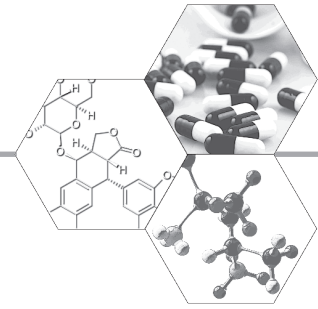

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Challenges and opportunities in developing novel drugs for TB

Mycobacterium tuberculosis is a difficult pathogen to combat and the first-line drugs currently in use are 40–60 years old. The need for new TB drugs is urgent, but the time to identify, develop and ultimately advance new drug regimens onto the market has been excruciatingly slow. On the other hand, the drugs currently in clinical development, and the recent gains in knowledge of the pathogen and the disease itself give us hope for finding new drug targets and new drug leads. In this article we highlight the unique biology of the pathogen and several possible ways to identify new TB chemical leads. The Global Alliance for TB Drug Development (TB Alliance) is a not-for-profit organization whose mission is to accelerate the discovery and development of new TB drugs. The organization carries out research and development in collaboration with many academic laboratories and pharmaceutical companies around the world. In this perspective we will focus on the early discovery phases of drug development and try to provide snapshots of both the current status and future prospects.

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Need for new drugs

One point often overlooked regarding existing TB drugs is that the standard four-drug combination is relatively inexpensive and works reasonably well in drug-sensitive (DS) TB patients. The four-drug combination (isoniazid, rifampin, pyrazinamide and ethambutol; **Table 1**) given daily over a period of 6 to 9 months can cure approximately 85% of DS TB patients if the treatment regimen is strictly followed [1]. Although cure rates as high as 95% have been reported, they are not typically observed. If the cure rate is 85%, a follow-up question might be why TB still kills 1.7–1.8 million people every year [2]. The most straightforward answer is that these drugs are far from ideal. However, the more complete answer is undoubtedly manifold and related to not only the current treatments but also rooted in socioeconomic factors. The almost universally accepted standard of care that involves long treatment times and multiple-drug combinations in treating TB patients attests to the fact that the current drugs are not exceedingly efficacious and TB's propensity to develop resistance to any single agent requires the use of combination chemotherapy. The four-drug combination treatment includes 2 months of these four drugs followed by 4 months of isoniazid and rifampin. The reason for this long treatment period is that it is exceedingly difficult to entirely eliminate the causative pathogen, *Mycobacterium tuberculosis* (Mtb), from a patient. Even if culture negativity (i.e., the absence of Mtb in sputum) is achieved in a period shorter than 6 months, should the treatment be discontinued, the organisms will often rebound in numbers causing a relapse. As discussed later, the pathogen Mtb is very well adapted to human infection, and can generally successfully evade the onslaught of human immunological attacks. The reason for combining drugs is that resistant mutants will emerge if only a single drug is used for a long period of time. The concept of TB drug combinations was empirically reached in the 1960s, and, in fact, these four first-line drugs and most of the drugs in the second-line treatment are at least 40 years old. From the 1960s until the present time, very few new TB drugs were introduced into the clinic. The second-line drugs include amikacin, capreomycin, ciprofloxacin, ethionamide, cycloserine and *p*-aminosalicylic acid **(Table 2)**. Second-line drugs, in general, tend to have more adverse effects, and a limited activity profile compared with first-line drugs. Some second-line agents, such as amikacin and capreomycin, have to be given by injection, making administration for an extended duration more difficult especially in resource-restricted regions of the world. As with other infectious diseases, it is inevitable that drug resistance occurs almost immediately after a new agent is introduced to clinic. Those strains of Mtb that are resistant to at least isoniazid and rifampin are termed multidrug resistant (MDR). For MDR-TB patients, the second-line drugs and/or other registered antibiotics are empirically used for approximately 12–18 months. In the past 5 years, Mtb strains resistant to at least isoniazid, rifampin,

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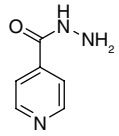
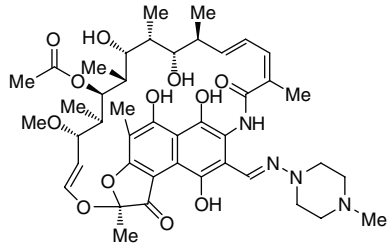
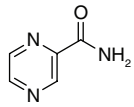
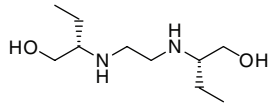
Table 1. First-line drugs.

Name	Structure	Structure number	Mechanism of action
Isoniazid		**1**	Cell wall (inhibition of InhA)
Rifampin (rifampicin)		**2**	RNA polymerase
Pyrazinamide		**3**	Multiple (including intracellular acidification, decrease of delta pH)
Ethambutol		**4**	Cell wall (inhibition of arabinosyl transferase)

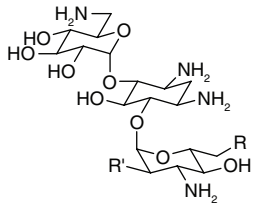
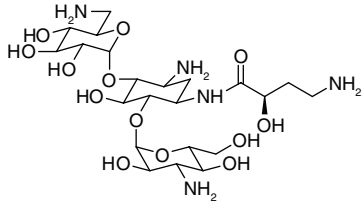
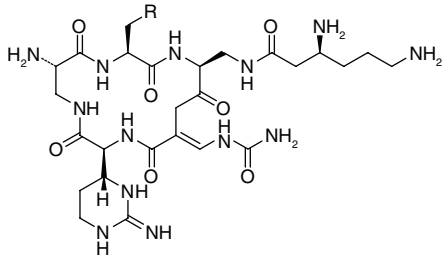
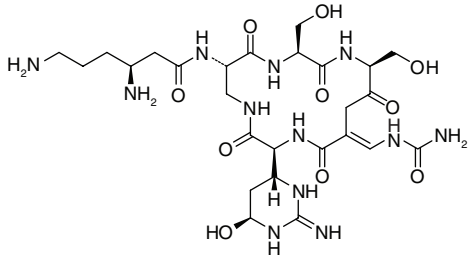
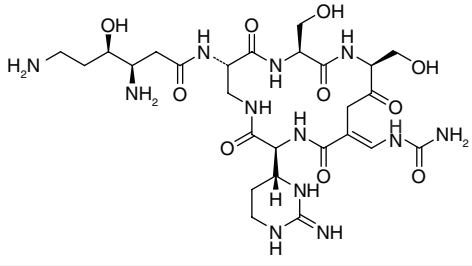
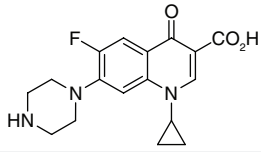
InhA: NADH-dependent enoyl-ACP reductase.

fluoroquinolones and one of the second-line injectable agents have been discovered and such strains have been defined as extensively drug resistant (XDR). In addition, large numbers of TB patients are co-infected with HIV, rendering more complex their treatment for these two infections that both require combination therapy. Ideally, TB drugs used to treat HIV co-infected individuals should have little potential for drug–drug interaction with co-administered antiretroviral drugs. However, this is not always the case; one prominent example of an agent causing drug–drug interactions is rifampin, which induces cytochrome P450 enzymes, leading to a reduction in systemic exposure of some commonly used antiretroviral agents.

