Challenges in tuberculosis drug research and development

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The present decade has seen a reawakening of tuberculosis (TB) drug research and development (R&D), spurred on by an urgent need to stem the tide of the disease globally and develop new, more effective treatments against drug-sensitive and resistant strains. As a result, there are now seven products in clinical development and the largest pipeline of early-stage projects and compounds in history. The primary goals of this resurgent activity are to shorten and simplify the treatment of active TB, provide safer and more efficacious treatments for drug-resistant TB, simplify treatment of TB-HIV coinfections by eliminating troublesome drug-drug interactions, and shorten treatment for latent TB infection. Successful development of new, safe and effective TB therapies faces a number of challenges, some unique to TB drug R&D, many with implications for other therapeutic indications.

In February 2000, TB and global health stakeholders gathered in Cape Town, South Africa and declared an urgent need for development of improved TB treatments[1]. The reasons were obvious. Two billion people worldwide are estimated to be infected with *Mycobacterium tuberculosis*, the bacterium that causes TB, although less than one percent of these have active tuberculosis at any given time. The rest are referred to as having latent TB infection (LTBI). Of the approximately nine million new cases of active TB each year, all but approximately 425,000 are estimated to be sensitive to current therapy, a regimen that routinely demonstrates greater than 95% efficacy in clinical trials. However, despite the potential effectiveness of standard therapy for drug-sensitive disease, there are close to two million deaths attributable to this disease each year. The urgency for improved treatments is driven by the fact that globally TB is not being controlled effectively with presently available treatment, particularly in parts of the world with limited public health infrastructure, high HIV incidence, or both[2,3]. The limited effectiveness of current therapy stems largely from the lengthy and complicated nature of first-line treatment for active TB: a six- to nine-month course of four drugs in combination (two months of isoniazid, rifampin, pyrazinamide and ethambutol, followed by four to seven months of isoniazid and rifampin). *M. tuberculosis* shows a still poorly understood ability to persist in very low numbers for long periods in human and animal hosts despite treatment with drugs to which it is genetically sensitive. This phenomenon, called phenotypic resistance or tolerance, is also commonly referred to as 'persistence', and the bacilli that remain in the host for relatively long periods despite appropriate drug treatment are referred to as 'persistors'. Investigators have demonstrated that isoniazid alone at the standard dose kills over 90% of the infecting mycobacteria in the first two days of treatment[4]. Yet, it takes months of isoniazid-containing combination drug therapy to eradicate the relatively few remaining persistors and ensure that patients won't relapse once therapy is stopped.

The difficulty in killing low infecting numbers of *M. tuberculosis* organisms is also highlighted in the treatment of LTBI. The present standard of care requires nine months of therapy with isoniazid to eradicate an extremely small population of cells, which are thought to exist in a 'latent' form. The relationship, if any, between persistors and latent organisms is not understood, although they share the common distinction of requiring lengthy periods of therapy despite genotypic sensitivity to the treating agents.

The most problematic issue with the current first-line TB regimen is that inadequate adherence to the treatment course, attributable to its length, complexity and associated adverse effects, is driving selection of much more difficult- and expensive-to-treat multi-drug-resistant tuberculosis (MDR-TB) strains. Inadequate adherence to treatment occurs despite extensive global efforts by the World Health Organization (WHO), ministries of health, and others to implement the highly labor-intensive TB treatment program known as DOTS, which includes direct observation of treatment by public health workers. The WHO has estimated that in 2004 there were 424,203 cases of MDR-TB globally; 181,408 occurred in patients who had already been treated with standard (first-line) therapy[3]. Treatment for MDR-TB typically requires 18–24 months of combination therapy with second-line drugs that are less efficacious, more toxic and much more expensive than the four first-line drugs. Recently, a subset of MDR-TB strains has been identified as 'extensively (or extremely) drug-resistant' (XDR-TB). These are now defined[5] as being resistant not only to isoniazid and rifampin, but also to fluoroquinolones and to at least one of three injectable drugs usually employed in second-line therapy of MDR-TB: capreomycin, kanamycin and amikacin. A recent survey conducted by the WHO and the US Centers for Disease Control and Prevention (CDC) on 2000–2004 data found evidence of XDR-TB strains in all regions of the world. XDR-TB was found most frequently in areas of the former Soviet Union and Asia, but even in the United States 4% of MDR-TB cases met the criteria for XDR-TB. In Latvia, a country with one of the highest rates of MDR-TB, 19% of MDR-TB cases met the XDR-TB criteria[6]. In some cases, XDR-TB has been shown to represent a particularly aggressive form of TB, causing very high mortality and, at least in one

