Challenges, successes and hopes in the development of novel TB therapeutics

Despite efficacious drugs for treatment, TB continues to affect enormous numbers of patients throughout the world. Failure to control TB may be related to the biological characteristics of Mycobacterium tuberculosis, the nature of susceptible hosts often impoverished and poorly supported by healthcare infrastructure and the complex treatment regimens that must be used. Challenges to anti-TB drug development include the organism’s slow replication, the ability of M. tuberculosis to survive in a dormant state and to persist despite therapy, its impregnable cell wall and its capacity to develop resistance to drugs. The need for extended therapy using combinations of drugs remains a practical obstacle to effective control in poor, malnourished and diseased communities most susceptible to TB. High-throughput screening of candidate agents and investigation of drugs already in use for other infections are yielding promising new candidates for TB treatment. New families of drugs entering clinical trials include 5-nitroimidazoles, diarylquinolines and ethylene diamines. Increasing funding initiatives, advances in the biology of TB and strategies for drug discovery have rejuvenated the pipeline of new drugs for TB, promising an expanding armamentarium of effective drugs with improved tolerability and potential to treat drug-resistant cases.

Despite the fact that TB has been recognized for thousands of years and despite the fact that the etiological agent has been identified since the earliest days of medical microbiology, the global burden of TB continues to loom as one of the largest among infectious diseases, with an enormous toll in morbidity and mortality. Paradoxically, available therapeutic agents are highly efficacious in TB, with cure rates exceeding 95% during clinical trials [1]. Why then has the control of TB been so problematic?

This has been ascribed to a diversity of challenges that include the socioeconomic status of affected communities, the indolent clinical nature of the disease, requiring prolonged complex therapy, and the unusual microbiological properties of the causative organism.

TB preys on impoverished or malnourished communities: individuals weakened by immunological deficiencies and situations where healthcare delivery is poor. Such communities are ill equipped to deal with the need for prolonged therapy with multiple different agents. In the absence of an infrastructure to support TB control programs, case finding is inefficient and patients present late in the disease, during which time the infection may be transmitted to many contacts. Supervision of complicated treatment regimens is inadequate, resulting in poor patient compliance. Among others, treatment interruptions, low rates of treatment completion, erratic drug supplies and poor healthcare infrastructure result in high rates of relapse, repeated attempts at treatment and the emergence of drug resistance. Transmission is enhanced by the susceptible nature of communities where HIV and malnutrition are prevalent. Incidence rates in Africa have been estimated to be as high as 363 per 100,000 individuals [2]. By contrast, in Western Europe and the developed world, the prevalence of TB has been significantly reduced using modern infection control strategies and chemotherapy – the incidence rate in the USA during 2006 was estimated at 4.6 per 100,000 individuals [3].

In some areas of poor control, the problem is now compounded by alarming rates of drug resistance. This is occurring not only in patients who have been treated for drug-sensitive TB with suboptimal therapy and go on to develop ‘secondary’ drug resistance, but also in new cases of TB who are initially infected with drug-resistant organisms that are now circulating in communities where control programs are failing. While the population-weighted mean of multidrug-resistant (MDR)-TB among all TB cases reported by the WHO is 5.3%, MDR-TB is much more frequent in areas with poor TB-control programs. For example, MDR-TB has been estimated to exceed 35% in some countries of the former Soviet Union [4].

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TB
The clinical disease caused by Mycobacterium tuberculosis

Multidrug-resistant TB
TB infection due to strains that are resistant to at least isoniazid and rifampin
Although standard short-course regimens and directly-observed therapy (DOTS) have improved response rates, MDR isolates and extensively drug-resistant (XDR) TB isolates continue to emerge. Once established, drug-resistant disease may enter susceptible communities with devastating effects. Rapid spread and mortality rates approaching 100% have been reported in highly immunocompromised patients with HIV disease [5]. The complexity of treatment is a major factor in the failure to control TB and prevent the emergence of drug resistance. The development of new drugs amenable to use in simpler, shorter regimens remains a central but challenging goal.

**Microbiological properties of M. tuberculosis**

Unusual microbiological characteristics of the organism include a long generation time, the ability to enter a dormant state, persistence in the face of appropriate therapy, the presence of a strong protective cell wall and the ability to develop genetic mutations conferring resistance to drugs. These present challenges for the development of new drugs and also contribute to the failure of TB-control programs.

In the company of a handful of rare infections, such as Whipple’s disease, bartonellosis and leprosy, the generation time for *M. tuberculosis* (~25 h [6]) is an order of magnitude greater than most common infections. Since many antimicrobials rely on the active multiplication of an organism to exert their effects, diseases caused by these slowly multiplying organisms typically require prolonged courses of therapy. By contrast, *Escherichia coli*, [7] *Neisseria meningitides* [8], *Streptococcus pneumonia* [9] and *Staphylococcus aureus* [10] all have a generation time of between 20 and 45 mins and successful treatment of infections caused by these organisms can be accomplished in a few days.