The long treatment duration, emergence of resistant strains, adverse effects from many of the existing drugs, and the need for treating HIV–TB co-infected patients make it apparent that there is a desperate need for new TB drugs. Considering the attrition rate and the length of drug development in general, the need for an even larger assortment of new TB drug candidates is equally urgent. The ideal new agent should be rapidly bactericidal (rather than bacteriostatic) and possess potent sterilizing activity to enable a stable cure to be achieved in a shorter time period than for the currently available therapy. As discussed later, there is growing consensus that we need to find drugs that kill nonreplicating as well as replicating bacteria to achieve overall treatment shortening. Ideal new drugs should demonstrate a novel mechanism of action (MOA) to avoid existing anti-TB drug resistance, and should be active against MDR and XDR strains. They should also be safe, well-tolerated and possess ADME properties suitable for co-administration with anti-HIV agents and appropriate for oral dosing, optimally within a fixed-dose combination. It is also imperative that the drugs are available at relatively low cost, especially if intended for DS TB patients in developing countries. The current cost of the first-line drugs is approximately US$20–40 for the full course of treatment [1] and a new drug should not change this substantially. It is also desirable that the drug should be orally efficacious for the ease of administration. Drugs intended for MDR and XDR TB patients should attempt to meet the above characteristics but may be given more leeway.

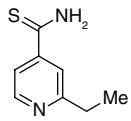
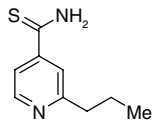
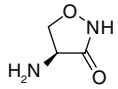
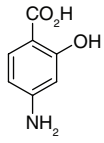
Recent advances in the knowledge of the molecular biology of Mtb have been significant, as spearheaded by the whole genome sequencing in 1998 [3]. More specifically, knowledge of the complete Mtb genome sequence has enabled the essentiality of genes to be established *in vitro* and *in vivo* [4,5]; the use of genome-wide DNA microarrays to study patterns of gene expression

Table 2. Second-line drugs.

Class	Name	Structure	Structure number	Mechanism of action
Aminoglycosides	Kanamycin	 Kanamycin A R=NH_2, R'=OH Kanamycin B R=NH_2, R'=NH_2 Kanamycin C R=OH, R'=NH_2	**4**	Protein synthesis inhibition (binding to 16S subnit of rRNA)
	Amikacin		**5**	Protein synthesis inhibition (binding to 16S subnit of rRNA)
Polypeptides	Capreomycin	 Capreomycin IA R=OH Capreomycin IB R=H	**6**	Protein synthesis inhibition (inhibition of translocation)
	Viomycin		**7**	Protein synthesis inhibition (inhibition of translocation)
	Enviomycin		**8**	Protein synthesis inhibition (inhibition of translocation)
Fluoroquinolones	Ciprofloxacin		**9**	DNA synthesis inhibition (inhibition of gyrase)

InhA: NADH-dependent enoyl-ACP reductase.

Table 2. Second-line drugs (cont.).

Class	Name	Structure	Structure number	Mechanism of action
Thioamides	Ethionamide		**10**	Cell wall biosynthesis (inhibition of InhA)
	Propionamide		**11**	Cell wall biosyntheis (inhibition of InhA)
D-cycloserine	D-cycloserine		**12**	Cell wall biosynthesis (alanine racemase and D-Ala-D-Ala ligase)
p-aminosalycylic acid	p-aminosalicylic acid		**13**	Thymidylate synthase inhibition and interference in iron acquisition

InhA: NADH-dependent enoyl-ACP reductase.

under various growth conditions [6,7] and more rapid target identification for new compounds via identification of the mutated genes of compound-resistant mutants [8]. Although big pharmaceutical companies have recently become more involved, there has been a lack of a concerted effort over the past 40 years,to develop drugs against TB in comparison to other more commercially attractive disease areas. Fortunately, a broad range of government and nongovernment organizations has taken the initiative to fill this gap, with a turning point occurring 10 years ago with the creation of a number of not-for-profit product-development partnerships (PDPs) such as The Global Alliance for TB Drug Development (TB Alliance). In this review, we highlight the challenges and promises of modern-day TB drug discovery and include our personal speculations on the prospects in this field.

The special challenges (Mtb biology)

The biggest challenge to TB drug research is one that is shared with general, broad-spectrum antibacterial drug research; identifying and developing a novel antibiotic is extremely difficult. The need for new antibiotics and the low rate of finding such species (only two new antibiotic series have been brought to market in the last 40 years) have been well publicized [9,10]. Aside from the fact that few pharmaceutical companies are currently working in the antibacterial domain, the current method of antibiotic discovery and development is inherently challenging such as the difficulty in advancing target-based hits [11].

Aside from the specific challenges listed below, several additional barriers in the TB area include: no well-established pharmacokinetic (PK)–pharmacodynamic paradigms; lack of validation and human-like pathology of animal models currently available for drug discovery; lack of adequate clinical laboratories for clinical trials; and lack of adequate research funds. The last point is quite pronounced as pharmaceutical companies have, in recent decades, paid little attention to TB drugs due to the poor return on investment. A very small fraction of global research funding is spent on TB as compared with other therapeutic areas.

■ A persistent pathogen

M. tuberculosis is an intracellular pathogen that upon entering the lung through inhalation is engulfed by highly phagocytic macrophages. It is capable of replicating until the ensuing interaction with the immune system arrests the organisms in a dormant, **nonreplicating state**. As a result of an active immune system, it is believed that a granuloma forms that effectively walls off the offending tubercle bacilli from the rest of the healthy lung, and limits the spread of the infection. Many of these granulomatous lesions eliminate all bacilli while a smaller percentage of others allow persistence of viable Mtb within this microenvironment [12,13]. These persistent organisms are endowed with an ability to avoid elimination even with antibiotic intervention, perhaps due to their restricted metabolism and replication, processes that are typically targeted by current antibiotics.

Key Terms

Nonreplicating state: Phase in which *Mycobacterium tuberculosis* is not growing in a logarithmic fashion and is not increasing in number although individual bacterial cell turnover continues. In animal models *M. tuberculosis* reaches this stage when its growth is held in check, for example, by the active immune system.

Latent TB: Where a patient is infected with *Mycobacterium tuberculosis*, but does not have active TB disease. Approximately 10% of these patients will go on to develop active TB at a later stage of their life. Especially if they develop a disease affecting the immune system (such as AIDS) or a disease whose treatment affects the immune system (such as chemotherapy in cancer or systemic steroids in asthma or Enbrel®, Humira® or Orencia® in rheumatoid arthritis) or in circumstances resulting in malnutrition.

With granuloma formation and an effective human immune response, most tubercle bacilli are killed and disease progression is halted [14,15]. The maturing granuloma exposes the pathogen to a harsh microenvironment characterized by limited nutrients, low oxygen and low pH, which does not support rapid growth but rather induces a state of dormancy in the bacteria [16,17]. **Latent TB** infection (LTBI), presumed prevalent in a third of the world's population, is the sustaining reservoir for this human scourge. This is because the dormant bacilli can inhabit the granuloma during the lifetime of the patient but are able to resume their growth if (or when) the immune response is compromised (reactivation TB). It is estimated that 5–10% of LTBI individuals will develop active TB disease during their lifetime [18]. It should be noted, however, that the risk of developing active disease is 5–10% every year, and the lifetime risk is approximately 50% in HIV co-infected individuals [19–21].

