using linezolid along with other drugs can favorably treat significant number of patients with XDR-TB or pre-XDR-TB.
Another oxazolidinone, named as PNU-100480, its structure shown on Fig. 6, has been demonstrated to have more potent bactericidal activity in vitro and in a murine TB model. Moreover, the incorporation of PNU-100480 dramatically improved the bactericidal activities of regimens containing current first-line anti-TB drugs and MFX. For instance, the addition of PNU-100480 (100 mg/kg/day) to the standard regimen of RIF, INH, and PZA resulted in an additional 2.0-log_{10} unit reduction in lung CFU counts during the first 2 months of treatment. The combination of PNU-100480, MFX, and PZA, which doesn’t contain either RIF or INH, was also more active than RIF, INH, and PZA. PNU-100480 also advances further sterilizing activity to the first-line regimen that is capable of shortening the duration of treatment necessary for cure.

In the study done on health volunteers, PNU-100480 was well tolerated, showed proportional increases in exposure to 1000 mg, adequately and predictably absorbed, and exhibited superior bactericidal activity compared with linezolid, independent of peak drug concentrations. In another study, the duration of dosing of PNU-100480 was extended to better appreciate potential oxazolidinone toxicities due to mitochondrial protein synthesis inhibition. No significant untoward hematologic effects were observed when it was dosed at doses of 600 mg twice daily. Besides, it shows additive or synergistic activity when it is added to standard doses of PZA. PNU-100480, TMC207, PA-824, SQ109, and PZA were examined singly and in various combinations, against intracellular MTB, using whole blood culture (WBA). Combinations of PNU-100480, TMC207, and SQ109 were fully additive, whereas those including PA-824 were less than additive or antagonistic. The most active regimens, including PNU-100480, TMC207, and SQ109, were predicted to have cumulative activity comparable to standard TB therapy. Susceptibility of clinical MTB isolates to PNU-100480 and linezolid was evaluated. The isolates had various susceptibilities to INH, RIF, EMB, and SM. The mean MIC for PNU-100480 was 3.2 times lower than that for linezolid. Therefore, PNU-100480 is a promising candidate to be developed further as an adjunct in the treatment of MDR-TB and XDR-TB.

AZD5847

A little further behind in development, AstraZeneca are developing an oxazolidinone, AZD5847. Healthy volunteer tolerability and pharmacokinetic studies were recently completed but results are not yet available. A phase II EBA study is in development. AZD5847, its structure shown on fig. 7, was originally intended as a broad-spectrum antibiotic, but has now been repurposed as an anti-TB agent. It is well recognized that with linezolid, treatment periods longer than 14 days may result in hematological adverse effects and since treatment for TB is considerably longer than 14 days, the degree and severity of this off-target activity with the next-generation oxazolidinone agents will be the key for their development.

Pyrrole (LL-3858)

The anti-TB activity of the pyrrole class was first described in 1998. BM212, the most potent pyrrole derivative studied so far, can be used as a lead for the preparation of new and more efficacious antimycobacterial drugs, with MIC that ranged from 0.7 to 1.5 mg/mL against several strains of MTB including the resistant strains. The mycobacterial target of LL3858, its structure shown on fig. 7, is not yet known having a MIC range of 0.06–0.5 mg/mL that was not affected by resistance to INH and RIF. Additive activity in combination with first line drugs in the murine model was also described. The target probably differs from the targets of currently used drugs.
Fluoroquinolones (Gatifloxacin and Moxifloxacin)

Of the new compounds being tested for their efficacy in TB treatment, the fluoroquinolones are the first novel drugs since the development of rifamycins to have been shown to have significant activity against MTB. Currently fluoroquinolones are used as anti-tuberculous agents in MDR-TB and, to a lesser extent, in the case of severe adverse reactions to the conventional anti-TB regimen. Fluoroquinolones are not included at present in the first-line treatment of TB, although that might change in the future, in order to shorten treatment duration. The newer fluoroquinolones, MXF and gatifloxacin have been shown to exert better activity and are associated with a lower probability of emergence of resistance.

Moxifloxacin (MFX)

The excellent in vitro activity of MXF translates into activity in the human host. Moxifloxacin, its structure shown on fig. 9, joins the small number of highly bactericidal anti-TB drugs and may contribute to the development of more effective regimens. The results of a comprehensive preclinical evaluation of the safety of MFX are consistent with those reported for other fluoroquinolones. Most of the findings (e.g. arthrotoxicity in juvenile animals and CNS toxicity) that have led to restrictions in the use of quinolones in general have also been observed with MFX. Besides, MFX has a good EBA against MTB in patients with drug-susceptible TB. Its extended EBA activity from days 2 to 7, a putative surrogate marker of sterilizing activity, may be slightly higher than for INH.

Gatifloxacin

Gatifloxacin, its chemical structure shown on Fig. 10, has excellent EBA, only slightly less than for INH, and greater extended EBA. Gatifloxacin was evaluated alone and in combination with ethionamide, PZA, and EMB. Ethionamide appeared to be the most promising single agent for use in
combination with ratifloxacin. Gatifloxacin-ethionamide-PZA and/or EMB would likely be an effective regimen for treatment of MDR-TB.  