Dormancy and persistence of *M. tuberculosis* may compound the problem of controlling the disease. Dormancy is the microbiological property of certain populations of *M. tuberculosis* to suspend active multiplication, possibly in response to environmental stresses such as nutritional or oxygen deprivation. Dormant organisms may sequester themselves in granulomatous or calcified tissues without active multiplication for months, years and sometimes lifetimes and may be responsible for the reactivation of TB years after a self-contained primary infection.

Persistence generally refers to the unusual capacity of some drug-susceptible organisms to survive treatment with effective antimicrobials without developing genetic resistance, particularly when treatment courses are short. Persistent organisms are thought to multiply slowly or sporadically and may be tolerant to TB therapy [11]. It is not yet well understood whether the biological processes accounting for dormancy also play a role in persistence.

The impregnable cell wall, fortified with mycolic acids and other lipids [12], presents an obstacle to the penetration of some antimicrobials. The cell wall also contributes to the durability of infectious organisms known to survive in the environment for weeks to months [13].

Emergence of drug resistance is another property of *M. tuberculosis* that has hampered control efforts. During treatment with antimicrobials that are generally effective against TB, strains emerge with genetic properties that allow them to survive in the presence of these agents. An early therapeutic trial with streptomycin mono-therapy in 1947 showed encouraging short-term success, but drug resistance was reported to have developed in the strains from 35 of the 41 streptomycin-treated patients and, after 5 years, death rates for streptomycin-treated patients and control patients converged (53 and 63%, respectively [14]). Subsequent studies showed that the development of drug resistance could be prevented using several anti-TB agents concurrently. The experience dictated a future of complex combination therapy for the treatment of TB.

**Successes**

Despite these obstacles, a very efficacious armamentarium of anti-tuberculous agents has evolved. Individuals with drug-susceptible disease who are able to comply with modern combination therapy (isoniazid, rifampin and pyrazinamide with or without ethambutol) can expect high cure rates, and the combination strategy, particularly when drug administration is directly observed, has improved global TB control [15] and prevented the emergence of resistant disease in compliant patients. In a US public health service trial, sputum culture conversion rates of 94.6% at 16 weeks and low relapse rates of 3.5% were observed, has improved global TB control and mortality rates approaching 100% have been reported in highly immunocompromised patients with HIV disease [5]. The complexity of treatment is a major factor in the failure to control TB and prevent the emergence of drug resistance. The development of new drugs amenable to use in simpler, shorter regimens remains a central but challenging goal.

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In the realm of drug-resistant TB, the advent of fluoroquinolones appears to have led to significant improvements in prognosis. In one retrospective study, comparing patients with MDR disease either before or after 1984, response rates
increased from 65 to 85%, respectively, believed to be due, at least in part, to the introduction of this family of drugs [17]. Fluoroquinolones have become the standard of care in this situation. Consequently, it is unlikely that prospective, randomized, double-blind, controlled trials will be performed to confirm this.

Treatment regimens have been simplified with the advent of intermittent therapy two- to three-times weekly instead of daily, and the use of fixed drug combinations has made it easier for patients to comply with therapy. DOTS by healthcare workers has improved treatment success rates [18]. However, intermittent therapy presents challenges when drugs with different half-lives are used together. The development of drug-resistance is a concern when drugs with short elimination half-lives fall to subtherapeutic levels, leaving other longer acting companion drugs as effective monotherapy during part of the dosing interval. Compliance with intermittent therapy may also be critical, and missed doses may result in drug resistance.

The tolerability of treatment has also improved since the days when para-aminosalicylic acid was administered in doses of 10–12 g per day and, at present, more potent agents are used at much lower doses. Injectable agents have been largely replaced by oral therapy in drug-sensitive disease and older toxic agents, such as thiacetazone and cycloserine, notorious for causing life-threatening skin rashes (particularly in HIV-infected individuals) [19] and neurotoxicity [20], have been relegated to ‘second-line’ use, primarily in the treatment of drug-resistant disease.

**Why continued development is needed**

Experience has taught us that that prolonged, complex regimens carry an inevitable burden of failure, particularly in communities struggling with poverty, poor infrastructure and limited medical facilities. Patients in developing countries are seldom protected by sick leave, and job security is a major concern, even during relatively short periods of absence. Thus, patients are often forced to choose between successful therapy and loss of livelihood. The identification and isolation of infectious cases are sporadic, compounding the burden of disease. A significant shortening of therapy remains one of the most pressing objectives in TB drug development. Although adding rifampin and pyrazinamide to existing INH-containing regimens revolutionized treatment and reduced the total duration of treatment to 6 months [16], further reductions in the duration of therapy are needed and will depend on a deeper understanding of the biology of the disease and the development of new agents.