■ Drug resistance

Besides co-infection with HIV, globally, TB is also complicated by increasing drug resistance that first develops against the highly effective first-line drugs. An estimated 5% of all TB cases are now MDR-TB (Mtb strains resistant at least to rifampin and isoniazid), and there were approximately 440,000 new cases of MDR-TB reported for 2008 [22]. In 2006, a new phenomenal level of drug resistance beyond MDR-TB, XDR-TB (MDR-TB strains additionally resistant to a fluoroquinolone and a second-line anti-TB injectable agent such as kanamycin, amikacin or capreomycin), was described, and has since been identified in 58 countries [23]. While MDR-TB has proven to be merely difficult and expensive to treat relative to the DS disease, XDR-TB is essentially untreatable in most of the developing countries, especially as it is usually associated with HIV co-infection [24].

■ Standard therapy

The current, WHO-recommended 'directly observed treatment, short course' standard chemotherapeutic regimen for treating smear-positive, newly diagnosed pulmonary disease consists of a multidrug combination of four first-line TB drugs (isoniazid, rifampin, pyrazinamide and ethambutol), administered for an initial intensive period of 2 months. This is followed by a continuation phase of an additional 4 months of isoniazid and rifampin. Therapy for drug-resistant disease is considerably more complex, and often has to rely on limited drug-sensitivity testing. It is also more costly requiring extended treatment durations with less effective and more toxic drugs. Therapy for LTBI is also protracted and comes in various regimens that may contain any combination of isoniazid [25,26], rifamycin [27–29], pyrazinamide [30,31] and an approved fluoroquinolone, in the case of drug-resistant LTBI [32], and is fraught with additional adverse effects.

Medicinal chemistry considerations (Mtb chemistry)

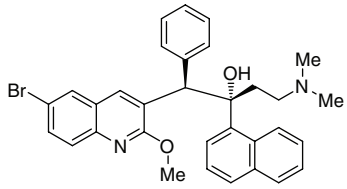
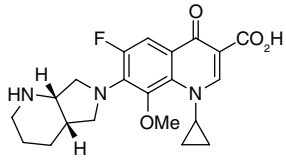
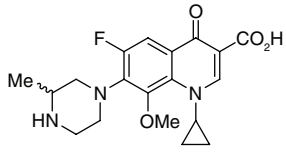
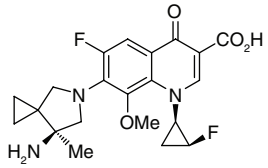
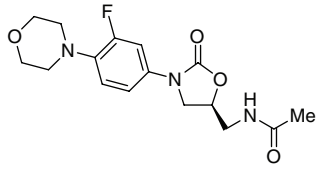
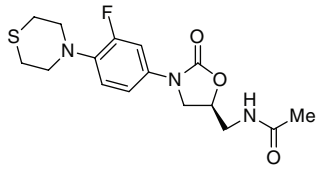
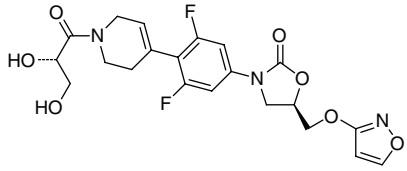
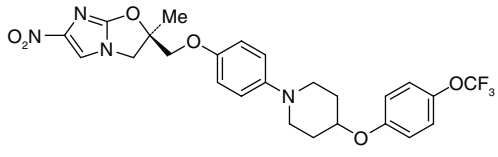
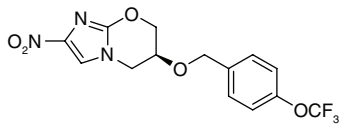
■ Compounds under development

The major drugs currently under development are listed in **Table 3** with their putative MOA. There are several excellent recent reviews of compounds currently under development for TB treatment [33–40] and, therefore, only salient features of these advanced development candidate compounds are summarized below.

TMC207

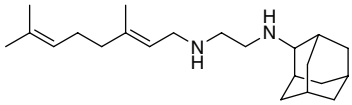
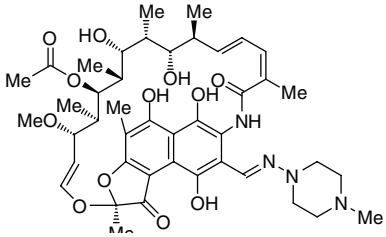
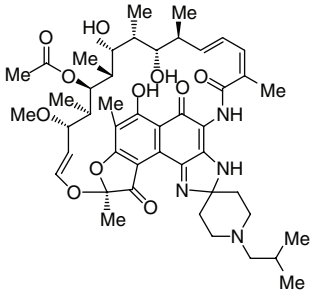
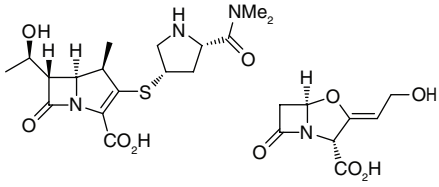
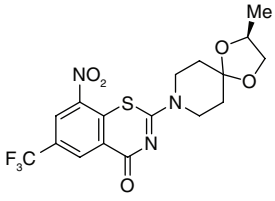
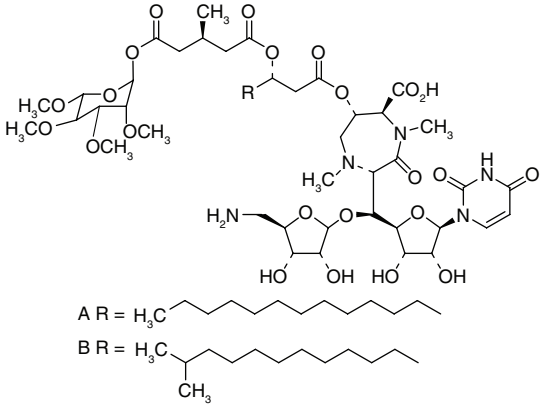
TMC207 **(14)** is a lead compound in the diarylquinoline series originally discovered by scientists at Janssen Pharmaceutica [41], and is currently undergoing Phase II clinical development for both MDR and DS-TB indications. Its MOA is unique among anti-TB agents, inhibiting Mtb ATP synthesis, by interacting with subunit c of its ATP synthase [42]. Subunit c forms an oligomeric structure AtpE of the transmembrane portion of ATP synthase (F_0). Previously, inhibition of ATP synthesis might not have been considered viable as a TB target because of concerns regarding selectivity between the pathogen and host targets. However, this compound has disproved this concern. TMC207 was discovered via whole-cell assay screening of approximately 70,000 compounds from Johnson & Johnson's (Janssen's parent company) corporate library against the surrogate organism, *Mycobacterium smegmatis*. The MOA was later established by analyzing the whole genome sequence of laboratory-generated drug-resistant Mtb mutants. This compound is effective against dormant Mtb organisms, even though ATP biosynthesis is significantly down-regulated in these bacteria [43]. Clinical development for use of TMC207 in MDR-TB treatment is being carried out by Tibotec, a subsidiary of Johnson & Johnson. In addition, TB Alliance has initiated clinical trials to investigate the utility of TMC207 for treatment of DS-TB. In MDR-TB patients, addition of TMC207 to the treatment regimen

Table 3. Advanced compounds under various stages of development.