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setting, killing HIV-positive patients within an average of 25 days from diagnosis[7,8].

TB treatment in HIV-positive patients is further complicated by drug-drug interactions between some of the antiretroviral agents (ARVs) and key antituberculous drugs, especially rifampin. It is very difficult, especially in resource-constrained settings, to accurately adjust dosages of the drugs in order to safely and effectively treat individuals affected by both TB and HIV[9].

Goals of improved TB therapy

Improving TB therapy can be conceived as having four primary goals: shortening and simplifying the treatment of active, drug-sensitive TB; improving therapy of drug- resistant disease; being able to simultaneously treat TB and HIV-AIDS; and shortening therapy of LTBI (see **Box 1**). The limitations of current TB treatment drive the need for shorter, simpler, yet still-affordable regimens to treat active, drug-sensitive TB. A markedly shorter treatment regimen for active TB would have an extraordinary impact on TB patients and public health systems, rendering treatment adherence much easier, potentially obviating the need for DOTS, and drastically reducing the selection drive leading to development of new drug-resistant strains of *M. tuberculosis*. Achieving this goal will, however, require overcoming the still perplexing phenomenon of persistence.

To effectively treat and control current MDR- and XDR-TB cases, physicians and national TB treatment programs require regimens based on safer, more tolerable and more efficacious drugs that also have new mechanisms of action. To eradicate the two billion–person reservoir of future cases comprising those who at present are latently infected with *M. tuberculosis*, there must be a treatment that is shorter than, and at least as safe and efficacious as, the presently recommended prolonged course of isoniazid. Whether new types of drugs that overcome the persistence phenomenon in active disease will also be effective against latent infections remains to be seen, but may be a key factor in markedly improving LTBI treatment in the foreseeable future.

The current TB drug pipeline

In February 2000, at the time of the Cape Town Declaration, there was a dearth of TB drug development activity. Largely owing to a lack of market incentives, no major pharmaceutical company was expending significant resources to develop new types of TB drugs, and only a handful of smaller, biotechnology companies had active programs[10]. Rifamycins, first introduced for TB treatment in 1966 and approved by the US Food and Drug Administration (FDA) in 1971, were the last new drug class added to the TB armamentarium. Despite the relatively short time since the Cape Town Declaration, there are now seven drugs in clinical trials being specifically developed for TB (**Table 1**). There are also a significant number of discovery and preclinical projects, with diverse sponsors contributing to these efforts. This represents the most active pipeline for TB drug development in known history.

The two furthest advanced of the seven clinical candidates are the 8-methoxy-fluoroquinolones gatifloxacin and moxifloxacin (see **Table 1**). Gatifloxacin, being developed by a consortium of public- and private-sector partners for an active, drug-sensitive, pulmonary TB indication, is now being evaluated in combination with isoniazid, rifampin and pyrazinamide in a four-month regimen (two months of daily gatifloxacin, isoniazid, rifampin and pyrazinamide followed by two months of thrice-weekly gatifloxacin, isoniazid and rifampin) versus standard six-month, first-line therapy, in an open-label, noninferiority, multicenter, phase 3, randomized, controlled trial in Africa. Patients are being followed for relapse during the two years following treatment completion for the primary efficacy endpoint. A particular issue for this program's ultimate success is the reported increased risk of dysglycemia with this compound compared to other fluoroquinolones, primarily but not exclusively in the elderly or diabetic[11]. In the United States and Canada, these data resulted in a change in label, contraindicating prescription of gatifloxacin for diabetic patients (February 2006).