In the Randomized Clinical Trial that was done by substituting EMB for new drugs in the standard regimen, the response at the end of treatment was uniformly high in all regimens, with 95% and 98% of the patients treated with a thrice-weekly 4-month gatifloxacin and MFX regimens, respectively, becoming culture negative at the end of treatment, compared to 97% in the standard regimen.

**New Targets Identified for the Development of Anti-TB Drugs**

It is reassuring that after many years, a TB drug pipeline is now developing. Moreover, several novel pathways essential for MTB survival are currently being explored, including the protein kinase G and coronin 1 pathways, which have potential to serve as novel drug targets for treatment of both drug-sensitive and drug-resistant TB. Whilst pressing on with the development of drugs and optimized combination and usage of existing drugs at the “outcome” end of the pipeline, concerted effort is needed to expand further the portfolio of novel drug targets and to identify novel leads.

The discovery that focuses on commercially available drugs, such as prescribed for the treatment of Parkinson’s disease, showed the potential to treat MDR and XDR-TB. These drugs, entacapone and tolcapone, are predicted to bind to the enzyme InhA and directly inhibit substrate binding. The prediction is validated by in vitro and InhA kinetic assays using tablets of Comtan, whose active component is entacapone, with MIC for MTB approximately 260µM. Moreover, kinetic assays indicate that Comtan inhibits InhA activity by 47.0% at an entacapone concentration of approximately 80µM. So, the active component in Comtan represents a promising lead compound for developing a new class of anti-tubercular therapeutics with excellent safety profiles.

Peptide-based antibiotics are attractive both to fundamental research and for their potential therapeutic applications. They are relatively small molecules, their action is fast and lethal to a large spectrum of pathogens, and they seem to escape many of the drug resistance mechanisms. Compared to classical antibiotics, peptides portray a highly modular synthetic antimicrobial system. Unlike classical antibiotics that must penetrate the target cell to act on it, antimicrobial peptides are believed to kill target cells by destroying their membrane. Theoretically, this mode of action should severely reduce microbial resistance and represents, therefore, a promising alternative in the treatment of raging MDR infectious diseases.

Mycobacterial persistence which refers to the ability of tubercle bacillus to survive in the face of chemotherapy and/or immunity is a new approach. Gene products involved in mycobacterial persistence, such as isocitratelyase (ICL), an enzyme essential for the metabolism of fatty acids; PcaA, required for cording and mycolic acid cyclopropane ring synthesis in the cell wall of both BCG and MTB; RelA<sub>Mtb</sub> (ppGpp synthase), critical for the successful establishment of persistent infection in mice by altering the expression of antigenic and enzymatic factors that may contribute to successful latent infection; and Rv3133c/DosR<sub>Mt</sub>, a transcription factor of the two-component response regulator class and the primary mediator of a hypoxic signal within MTB, used to control a 48-gene regulon involved in MTB survival under hypoxic conditions, have been identified and could be good targets for development of drugs that target persistent bacilli.

In MTB, the energy production pathways are not well characterized. But, the findings that showed as PZA acts by disrupting membrane potential and depleting energy in MTB lead to focus on energy pathway as a drug target. Energy production or maintenance is important for the viability of persistent non-growing tubercle bacilli in vivo. The recent discovery of the highly effective TB drug
TMC207 also highlights the importance of energy production pathways for MTB. It is likely that energy production pathways, such as the electron transport chain, glycolytic pathways and fermentation pathways, could be good targets for TB drug development.2 Another target is toxin-antitoxin modules. Inappropriate or uncontrolled expression of the toxin or a decrease in the expression of antitoxin can cause bacterial cell death.3 It is interesting to note that the MTB genome has recently been found to contain at least 38 toxin-antitoxin modules including three relBE and nine mazEF loci. RelE and MazF are toxins that cleave mRNA in response to nutritional stress. Evidence now indicates that these loci provide a control mechanism that helps free-living prokaryotes cope with nutritional stress.4 Hence, the toxin-antitoxin modules are attractive targets in MTB for designing drugs that either induce the production of the toxin or inhibit the expression of the antitoxin.2

Essential mycobacterial genes can also be good targets for TB drug development. Systematic analysis of essential genes by transposon mutagenesis and targeted knockout of specific genes are valuable approaches to identify essential genes because whose disruption leads to non-viability of the bacilli. 3 Around one-sixth of the total number of genes (614 genes) in MTB, were found to be essential for in vitro growth.5 While 194 genes were demonstrated to be essential for in vivo MTB survival in mice. A surprisingly large fraction of these genes are unique to mycobacteria and closely related species, indicating that many of the strategies used by this unusual group of organisms are fundamentally different from other pathogens.6

It has been demonstrated that the ClpP proteins of MTB, which are essential for growth in vitro using genetic means but its activators are effective against mycobacteria, are exciting new drug targets for exploration using genetic and chemical validation approaches. Targeting the Clp series has the added advantage that both inhibitors and activators have the potential to kill the cell. Further work to develop the acyldepsipeptides (ADEP) activator series or to identify new activators or inhibitors could lead to the development of new drugs in the long term.7