Class	Name	Structure	Structure number	Mechanism of action	Clinical phase	Ref.
Diarylquinolines	TMC207		**14**	ATP synthesis inhibition (subunit c of ATP synthase)	For MDR-TB, Phase II–III; for DS-TB, Phase II	[221]
Quinolones	Moxifloxcin		**15**	DNA synthesis inhibition (inhibition of gyrase)	Phase III	[222]
	Gatifloxacin		**16**	DNA synthesis inhibition (inhibition of gyrase)	Phase III	[222]
	DC-159a		**17**	DNA syntheis inhibition (inhibition of gyrase)	Preclinical	[109]
Oxazolidinones	Linezolid		**18**	Protein synthesis inhibition		[223]
	PNU100480		**19**	Protein synthesis inhibition	Phase I	[224]
	AZD5847		**20**	Protein synthesis inhibition	Phase I	
Nitroimidazoles	OPC-67683		**21**	Multiple	Phase II–III	[62]
	PA-824		**22**	Multiple	Phase II–III	[225]

DS-TB: Drug-sensitive TB; MDR-TB: Multidrug-resistant TB; MOA: Mechanism of action; MraY: Phospho-MurNAc-pentapeptide translocase.

Table 3. Advanced compounds under various stages of development (cont.).

Class	Name	Structure	Structure number	Mechanism of action	Clinical phase	Ref.
Ethylenediamines	SQ109		**23**	Cell wall biosynthesis but its exact MOA not established	Phase I	[85]
Rifamycins	Rifapentine		**24**	RNA synthesis inhibition (inhibition of RNA polyerase)	Phase II–III (for high-dose admin-istration)	
	Rifabutin		**25**	RNA synthesis inhibition (inhibition of RNA polymerase)		
β-lactams	Meropenenm plus clavulanic acid		**26, 27**	Cell wall biosynthesis (inhibition of transpeptidase and inhibition of β-lactamase by clavulanic acid)	Preclinical	[91]
Benzothiazinones	BTZ043		**28**	Inhibition of cell wall biosynthesis (inhibtion of decaprenyl-phosphoryl-β-D-ribose-2'-epimerase)	Preclinical	[226]
Lipo-uridine antibiotics	Caprazomycin		**29**	Inhibition of cell wall biosynthesis (inhibition of MraY)	Preclinical	[104]

DS-TB: Drug-sensitive TB; MDR-TB: Multidrug-resistant TB; MOA: Mechanism of action; MraY: Phospho-MurNAc-pentapeptide translocase.

Key Term

Colony-forming unit: Measure of the number of bacteria, for example, in sputum (per ml) or in an organ (per gram). Since it is not always easy to count the number of microorganisms, this method estimates the number of microorganisms by counting the colonies formed after a certain period of culturing on solid media.

reduced the time required to convert to sputum culture negativity and increased the proportion of patients with conversion of sputum culture at 8 weeks (48% vs 9%) [44]. A fairly large number of TMC207 analogues have been prepared by Tibotec (see the structure–activity relationships [SARs] paper in this issue by Guillemont [45]) and a preliminary review has been published [46]. The synthesis and biological characterization of newer analogues are being jointly carried out by Tibotec and the TB Alliance. TMC207 helped to validate ATP synthesis inhibition as a new pharmacologically attractive Mtb target, and it is extremely potent compared with other TB drugs. One challenge might be how to address the very lipophilic nature of the compound (Clog P of TMC207 is 7.25) as this may affect PK, drug formulation, and potentially have other consequences.

PA-824 & OPC-67683

PA-824 **(22)** was discovered by scientists at Pathogenesis [47] as a derivative of CGI-17341 **(30)** whose anti-TB activity had been reported several years earlier [48]. It is currently in Phase II clinical trials conducted by the TB Alliance and following 14-day dosing in DS-TB patients, PA-824 (at 200, 600, 1000 and 1200 mg/day) demonstrated bactericidal activity similar to that demonstrated by the standard first-line drug combination [49]. In terms of SAR, the nitro group is essential for anti-TB activity. In addition to the oxazine ring, other six-membered heterocycles were prepared but the anti-TB activity remains essentially only with anoxazine ring and, to a lesser degree, with a thiazine ring [50–52]. A diverse range of 'tail' variations have been synthesized [53–55], and some of them exhibit increased *in vitro* and *in vivo* potency, and are currently in the preclinical candidate stage of development. For its MOA, characterization of PA-824-resistant mutants has revealed that the first step is reduction of the nitro-imidazole moiety by an F420-dependant enzyme (Rv3547, deazaflavin-dependent nitroreductase) in Mtb cells [56]. Interestingly, an extraordinary abundance of F420-based enzymes in mycobacteria has been described [57]. Two-electron reduction actually takes place not at the nitro group but at the imidazole ring and it has been hypothesized that the resulting intermediates generate reactive nitrogen species, including nitric oxide [58,59]. Under anaerobic conditions, a correlation between the amount of des-nitro intermediates and cell killing suggests this as the bactericidal mechanism under these conditions. However, under aerobic conditions the exact MOA is not known although inhibition of mycolic acid biosynthesis may be involved [60]. The apparently multiple MOAs for this class of compounds render optimization of their anti-TB activity rather challenging. At present, a combination therapy including PA-824, moxifloxacin and pyrazinamide is being evaluated in DS-TB patients.

OPC-67683 **(21)** is another analogue of CGI-17431, discovered and currently under development by Otsuka Pharmaceutical Company [61]. This compound contains a fused oxazoline ring instead of an oxazine ring. After treatment with *Mycobacterium bovis*, des-nitro-imidazooxazole was obtained indicating a similar MOA to that of PA-824 [62]. The same paper indicates inhibition of mycolic acid biosynthesis as a potential MOA. A large number of analogues have been prepared by varying the tail portion of the molecule [301]. OPC-67683 is 4–16 times more potent than PA-824 *in vitro* and it does not show cross-resistance with other first-line TB drugs. It is currently in Phase II clinical trials for MDR-TB patients.

A 48-patient study of early bactericidal activity of OPC-67683 was recently published [63]. More patients receiving 200 or 300 mg daily experienced a response of greater than or equal to 0.9 log **colony-forming unit**/ml decline in sputum over 14 days than those receiving 100 or 400 mg. There appears to be a plateau in plasma exposure at 300 mg. It was safe and well-tolerated.

Moxifloxacin & gatifloxacin

Being in Phase III clinical trials, both moxifloxacin **(15)** and gatifloxacin **(16)** are the most advanced compounds currently under development for TB. They are both 8-methoxyquinolones originally developed as broad-spectrum antibiotics and now being repurposed as anti-TB agents. Fluoroquinolones target gyrase and topoisomerase IV in most bacteria but in Mtb it is assumed they target solely gyrase since there is no evidence of topoisomerase IV present in Mtb [64]. Other quinolones, such as ciprofloxacin and ofloxacin, have been used as second-line TB drugs, but moxifloxacin and gatifloxacin are more potent *in vitro* than these older quinolones [65–68]. They are both currently in Phase III clinical trials in DS-TB patients replacing either ethambutol or isoniazid in standard RHZE, first-line treatment [69–72]. Moxifloxacin is being investigated by a large number of organizations

while gatifloxacin is sponsored by Oflotub consortium [69]. The potential adverse effects that have been reported for these drugs are dysglycemia with gatifloxacin and QT prolongation with moxifloxacin [71,72]. In some regions of the world, there is a fairly high incidence of quinolone resistance, (e.g., in India), and use of either of these compounds in TB regimens might prove to be limited in such circumstances.