Moxifloxacin, being evaluated under the umbrella of a Bayer Healthcare–Global Alliance for TB Drug Development (TB Alliance) partnership (see **Table 1**), is being tested in two different combination regimens. The first regimen, whose evaluation began as an academic exercise by investigators before animal model data were available, substitutes moxifloxacin for ethambutol in the standard first-line regimen. In the phase 2 Tuberculosis Trials Consortium (TBTC) Study 27 (ref. 12), this regimen was demonstrated to be just as efficacious as the standard control regimen in converting patient sputum to culture-negative after two months' treatment and showed a favorable safety and tolerability profile. A *post hoc* analysis further demonstrated that moxifloxacin substituted for ethambutol resulted in a greater rate of sputum culture conversion at early time points (four and six weeks) than the control treatment. A second, similar phase 2 trial of this regimen is now underway and has almost completed enrollment in Brazil (R. Chaisson, Johns Hopkins University, personal communication). Data from the mouse model, acquired after these trials were initiated, are entirely consistent with the Study 27 results and indicate that moxifloxacin is likely to be most efficacious when substituted for isoniazid rather than for ethambutol in the standard first-line regimen[13]. This combination treatment is now under evaluation in a double blind, randomized, controlled phase 2, two-month treatment trial (TBTC Study 28). A phase 3 trial, to include both these regimens if Study 28 data are supportive, is being planned for initiation in the second half of 2007 (REMox TB trial). This trial will evaluate the ability of moxi-

Box 1 Goals and challenges of TB therapy

Goals

1. Shorten and simplify the treatment of active, drug-sensitive TB.
2. Improve efficacy and safety and shorten duration of therapy for drug-resistant disease.
3. Develop drugs for those with TB who are coinfected with HIV that can be readily coadministered with ARVs.
4. Shorten therapy of latent TB infection.

Challenges in reaching these goals

1. Elucidate the biological mechanisms of mycobacterial persistence and latency.
2. Discover and develop new drugs that have novel mechanisms of action and are effective against persistent bacilli.
3. Develop and validate animal models that reliably predict human treatment duration.
4. Develop and validate biomarkers and surrogate endpoints that predict efficacy and thereby shorten clinical trial duration.
5. Develop new preclinical approaches to identifying optimized drug combinations and new clinical and regulatory approaches to testing drug combinations in phase 2 and 3 clinical trials.
6. Enhance capacity to conduct clinical trials in high-burden countries.

floxacin-based regimens to shorten treatment to four months with safety and efficacy not inferior to the current standard six-month therapy for active, drug-sensitive, pulmonary TB in HIV-positive and HIV-negative adults.

TMC207 (Tibotec), the next-most-advanced compound in development, is a diarylquinoline, now in phase 2 clinical development. First identified in a whole-cell screen[14], TMC207 represents a new drug class for TB, is equally potent *in vitro* against drug-sensitive and drug-resistant strains of *M. tuberculosis*, and has been demonstrated to inhibit the bacilli's ATP synthase. As recently reported[15], although the compound is extremely potent with excellent *in vitro* and mouse *in vivo* activity, there are at least three potential obstacles to its successful registration as a first-line TB treatment: first, it demonstrates a two-fold increase in serum concentration when delivered in the fed versus the fasted state, which could complicate its appropriate delivery to TB patients in a variety of settings globally; second, it is metabolized by the cytochrome P450 3A4 enzyme, and its serum concentration is therefore reduced by rifampin, one of the cornerstone drugs for first-line TB treatment (by 50% percent in a recent trial); and third, TMC207's early bactericidal activity (EBA, a measure typically used as a proof of concept in TB drug development for lack of other biomarkers—see discussion below) in adult patients with active, drug-sensitive, pulmonary TB, is minimal for at least the first four days compared to either isoniazid's or rifampin's. However, in data from Days 5 to 7 of this study, TMC207 at 400 mg per day demonstrated an EBA similar to that of either isoniazid or rifampin during this same time period. Promisingly, TMC207 has demonstrated a good safety and tolerability profile so far, with linear pharmacokinetics in humans. Tibotec is planning to further evaluate the activity of TMC207 in a phase 2 trial in MDR-TB patients. This setting is expected to provide an optimized opportunity to demonstrate potential efficacy, owing to both the absence of rifampin in MDR-TB treatment regimens and the relatively low efficacy of the control, standardized MDR-TB regimen.