**Drug development at the crossroads**

The daunting costs of new drug development continue to plague the field. Unlike other antimicrobials used in developed societies for common infections, anti-TB drugs promise limited financial return from the indigent communities where they are most needed. Mired in this discouraging economic environment, TB drug development has languished for more than 30 years. Rifapentine, approved by the US FDA in June 1998, was the only novel TB drug to reach the US market since rifampin was introduced in the 1970s. Like rifampin, rifapentine targeted the bacterial RNA polymerase. Similarities in microbiological activity made it unlikely to be of value in shortening the current duration of therapy or in treating rifampin-resistant infections. Rifapentine has offered limited new benefits, primarily the consequence of a long 10–15-h half-life and a longer dosing interval. However, the lack of companion drugs with equally long half-lives has resulted in the development of rifamycin mono-resistance in some rifapentine-treated patients with concurrent HIV infection [21].

Within the past few years, several factors have come together, resulting in a resurgence of TB drug development. Amid growing international concerns about untreated drug resistance, an infusion of new research and development support from the Bill & Melinda Gates Foundation, the Rockefeller Foundation, several developed economy governments and other sources have reshaped the practical landscape. Collaborative partnerships have been formed that rely on public and private funding, such as the Global Alliance for TB drug development, and pave the way for integrated research programs and opportunities for drug discovery [22]. For the first time in decades, investigative drugs for TB have begun to enter the pipeline [23].

Advances in the understanding of the biology of the organism have also bolstered the discovery of new drugs. Elucidation of the mycolic acid [24] and other synthetic pathways critical to the survival of *M. tuberculosis* has produced a new array of potential microbiological targets. Among others, novel assays, such as flux balance analyses [24] and microarray hybridization, used to explore changes in gene expression [25], may be of use in screening for drug activity and drug targets.
Strategies for drug discovery

- Investigating antibiotics used for other infections

*M. tuberculosis* shares several targets for antimicrobial activity with other bacteria. This has prompted investigation of the antitymocobacterial activity of antimicrobials already in use for other infections. Fluoroquinolones targeting the DNA gyrase and topoisomerase of other bacteria have already assumed a major role in the therapeutic armamentarium against drug-resistant TB, and ongoing studies are investigating the use of the 8-methoxyquinolones moxifloxacin and gatifloxacin in the treatment of drug-sensitive TB. Among other products that have been investigated, amoxicillin/clavulanate, representing the combination of a penicillin and a β-lactamase inhibitor, has shown some early bactericidal activity when used alone for 7 days in patients with TB [26]. Linezolid, representing the class of oxazolidinones, has shown *in vitro* activity against *M. tuberculosis* and possible *in vivo* efficacy in patients with MDR-TB, although substantial neurotoxicity and hematological toxicity were seen during extended therapy [27]. *In vitro* experiments on dormant *M. tuberculosis* in anaerobic culture suggest that metronidazole may have bactericidal activity in this setting [28].

- Empirical discovery

This approach involves the empirical screening of existing compounds for activity against *M. tuberculosis*. Various high-throughput screening techniques using whole cells have been applied to large libraries of compounds [29], resulting in the identification of thousands of potentially active agents. Promising compounds face a battery of testing to characterize potency, mechanism of action, pharmacokinetic performance, safety and efficacy *in vitro* and in animal models, for example. Our existing anti-tuberculous drugs have all been developed using this screening approach.

- Targeted discovery

While the current TB drugs were discovered simply by screening for whole-organism killing, targeted discovery is a strategy that exploits the growing database of mycobacterial enzymes and other critical biological drug targets. Genes, such as *icl*, *relA*, *dnaE2* and *pcaA*, with a possible role in mycobacterial persistence have been identified and may facilitate the development of drugs to combat persistent organisms. Active agents can be ‘custom designed’ for their interaction with target molecules and interference with the function of the target may form the basis of an *in vitro* ‘readout’ on the performance of new agents. Leading compounds can then be synthetically altered to maximize potency or other desired properties [30,31].

Hopes for new drugs

These converging approaches have yielded a handful of novel compounds in various phases of human study (Figure 1).