High dose rifampin & rifapentine

Rifampin (rifampicin) is an important component of the first-line, four-drug combination treatment owing to its potent sterilizing ability. In recent years, there have been efforts to investigate if higher doses of rifampin might contribute to treatment shortening [64,65]. Earlier trials of high-dose rifampin were recently reviewed and it was concluded that higher than standard rifampin dosing results in improved culture conversion rate [66]. High-dose rifampin is being investigated in several clinical trials [401]. In evaluating such an approach, one has to be concerned with potential adverse effects, the most critical being a flu-like syndrome that does not appear to be easily predicted by animal models [67]. Rifapentine **(24)**, a newer analogue in the rifamycin class, is more potent against Mtb and has a longer half-life in human compared with rifampin [68–70]. Rifapentine is currently being examined in Phase II trials by Sanofi-Aventis. The rifamycin class of compounds inhibits Mtb RNA polymerase and mutations in RNA polymerase are the major cause of rifamycin resistance [71]. Among TB drugs, another prominent feature of this class is that it induces cytochrome P456 expression, which can affect the plasma levels of co-administered drugs [72].

Oxazolidinones (PNU-100480 & AZD5847)

Linezolid **(18)**, which represents the oxazolidinone class of antibiotics, is one of only two new antibiotics introduced onto the market in the past 40 years (the other being daptomycin, representing lipopeptide antibiotics), with the main target pathogens being Gram-positive bacteria including MRSA and vancomycin-resistant Enterococci). It has also been shown that linezolid is active against Mtb *in vitro* and in animal models [73,74]. Its target is the Mtb ribosome and it exhibits potent activity against MDR-TB clinical isolates. Linezolid has been used for MDR-TB (and XDR-TB) and additional Phase II clinical trials are ongoing [75,76]. Two additional oxazolidinones are also undergoing clinical development for TB. Pfizer's PNU-100480 **(19)** was actually one of the earliest oxazolidinone antibiotics synthesized, exhibiting increased antimycobacterial activity compared with linezolid, but its potential as anti-TB agent has been recognized and pursued more only recently [77]. Another oxazolidinone, AstraZeneca's AZD5847 **(20)**, was originally intended as a broad-spectrum antibiotic, but has now been repurposed as an anti-TB agent [78]. It is well recognized that with linezolid, treatment periods longer than 14 days may result in hematological adverse effects [79] 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. Phase I studies with PNU-100480 have been completed and a Phase II study is in the planning stages. Two Phase I studies of AZD5847 have also been completed.

SQ109

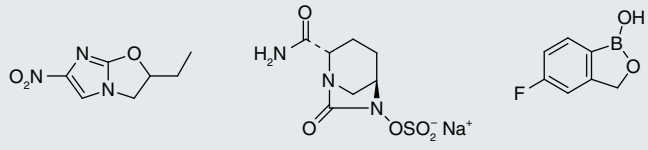
SQ109 **(23)** was identified as an anti-Mtb lead among analogues of ethambutol prepared through combinatorial chemistry [80]. Ethambutol is known to inhibit the synthesis of arabinogalactan, which is a component of the Mtb cell wall [81]. SQ109 is implicated in affecting cell wall biosynthesis, but it is also active against ethambutol-resistant strains, and its precise MOA has not been elucidated and is potentially novel [69,82]. It nonetheless produces a remarkable synergy *in vitro* and in animal models when combined with rifampin, isoniazid, or TMC207 [83–85]. Relatively low bioavailability might be a problem [86], but Phase I studies have been completed and recruitment has begun for a pivotal Phase II study.

Meropenem & clavulanic acid

β-lactam antibiotics have not been used widely against TB mainly due to their lack of efficacy. Only recently has there been some effort to reinvestigate this phenomenon, resulting in important work indicating that deletion or inhibition of the major β-lactamase of Mtb, BlaC, results in activity of β-lactam antibiotics against Mtb [87–90]. A combination of clavulanic acid **(27)**, a β-lactamase inhibitor, and meropenem **(26)**, a carbapenem antibiotic, was shown to have potent activity *in vitro* killing XDR Mtb under aerobic as well as anaerobic conditions [91]. Meropenem was selected because it itself is quite resistant to Mtb BlaC β-lactamase. Very

recently, a much needed *in vivo* study of carbapenems combined with clavulanic acid was published [92]. In *in vitro* assays, combinations of any of the carbapenems (imipenem, meropenem and ertapenem) with clavulanic acid reduced the minimum inhibitory concentration (MIC) of the carbapenem from 8–16 to 1–4 μg/ml. In the *in vivo* study combinations of imipenem or meropenem with clavulanic acid significantly improved survival of mice but the colony-forming unit count reduction was less than that of isoniazid after 28 days of treatment. It is possible that optimal dosing was not achieved since mice were given β-lactams subcutaneously once a day while in humans they are usually given three times a day. There appear to be four successful human cases for use of the carbapenems in treating XDR-TB patients [93,94]. One challenge might be to identify an orally bioavailable penem or carbapenem for ease of administration, since meropenem can be given only by injection. In addition to meropenem and clavulanic acid, there are other novel β-lactam antibiotics and β-lactamase inhibitors [95], and it may be necessary to try various combinations of such agents. One β-lactamase inhibitor in particular, NXL104 **(31; Figure 1)** may be of interest since it is a non-β-lactam β-lactamase and is reported to be more potent than clavulanic acid or tazobactam [96]. It is currently being developed by Novexel for combination with ceftazidime or aztreonam against serious Gram-negative pathogens [97,98]. On the other hand, since inhibition of Mtbβ-lactamase by clavulanic acid is irreversible [99], the focus of this approach should be on the selection of β-lactam antibiotics rather than β-lactamase inhibitors.

In addition, β-lactams are generally considered to inactivate D,D-transpeptidases, but quite recently L,D-transpeptidases have been identified as potential targets of carbapenems in Mtb [100,101]. These are cysteine-based transpeptidase as opposed to serine-based enzymes and they form different peptidoglycan cross-links (3–3 vs 4–3). The relative significance of inhibition of these two kinds of transpeptidases in Mtb needs to be established.



33: CGI17341 **31**: NXL104 **32**: AN2690

Figure 1. CGI17341, NXL104 and AN2960.

Benzothiazinones

Although still in the preclinical stage, BZT043 **(28)** is an extremely potent new class of antimycobacterial agent (MIC against Mtb = 0.004 μg/ml) [102]. *In vivo* this compound at 37.5 mg/kg reduced the bacterial burden by 1 and 2 logs in the lungs and spleen, respectively. Subsequently by sequencing the mutant strains, its target was determined to be the enzyme decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1), which is involved in the biosynthesis of cell-wall component, arabinogalactan. The proposed MOA involves reduction of the nitro group to a nitroso residue, which reacts with the cysteine group of DprE1 [103].

Caprazamycins

Caprazomycins are closely related natural products of complex structures **(29)** [104,105]. They are active against both DS and DR strains of Mtb with a MIC value of 3.13 μg/ml. Due to the similarities to other known lipo-uridine antibiotics, the MOA is believed to be inhibition of lipid biosynthesis, more specifically inhibition of phospho-MurNAc-pentapetidetranslocase (MraY, translocase I) [106]. Subsequent analogue syntheses have indicated that the complex side chain can be replaced with a simpler fatty acid (palmitic acid), and the diazepine ring can be converted to an acyclic system without significant reductions in potency [107,108]. There is little information on their PK properties, and the compounds would be dosed by injection only, making them possibly targeted only for DR TB patients. Caprazamycins are still in the preclinical development stage.