Another exciting class of compounds that contributes two candidates to the list of products now in development is the nitroimidazoles. Although they are known to act through a new mechanism of action for TB treatment, the exact intermediaries of activity have not yet been fully elucidated. These two compounds have been demonstrated to be prodrugs whose nitroreductive activation is likely to lead to formation of a number of radicals[16,17], in turn leading to bactericidal activity, presumably through negative effects on a range of critical bacillary functions.

Otsuka Corporation is at present evaluating its nitroimidazo-oxazole, OPC-67683, and the TB Alliance is evaluating the nitroimidazo-oxazine PA-824, originally discovered at Pathogenesis and subsequently outlicensed from Chiron Corporation (now Novartis). OPC-67683 is reported[18] to have completed phase 1 safety, tolerability and pharmacokinetic testing and a 7-day, EBA, proof of concept study at a dose of 400 mg. A new formulation, intended to minimize a fed-versus-fasting effect on drug pharmacokinetics, is being tested in an extended (14-day), multiple-dose, multicenter, EBA study now in progress in South Africa with a standard four-drug control regimen. PA-824 has been tested for safety, tolerability and pharmacokinetic parameters under an investigational new drug application in the United States and at present is being planned for evaluation in a 14-day, EBA study, to be conducted in South Africa. If either or both compounds demonstrate significant EBA with an acceptable therapeutic window between efficacious dose and maximum tolerated dose, the next step will be longer-term efficacy trials—most likely two-month, combination-regimen phase 2 studies to assess superior ability to convert patient sputum to culture-negative, as well as to further assess longer-term safety.

Lupin Ltd. is developing a new pyrrole, known as sudoterb or LL-3858. In a mouse model, this compound, when administered in combination with first-line drugs, is reported to have sterilized lungs and spleens in less time than the standard first-line regimen[19]. It is now in multidose phase 1 clinical development.

The seventh compound known to be in clinical development is Sequella Inc.'s SQ-109 (ref. 20). This compound, an ethylenediamine, is believed to have synergistic interactions with both isoniazid and rifampicin[21]. It has recently entered phase 1 evaluation, and results of the first-in-human study are expected in the first quarter of 2007 (ref. 22).

The current discovery and preclinical pipeline feeding the clinical development portfolio must be robust, even more so than it is today, if it is to adequately support the goals for improved TB therapy described above. To that end, the Bill & Melinda Gates Foundation has recently initiated a TB drug development 'accelerator' program to stimulate research

Box 2 Factors contributing to the long duration of TB drug clinical development

1. Limited biomarkers of drug efficacy for use in early clinical development. Well validated efficacy predictors that could be used in phase 1 and 2 to decide whether to advance a compound to late-stage trials would help streamline clinical development. At present, the best substantiated and most broadly accepted biomarker for assessing efficacy in a phase 2 trial is the two-month sputum culture conversion rate. Serial sputum colony counts (SSCC) with nonlinear mixed-effects modeling may prove to be a better biomarker, in that statistically significant differences appear to be achievable with considerably fewer patients[29]. There are, however, no data yet that compare SSCC results with the gold-standard efficacy endpoint of treatment failure and relapse rates in the one to two years following treatment completion. The ongoing gatifloxacin phase 3 trial will provide the first such data.