- 5-nitroimidazoles

This class of agents, including the US-approved metronidazole and tinidazole, has generated interest based on activity in anaerobic environments and a novel target unrelated to the targets of existing antimycobacterial drugs. The mechanism of action includes an effect on the late stages of mycolic acid synthesis in the mycobacterial cell wall. Early *in vitro* data demonstrated activity of investigational nitroimidazoles (nitroimidazopyran and nitrodi-hydro-imidazooxazole) against drug-sensitive and drug-resistant isolates of *M. tuberculosis*, and a dose-related effect on tissue burden and survival in animal studies [32,33]. Activity has been demonstrated in dormant cultures and in the latter months of experimental therapy in animals, suggesting that these drugs may act on dormant and possibly persistent organisms in humans [34]. Preliminary human studies have been conducted with two new candidate compounds in this class, PA-824 and OPC-67683 [35,36].

- Diarylquinolines

TMC 207, the prototype of this unique class, acts by inhibiting mycobacterial ATP synthase [37]. *In vitro* studies have confirmed activity against drug-susceptible and drug-resistant isolates of *M. tuberculosis*, and early bactericidal studies in humans have paved the way for the first clinical efficacy study. The drug has shown notable efficacy in hastening the sterilization of sputum cultures when studied in a small number of patients with MDR-TB [38].

- Ethylenediamines

SQ109 (identified by high-throughput screening of 63,238 synthetic ethambutol analogs) is active *in vitro* against drug-sensitive and drug-resistant strains of *M. tuberculosis*, including strains resistant to ethambutol, which suggests a novel
mycobacterial target. In mice, this compound decreased the concentration of viable mycobacteria with potency similar to ethambutol [39]. An early study in humans has been performed to examine the safety and pharmacokinetics of the drug [40].

LL-3858 is a pyrrole derivative shown to have in vitro and in vivo activity against drug-sensitive and drug-resistant strains of M. tuberculosis and a dose-escalation trial in 156 healthy volunteers has been performed [41].

**Safety**

The need for prolonged use in humans at effective doses raises concerns about the long-term safety of these new compounds, requiring characterization in adequate numbers of human subjects for representative durations of therapy. Drugs

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**Figure 1. New drugs in clinical development for TB.**
for TB are used in combination, and patients are often receiving simultaneous treatment for HIV and other conditions. Hence, a thorough understanding of the drug–drug interactions is integral to the safe use of these products.

**Challenges in translating microbiological efficacy into clinical efficacy**

Demonstrating activity in actively dividing microbiological cultures is a first step in characterizing a drug’s clinical efficacy. However, the challenge unique to TB is to characterize the efficacy of the drug in the face of persistent organisms; dormancy may be one of the factors allowing persistence of some organisms despite therapy. Although several models have been developed for bacterial dormancy, including oxygen starvation [42], nutrient deprivation [43] and rifampicin conditioning [44], the biology of dormancy is not well understood and the relationship between bacterial dormancy and persistence is not clear. None of these models has yet been shown to reliably predict a new drug’s sterilizing activity: the ability of a new drug to eradicate persistent bacilli or prevent persistence in humans. Demonstrating these properties still relies on lengthy clinical trials designed to examine the rates of disease relapse in the 1–2 years following the completion of therapy.

Animal models of disease may fail to reflect how therapies will perform in the settings of cavitation, fibrosis and calcification. These hallmarks of human pathology present their own challenges to the activity of new drugs, since the blood supply to such lesions may be poor and drug delivery may be compromised. Various bacteriological surrogate end points for the cure of TB are being explored in human subjects but, until these have been validated to reliably predict relapse, any attempts at treatment shortening are destined for arduous and prolonged clinical trials that compare regimens incorporating new agents with existing regimens.

**Future perspective**

Rejuvenation of the drug-development pipeline for TB is a source of enormous encouragement for the millions of individuals infected with *M. tuberculosis* and the institutions that take care of them. It is hoped that advances in the discovery of new agents will carry in their wake new initiatives to develop biomarkers for treatment responses and *in vitro* models for the screening of novel compounds. Novel markers for drug efficacy and safety would expedite the discovery of new drugs and could reduce the scale of clinical trials needed to develop these products. New drugs with novel targets would also bring hope to combating the growing scourge of drug-resistant disease. An expanded armamentarium may simplify treatment in HIV-infected patients, avoiding problematic drug interactions with antiretroviral agents. New drugs may supplant the toxic drugs currently used to treat drug-resistant disease. Finally, new drugs may also carry the prospect of a significant reduction in treatment duration in both drug-sensitive and -resistant disease, which will constitute the most important step toward eradication of this global problem.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Bibliography

Papers of special note have been highlighted as:
- of interest
  - of considerable interest

15. Comprehensive review of the successes and failures in clinical anti-tuberculous drug development over 40 years.
27. Genetic markers and their functional correlates in Mycobacterium tuberculosis are discussed in relation to new and existing drug targets.
33. Provides a summary of strategies for drug discovery and relevant considerations specific to TB.
35. Reviews techniques for screening the activity of new drugs.