DC-159a

DC-159a **(17)** is an 8-methoxy-fluoroquinolone originally developed as a broad-spectrum antibiotic, but its potential use in TB patients has recently been investigated by Daiichi-Sankyo. It was shown to be fourfold more potent *in vitro* than moxifloxacin and gatifloxacin against DS Mtb, and to retain activity against 11 strains of quinolone-resistant DR Mtb [109]. In mouse efficacy studies, it was shown to be more potent than moxifloxacin in the rapidly growing phase as well as in the non- (or slow-) replicating phase [110]. The results seem to warrant its study as part of drug combinations in animal models

of TB. Possessing three chiral centers, one challenge for this compound's development might be the cost of goods. This compound is still in the preclinical development stage.

Although the list mentioned earlier may give the appearance that there are ample development candidates in the current global drug pipeline for TB, if one considers the attrition rates in drug development, this number of compounds and classes is far too small and narrow in scope to ensure a series of robust new TB drugs in the next few years. We do not have sufficient data to estimate the attrition rates for TB drugs but if we assume the rates seen for antibiotics [11], (although attrition rates in other therapeutic areas vary considerably [111,112]) it becomes obvious that we do not have enough candidates or discovery programs to provide a steady stream of new TB drug approvals. Therefore, it may be useful to examine where else we may find the next set of compounds to develop and how to increase the odds of success, as discussed in the next section.

■ Potential sources of future leads

As discussed earlier, it may be of interest to speculate where else one can search for leads to novel TB drugs. It is also important to remember that TB therapies, however unique they might be, are still within the field of antibacterial therapeutics, and many of the characteristics of antibacterial research still apply. Indeed, some of the compounds under development are reapplied from the broad-spectrum antibiotics arsenal.

Libraries of compounds beyond 'drug-likeness'

As described earlier, TMC-207 was discovered by screening a relatively small number of compounds in a corporate library in a whole-cell assay using *M. smegmatis*. Although it is not the same species as Mtb and some compounds can be missed [113], use of the faster-growing and nonpathogenic *M. smegmatis* has the advantages of not requiring a biosafety-level III laboratory and the increased speed of assays. Benzothiazinone BTZ043 was also discovered by a whole-cell assay of a rather unique set of sulfur-containing synthetic compounds [113]. In retrospect, both of these compounds have unusual structures that might not be found in typical libraries of a pharmaceutical company or of commercial libraries. Furthermore, these compounds might otherwise be rejected for violating the 'drug-likeness' rules because the former has a rather high MW (554) and clogP (7.25), and the latter has an aromatic nitro- and isothiourea-moieties that would be eliminated by structure-alert filters such as SMARTS [114]. As pointed out by R Elliott of the Bill and Melinda Gates Foundation (BMGF) in a recent meeting, it might be necessary to loosen the medicinal chemistry structural criteria to find molecules that work against Mtb and parasitic diseases [115]. The key question is what a chemical moiety typically considered 'ugly' is doing in the molecule. If it is critical for the molecule to have the intended biological effect and it cannot be replaced by a less 'objectionable' group, then we might need to modify our impressions of ideal compound libraries for these diseases. As long as we are aware of the potential risks of including such a group and try to manage them from the earliest stage of drug development, it may be acceptable or prudent to include such compounds. It has been pointed out earlier that antibacterial agents appear to have quite different physicochemical properties when compared with drugs from other therapeutic areas [116]. More recently the physicochemical properties of TB drugs were analyzed and again it shows that the properties are more widely spread compared with the non-antibacterial drugs [39]. This may be supported in part by the observation that we have not witnessed large numbers of lead chemical series arising from fairly extensive screens conducted with commercial libraries designed predominantly on drug-likeness [117,118]. This is also based on our own experience from screening commercial libraries where we have typically identified compounds with relatively low *in vitro* potencies, poor therapeutic indices and/or poor PK properties [TB Alliance, Unpublished Data].

PA-824, on the other hand, was discovered by preparing analogues of CGI-17341, a compound known to have *in vitro* and *in vivo* anti-TB activity. PA-824 contains a nitro-imidazole group that might have been rejected based on its structural alerts. These examples indicate that compounds that may be beyond typical drug-likeness criteria are quite potent against Mtb. It suggests we should seek new leads in much more chemically diverse space, not necessarily groomed by the drug-likeness rules, and among compounds that can be activated by an Mtb specific physiology. In reality, it is the interplay of chemistry and biology that it important; often a very potent inhibitor such as TMC207 helps identify a new drug target, and a new target identified in turn reveals additional compounds against the same target [119]. Another dramatically different chemical space is the burgeoning field of boron-containing

compounds [120]. For example, AN2690 **(32)** is an unusual of oxaborole compounds exhibiting antifungal activities and its MOA is shown to be inhibition of aminoacyl-tRNA synthase by trapping tRNA in the editing site [121].

Taking advantage of activating mechanisms

It is also striking how many of the current TB drugs or drugs under development have MOAs that require activation within an Mtb cell. Isoniazid requires oxidation by KatG to generate an acyl radical [122]. Pyrazinamide requires hydrolysis of the amide moiety by Mtb nicotinamidase/pyrazinamidase to pyrazinoic acid. As mentioned, PA-824 and OPC-67683 require reduction of the nitroimidazole moiety by a F420-dependent nitroreductase [56]. Benzothiazinone BTZ043 and ethambutol share a target of arabinogalactan biosynthesis [123], and benzothiazinone BTZ043 also requires its initial reduction to a nitroso intermediate that traps a cysteine residue of DprE1 [124]. One of the second-line drugs, ethionamide, has to be activated by flavin-containing monooxygenase [125]. Although their mechanisms of action were established after their anti-Mtb activity was identified, these activation mechanisms can be in turn exploited to design prodrugs that can release an entirely different active 'war heads'. Utilizing such a prodrug approach, new chemical entities could be designed to release unique bioactive moieties (e.g., covalent modifiers of certain macromolecules) that are not present in the original prodrugs themselves. Since activation takes place within the Mtb cell, one could be less concerned about systemic toxicity. These types of prodrug designs have been used extensively in the anticancer field [126]. For example, the hypoxic and low pH conditions of tumor tissues have been employed to prepare prodrugs of an antitumor nucleoside ECyd, which were shown to be selectively activated by bioreduction and hydrolysis mechanism [127]. It is of interest to note that hypoxic and low pH conditions also exist in the TB granuloma. Another example is the design of water-soluble camptothecin derivatives that can be activated by reduction of the nitrofuran or nitrobenzene moiety [128]. Thus, rational design of new drugs may be possible based on this concept of utilizing the Mtb-specific activating mechanisms. Specifically, the use of Baeyer–Villiger monooxygenase (ethionamide), nicotinamidase (pyrazinamide) or nitro-reductases (nitroimidazoles) appear to be promising at this stage.