2. Long doubling time of *M. tuberculosis*. The 24-hour doubling time of *M. tuberculosis* means that standard microbiological endpoints based on culture of mycobacteria from sputa require at least six weeks of growth for solid media–based techniques and typically three to four weeks for liquid culture–based techniques.

3. Lengthy treatment periods. Current TB treatment for active, drug-sensitive disease is highly efficacious and therefore must be included in any pivotal trial as a control arm. This necessitates a minimum six-month treatment period in any phase 3 trial.

4. Requisite long patient follow-up times. Presently, the only validated efficacy endpoint for pivotal, phase 3 trials requires following patients for one to two years after completion of the full treatment regimen to measure the combined failure and relapse rate of a TB therapy. Data indicate that six-month post-treatment follow-up rates might be an acceptable surrogate, as the majority of relapses occurring in the first two years occur in the first six months of follow-up after treatment completion[30,31]. Recently, efforts have begun to identify surrogate endpoints that could be measured at the end of treatment, or even earlier, and would accurately predict long-term relapse rates. Such a surrogate, once validated, would dramatically decrease pivotal trial timelines.

5. Relatively large patient numbers. Current TB treatment's high efficacy means that demonstrating even noninferiority of a new regimen requires a relatively large number of patients per arm, and hence relatively long enrollment periods.

Table 1 TB drug candidates in clinical development

Compound	Development stage	Sponsor or coordinator
Gatifloxacin	Phase 3	OFLOTUB Consortium; European Commission; WHO TDR; Lupin Ltd.
Moxifloxacin	Phase 2, 3	Bayer; TB Alliance; CDC; University College of London; Johns Hopkins University
TMC207	Phase 2	Tibotec
OPC-67683	EBA	Otsuka Pharmaceutical Co., Ltd.
PA-824	Phase 1	TB Alliance
LL-3858	Phase 1	Lupin Ltd.
SQ-109	Phase 1	Sequella, Inc.

WHO TDR, World Health Organization Tropical Disease Research; CDC, US Centers for Disease Control and Prevention.

that would lead to identification of persistence targets, and to further develop and validate relevant animal models. Positive outcomes from this initiative and related research funded by other sources such as the US National Institutes of Health and the European Commission would be a crucial stimulant to successful TB drug development.

Challenges for drug development

Identification of drugs that will shorten treatment and thereby improve adherence is key to radically improving active TB treatment, decreasing demands on national TB control programs, and preventing further selection of resistant strains. Ideally, finding such drugs would be based on knowledge of the underlying mechanisms of mycobacterial persistence, enabling identification of crucial targets. At present, both a clear understanding of persistence mechanisms and fully validated animal models that reliably predict human treatment duration are lacking, and thus so is an efficient path to developing drugs for shortening treatment. The mouse model[23] appears to reflect human treatment results in most but not all circumstances, but it lacks adequate prospective data to be considered truly validated at this time. In the absence of fundamental biological understanding of persistence, shortening therapy of active disease to days rather than months is likely to remain a distant goal. Realistically, current animal model evidence and clinical data indicate that shortening treatment to three to four months should, however, be achievable even with combinations of current and new drugs already in the pipeline.

A second challenge for TB drug R&D is the long timeline of clinical trials. Phase 2 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. These relatively long periods result from a number of factors (see **Box 2**).

The requirement for multidrug therapy represents one of the crucial challenges for TB drug R&D, as it has several repercussions that affect the R&D process. First, because as little as a few weeks of monotherapy may lead to the development of drug resistance[24–27], it is not ethical to test single drugs beyond the stage of EBA studies (which have maximally involved 14 days of treatment). Furthermore, current therapy, being highly efficacious, should not be withheld for any longer than necessary. This means that any new drug must be evaluated for efficacy in the context of combination therapy. Second, the need for combination therapy necessitates extensive drug-drug interaction studies, not only with potential concomitant medications, such as antiretroviral agents, but also with other drugs in the proposed TB treatment regimen. The fact that people must be treated at present 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 (an estimated one year for phase 1, two years for phase 2, and three years for phase 3 as an aggressive clinical development timeframe), but at least four × six years—over two decades—just for the clinical phase of development. Clinical development for TB drugs would be much more efficient if the unit of drug development were considered to be the combination regimen rather than the single new agent. This strategy can be thought of as analogous to vaccine development approaches, in which a new vaccine may include a number of distinct antigens, all of which are developed together as a single 'candidate'. Identifying and developing a three- or four-drug regimen as a single unit would cut development time dramatically to within a timeframe commensurate with the urgency of the need for improved TB treatments.