Challenges in the Development of New Drugs

Along with the socioeconomic and host factors that underlie the serious global burden of TB, a fundamental problem that hinders more effective TB control is the ability of MTB to persist in the host and to develop drug resistance, often because of poor adherence to lengthy therapy.8 Otherwise, the resurgence of TB, development of new drugs to treat the disease has stagnated in the face of numerous scientific and economic obstacles. Showing of the superiority of new agents constitutes the most convincing clinical evidence of drug efficacy, but in the case of drug-sensitive disease this may be infeasible given the high efficacy rates of existing regimens, the need for extended follow-up, and the large number of participants required supporting statistical conclusions.9

Primarily, overall funding for TB research in general, and drug discovery in particular, remains alarmingly inadequate. TB research is funded in competition with all other areas of biomedicine and is clearly not receiving funds commensurate with the global dimension of the disease and the probability that untreatable forms of TB will become increasingly widespread.90 Besides, the TB drug market is associated with insufficient profit opportunity or investment return to instigate pharmaceutical industries to develop new drugs.91 Secondly, it is the lack of access to information, pharmaceutical expertise, compounds, and research tools. There would be great value, for example, in a publicly accessible database that collected thorough information about screenings of compounds and about analyses that indicate which targets in MTB appear to be “druggable.” Considering the limited resources for TB drug development, it is critical to avoid repetitive efforts, particularly multiple independent journeys to a dead-end.88 However, the molecular mechanisms responsible for mycobacterial dormancy, persistence, and drug resistance are not yet fully understood.92 Thirdly, there are a number of constraints that have companies from investing in new anti-TB drugs. The research is expensive, slow and difficult and requires specialized facilities for handling MTB. There are few animal models that closely mimic the human TB disease. Development time of any anti-TB drug will be long. In fact minimum six month therapy will require with a follow up period of one year or more.93 Lastly, the challenge of TB drug Research and Development is the long timeline of clinical trials. Phase II studies for TB drugs typically require at least two years, and pivotal trials a minimum
of three years from beginning patient enrollment to finalized study reports. Furthermore, the fact that people must be treated with a combination of four drugs, rather than with a single drug, means that to replace the current regimen with a totally new three- or four-drug regimen by testing the substitution of one drug at a time into the standard regimen will require not a minimum of six years, but at least four times six years - over two decades - just for the clinical phase of development.  

**FUTURE PERSPECTIVES**

A promising new era in TB drug development has begun. It is now critical to consolidate recent progress and ensure that new drugs/regimens for treatment of all forms of TB are suitably introduced in countries in a way that guarantees access to best treatments for all those in need and avoids inappropriate use of new drugs. Moreover, one can certainly strive to discover new drugs with improved efficacy and tolerability and with the ability to be used in new combination therapies to shorten the current, protracted treatment duration. So, it is important to identify essential TB targets based on the increased knowledge of the pathogen and the physiology of the disease, to develop smarter screening assays, and to prepare sets of compounds designed to give improved leads for antibacterial activities. However, it requires the wide involvement of all relevant parties (industry, academia, drug regulatory agencies, and international policy-making agencies) who must collaborate effectively to deliver optimal future therapies for TB.

The emergence of multi-drug-resistant strains of MTB makes the discovery of new molecular scaffolds a priority, and the current situation even necessitates the re-engineering and repositioning of some old drug families to achieve effective control. In this condition, smaller studies may suffice if large differences in efficacy between experimental and comparator regimens are likely. Use of preliminary endpoints may be possible, resulting in accelerated drug approval. In a situation of dire medical need, large potential benefits may outweigh minor risks. But most important, given the pressing need for new drugs to treat resistant TB, this approach will bring the promise of new drugs to an area of major public health concern.

Finally, ways to shorten clinical trials with new TB drugs should be explored. The evaluation of surrogate biomarkers that predict the likelihood of relapse, such as serial sputum colony counts and molecular markers, should be incorporated into clinical trials as much as possible. Validated surrogate markers will reduce the time it takes to assess the efficacy of new agents. Besides, it is important to develop novel drugs that avoid the manifestation of drug resistance in bacterial cells. Control programs that implemented have also been less effective than expected in reducing the occurrence of TB transmission, mainly because patients are not diagnosed and cured quickly enough.

**CONCLUSION**

In the past ten years, the development of anti-TB drugs were extremely increasing and expecting to improve the current treatment protocol to life saving and optimum status. Consequently, the death and being infectious resulted from TB will stop or exponentially decrease in near future. However, to reach this level, it needs hand-to-hand work of different sectors that are responsible for the development of new drugs. In addition, the research area should look for the potential targets that can bring paradigm shift in the history of treatment of TB.

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Current Research Activities:
Antiglycation activities of extract of Moringa stenopetala leaves in fructose induced protein glycation
Antidiabetic activities of aqueous ethanol and n-butanol fraction of Moringa stenopetala leaves in streptozotocin-induced diabetic rats Under submission
Antiinflammatory activity of Moringa stenopetala leaves in carreggen in induced mice.
Evaluation of treatment outcome in antiretroviral therapy in Hawassa Referral Hospital.