Antiparasitic leads

There seems to be some commonality between compounds active against Mtb and compounds active against parasites. For example, nitroimidazoles related to OPC-67683 were recently shown to be active against *Leishmania* parasites. There may be several reasons for this phenomenon. Although they are eukaryotes, the apicomplexan family of protozoa parasites, such as *Plasmodium* and *Toxoplasma* species, contains plastid-like organelles. This organelle is the product of a second endosymbiosis event when the ancestral apicomplexan ingested an alga and has since retained many prokaryotic metabolic pathways such as fatty acid biosynthesis type II (FAS II), isoprenoid, iron–sulfur clusters and haem biosynthesis [129]. These pathways are also the targets of many drugs with anti-Mtb activity. For example, FAS II inhibitors thiolactomycin and triclosan are known to be effective against Mtb as well as against *Plasmodium falciparum* [130]. Another example of shared targets is peptide deformylase, which exists in eukaryotic protists, including the causative agents of malaria, sleeping sickness, Chagas disease and leishmaniasis, as well as Mtb [131]. Last year GlaxoSmithKline (GSK) disclosed more than 13,000 hits from their anti-*Plasmodium falciparum* high-throughput screening (HTS). Considering the overlap of essential biochemical targets present in both *P. falciparum* and Mtb, this set of HTS hits may serve as an excellent source for novel Mtb-active compounds as well.

The second reason for the common activity may be from a symbiotic relationship between the parasite and bacteria it harbors, as found in filarial nematodes. In this case, the *Wolbachia* bacteria are necessary for proper functioning of adult worms and it has been shown that killing bacteria with doxycycline can lead to a filariacidal activity [132]. The drugs rifampicin and clofazimine, which have anti-Mtb activity, were shown to have antifilarial activity *in vitro* by killing *Wolbachia* bacteria [133]. It is almost certain that there are other symbionts in additional multicellular parasites and killing such bacteria by anti-TB antibiotics would lead to killing the parasite.

The third reason for the common activity is that biochemically fundamental processes are shared between parasites and Mtb. This is the case with topoisomerase inhibitors and some of the protein synthesis inhibitors [134]. For those various reasons, it seems quite interesting to screen for Mtb activity among the compounds that have shown activity against protozoan and

multicellular parasites. This can be achieved by using published data such as those from GSK, and cross-screening compounds identified by the researchers in their fields and organizations such as Medicines for Malaria Venture and Drugs for Neglected Diseases *initiative*. The oxaborole class mentioned earlier shows activity as antimalarial and antitrypanosomal agents [135], but their congeners also are active as antibacterials against Gram-negative pathogens [136].

Structure-based drug design

Our knowledge of the structures of targets is growing rapidly with the advancement of x-ray crystallography and chemoinformatics techniques, and this will certainly aid our ability to design molecules that can bind to such targets. Recently, there have been several excellent reviews on structure-based drug design of anti-infective agents [137–139]. For a target to be suitable for structure-based drug design, there are several requirements:

- The protein being essential not only *in vitro* but also *in vivo*;
- A bioassay being available to check potential inhibitors;
- Although not absolutely necessary but preferable, availability of a positive control compound indicating its pharmacological validation; and some structural information on the Mtb protein or at least its homologous protein in other bacteria.

Some potential targets are listed in **Table 4** although they are by no means all inclusive. As an example, DNA gyrase-B subunit in the table is essential, there is a known inhibitor, novobiocin, and the structure of Mtb DNA gyrase-B subunit is available. ATP synthase is also essential and, as mentioned earlier, TMC207 is a known inhibitor.

Natural products

The popularity of natural products as a source of new leads has waxed and waned over recent decades, but the fact remains that many antibacterial agents are derived from natural products. By some accounts more than 70% of commercialized anti-infective drugs are based on natural products [140]. It has been theorized that in the case of microbial natural products, they exist to give the producing microorganism some evolutionary advantage by controlling other microorganisms [141]. Therefore, the major advantage of natural products should be that they have gone through the evolutionary process over millennia to engender properties to enter and affect competing microbial organisms. Since our knowledge in this regard is still limited to designing totally novel molecules that can enter microorganisms and cause physiological changes, taking advantage of the evolutionary process seems to be reasonable. With the advancement of analytical instruments, increasing knowledge of the physiology of producing organisms, and expansion of potential source organisms, some of the past criticism of natural products can be overcome. They can provide 'unexpected' structures and, as mentioned previously, they can provide diversity beyond 'the rule of five'. Even if a newly discovered natural product may not be developable, it can provide a new structural theme or identify a new target. There are several recent reviews discussing the new focus on antibacterial natural products [142,143].

■ Biology considerations (novel targets & novel models for TB drug development)

Clearly TB poses a unique problem compared with other bacterial diseases. The WHO has declared that new preventative, diagnostic and therapeutic measures are required to control TB [144]. Novel diagnostic tools need to be developed to improve drug-sensitivity testing and to reduce transmission. Also, novel bacterial persistence targets need to be identified that are required by the organism for the prolonged nonreplicating survival within the various stages of the granuloma. Just as importantly, relevant *in vitro* and *in vivo* models need to be developed to replicate, as closely as possible, the conditions in a human infection to assist the progression and development of the most effective drug candidates.

■ Surrogate models

M. tuberculosis is highly host-specific, with no known nonhuman hosts, making it ethically and practically unfeasible to study it in its natural host. Various *in vitro* and *in vivo* experimental models have been used for the development of TB drugs. While these models are limited by their inability to replicate the human–pathogen relationship precisely, they have been useful for their inherent safety, affordability and speed.

Surrogate organisms

Instead of using the highly infectious, and slow-growing pathogen itself, various members of less pathogenic, faster-growing species of

Table 4. Structure-based drug design.

Gene product name	Annotation number	*In vivo* expression	Genetic validation	Pharmacological validation	Structure of Mtb protein	Structure of other microorganism protein	Ref.
GyrB	Rv005	Yes	Essential	Yes; novobiocin	Available	Available	[227]
AtpA-H	Rv1304–1311	Yes; inferred due to pharmacological validation	Essential *in vitro*	Yes; TMC207	Not available	Available	[228]
RpoB	Rv0667	Yes	Essential	Yes; rifamycins	Not available	Available; *Thermus thermophilus*	[229]
RpoC	Rv0668	Yes	Essential	Yes; rifamycins	Not available		[230]
Ndh	Rv1854c	Yes	Essential; unclear	Yes; phenothiazines	Not available	Yes; *Acidianus ambivalens*	[231]
NdhA	Rv0392c	Yes	Essential; unclear	Yes; phenothiazines	Not available	Yes; *A. ambivalens*	[231]
TopA	Rv3646c		Essential *in vitro*	Yes; for Topo IB and II	Not available	Available	[232]
Icl1, Icl2		Yes; Icl1: proven expression in TB in mouse and human lung tissue	Not essential *in vitro*; essential together *in vivo* (mice) but redundant; have to inhibit both enzymes	Yes, *in vitro* only; 3-nitropropionate	Available for both Icl1 and Icl2	Available	[233, 234]
GlcB	Rv1837c	Unclear; studies suggest much lower *in vivo* expression (mice) than for Icl1		No	Available	Available	[233, 235]
InhA	Rv1484	Yes – inferred due to pharmacological validation	Essential *in vitro*	Yes; INH/ ethionamide	Available	Available	[236]
MenA	Rv0534c	Yes	Essential	Yes; suspected	Not available	Not Available	[237]
DfrA	Rv2763c	Yes	Essential; unclear	Yes; trimethoprim	Available	Available	[238]
FolB	Rv3607c	Yes	Essential	Yes; pteridine derivatives	Available	Available	[239]
	Rv0211	Yes; in human lung tissue and in mice and inferred due to *in vivo* essentiality	Essential during acute and chronic infection in mice	Diabetes target	Available	Available	[233, 402]
ClpP1	Rv2461c	Yes	Essential *in vitro*	Yes; acyldepsipeptides	Model	Yes; *Escherichia coli*	[240]
ClpP2	Rv2460c	Yes	Essential *in vitro*	Yes; acyldepsipeptides	Model	Yes; *E. coli*	[240]