New regimens that take maximal advantage of even the existing TB drug pipeline and dramatically shorten the treatment duration for active disease will most probably require more than one new drug. In order to develop a new regimen that contains more than one new chemical entity, we propose preclinical and full phase 1 safety, tolerability and pharmacokinetic testing of each individual drug, in parallel with *in vitro* and *in vivo* preclinical evaluation of potential drug combinations to identify optimized candidate regimens. A preclinical approach has been proposed by the FDA in draft guidance for nonclinical testing of new drug combinations[28]. An optimized candidate combination regimen could then be advanced into phase 2 testing as a development unit, perhaps both in EBA and in two-month treatment trials for proof of concept and dose finding, and then, if the data are supportive, into full, phase 3, safety and efficacy testing. Although it would be intellectually gratifying and regulatorily preferable to understand the contributions to safety and efficacy of each individual drug in a combination regimen based on comparative human data, this is not possible for TB drugs. The current first-line regimen was developed in an era where such regulatory requirements did not exist and the necessary trials were never conducted to acquire most of the relevant data for current first- or second-line TB drugs. As it is now unethical to test these drugs individually in TB patients for safety and efficacy beyond one to two weeks of treatment, it is not possible to document clinically the individual contributions of each component in the context of a combination regimen in TB.

Another challenge in TB drug development is presented by the very high efficacy of the current standard regimen for active, drug-sensitive disease—routinely over 95% under trial conditions. As a result, a new regimen must be tested for noninferiority rather than superiority compared to a standard control arm to avoid impractically large patient numbers and the very small 'window' in which a new regimen could possibly be shown to be 'superior' to standard treatment. However, superiority of a new TB regimen may be demonstrated by providing convincing data that clinically significant shortening of treatment duration with the new drug combination is 'noninferior' to standard therapy.

TB clinical development may be impeded further in coming years by a lack of adequate clinical trial capacity for the conduct of both EBA and late-stage clinical trials to registration and internationally accepted standards of good clinical practice and good laboratory practice. As most TB patients are in low- and middle-income countries, late-stage clinical trials must and should be conducted in these high-burden settings.

The dearth of new TB drug development, particularly of trials to support regulatory registrations, over the past 30 to 40 years means that relatively few sites are experienced in the appropriate conduct of clinical trials. The TB Alliance has recently conducted a baseline assessment of over fifty clinical sites, including their mycobacteriology and safety laboratories, in 25 countries on five continents. Although the data from this study are still being analyzed, it is clear that developing adequate capacity to fully evaluate even the seven compounds now in the clinic will require significant capacity-building over the next few years at a number of sites throughout the world. Although approximately one-third of all TB patients are now in India and China, the WHO-identified 22 high-burden countries for TB are scattered throughout Asia, Africa, Eastern Europe and South America, and the disease has been identified in every country investigated. Global registration of new, improved treatment regimens will therefore require safety and efficacy testing in appropriately diverse populations.

Last, despite the recent, significant contributions of major funders such as the Bill & Melinda Gates Foundation, the Rockefeller Foundation and a number of governments throughout the industrialized world, a key challenge for this field remains increased and sustainable funding to meet the demands of research to fight TB. Improved therapies for this disease must not only be discovered and developed, but must also be made affordable, accessible and adopted throughout the world to help eliminate TB as a major public health problem and leading infectious killer.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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