AtpA-H: ATP synthase-E subunit; BioA: Adenosylmethionine aminotransferase; ClpC2: Clp protease ATP binding subunit; ClpP1: Clp protease proteolytic subunit 1; ClpP2: Clp protease proteolytic subunit 2; DfrA: Dihydrofolate reductase – DHFR; DlaT: Dihydrolipoamide Acyltransferase; FolB: Dihydroneopterin aldolase; GlcB: Malate synthase; GyrB: DNA gyrase-B subunit; Icl1: Isocitrate lyase 1; Icl2: Isocitrate lyase 2; InhA: NADH-dependent enoyl-ACP reductase; LeuRS: Leucyl-tRNA synthetase; Lpd: Lipoamide dehydrogenase; MenA: 1,4-dihydroxy-2-naphthoate prenyltransferase; Mtb: Mycobacterium tuberculosis*; Ndh: NADH dehydrogenase II; NdhA: NADH dehydrogenase II A; PckA: Phosphoenolpyruvate carboxykinase – PEPCK; RpoB: RNA polymerase* β *chain B; RpoC: RNA polymerase* β *chain C; TopA: DNA topoisomerase I.*

Table 4. Structure-based drug design (cont.).

Gene product name	Annotation number	*In vivo* expression	Genetic validation	Pharmacological validation	Structure of Mtb protein	Structure of other microorganism protein	Ref.
ClpC1	Rv3596c	Yes	Essential	Yes	Not available	Not Available	[241]
ClpC2	Rv2667	Yes	Essential	Yes	Not available	Not Available	[242]
Acetyl-CoA carboxylase; biotin carboxylase +BCCP AccA2	Rv0973c	Yes	Essential	Yes		Yes; *E. coli* and *Staphylococcus aureus*	[243]
Acetyl-CoA carboxylase; biotin carboxylase +BCCP AccA3	Rv3285	Yes	Essential	Yes		Yes; *E. coli* and *S. aureus*	[243]
Acetyl-CoA carboxylase; carboxytranferase AccD4	Rv3799c	Yes	Essential	Yes; andrimid and moiramide		Yes; *Corynebacterium glutamicum*	[244]
Acetyl-CoA carboxylase; carboxytranferase AccD6	Rv2247	Yes	Essential	Yes; andrimid and moiramide		Yes; *C. glutamicum*	[245]
LeuRS	Rv0041	Essential	Essential	Yes; benzoxaboroles	Not available	Yes	[246]
BioA	Rv1568	*In vivo* essential	Not essential *in vitro*	Yes; amiclenomycin	Yes	Yes; *E. coli*	[247]
DlaT	Rv2215	Yes	Attenuated *in vitro/vivo*	Yes; rhodanines	Not available		[248]
Lpd	Rv0462	Yes	Highly Attenuated *in vivo*	Yes; triazaspirodi-methoxybenzoyls	Yes		[249]

AtpA-H: ATP synthase-E subunit; BioA: Adenosylmethionine aminotransferase; ClpC2: Clp protease ATP binding subunit; ClpP1: Clp protease proteolytic subunit 1; ClpP2: Clp protease proteolytic subunit 2; DfrA: Dihydrofolate reductase – DHFR; DlaT: Dihydrolipoamide Acyltransferase; FolB: Dihydroneopterin aldolase; GlcB: Malate synthase; GyrB: DNA gyrase-B subunit; Icl1: Isocitrate lyase 1; Icl2: Isocitrate lyase 2; InhA: NADH-dependent enoyl-ACP reductase; LeuRS: Leucyl-tRNA synthetase; Lpd: Lipoamide dehydrogenase; MenA: 1,4-dihydroxy-2-naphthoate prenyltransferase; Mtb: Mycobacterium tuberculosis*; Ndh: NADH dehydrogenase II; NdhA: NADH dehydrogenase II A; PckA: Phosphoenolpyruvate carboxykinase – PEPCK; RpoB: RNA polymerase β chain B; RpoC: RNA polymerase β chain C; TopA: DNA topoisomerase I.*

mycobacteria (including *Mycobacterium phlei*, which was instrumental in the discovery of streptomycin in the 1940s [145], *Mycobacterium marinum* [146], and *Mycobacterium fortuitum* [147]) have been used for compound screening and other studies. The most widely used of the fast growers is *M. smegmatis*, which has proven amenable to genetic manipulations including target validation, and has also served as a model for rapid screening of compound libraries. The recent discovery of TMC207 from HTS against this organism has firmly established its usefulness for this purpose [41].

When there has been a need to stay within the Mtb complex without sacrificing safety, nonpathogenic strains have been used including H37Ra and the vaccine strain of the related animal pathogen *M. bovis*, BCG. Recently, a number of Mtb strains have been designed with targeted multiple deletions [148–150] that ensure auxotrophy and a reduced biohazard level to allow their use in an environment commensurate with drug discovery instead of the restrictive biosafety-level III conditions required for the wild-type pathogen. A recent publication compared hits discovered using *M. smegmatis*, *M. bovis* BCG and Mtb using three different sets of compound [124]. For example, with the NIH diversity set, 48% of Mtb hits were not detected by *M. smegmatis* while 7% of Mtb hits were not detected by *M. bovis* BCG, indicating that *M. bovis* BCG is a better surrogate model for HTS.

In vitro growth conditions

Typically, antibiotic discovery involves screening of compound libraries against organisms maintained in nutrient-rich media with continuous agitation that permits enough oxygen to support rapid growth. For Mtb, researchers have observed that the pathogen persists under the harsh hypoxic environment of the human granuloma, exhibits an altered morphology (a loss of acid-fastness) [151], and is not in a state of rapid replication [152] perhaps due to a shortage of nutrients. A number of groups have more

recently examined and exploited various *in vitro* growth conditions that attempt to replicate the environment in the diseased lung including the Wayne model [153,154], which allows a bacterial culture in a deep liquid medium to gradually deplete its oxygen content. Under these conditions, Mtb gradually shuts down replication while maintaining viability. Mtb first enters a microaerophilic and then an anaerobic, nonreplicating, persistent state in which it remains dormant for long periods while retaining the capacity to resume rapid replication when oxygen and nutrients are replenished. Since its introduction, the Wayne model (including modified versions of the original model design) [155–157] has been utilized by a number of TB research groups for the screening and early development of drugs that have the potential to kill the pathogen in different stages of nonreplicating persistence [158]. Growth conditions have been utilized that expose the bacteria to either low oxygen [159,160], reduced nutrients [161–163], low pH [163], reactive nitrogen intermediates [164–167] or, recently, all these conditions combined [168–171] to completely shut down *in vitro* bacterial replication and mimic *in vivo* conditions. A recent addition to TB drug development has been the introduction of the hollow fiber model for dose selection and pharmacodynamic antibiotic-exposure targets associated with maximal microbial kill and complete suppression of drug resistance [172,173].

Animal models

Animal models are what connect *in vitro* microbiological observations to human clinical trials, allowing the demonstration of drug activity against the infectious agent in the context of an animal host, and can therefore help inform and guide subsequent clinical trial designs. The guinea pig was the original animal model for the study of TB and later for TB drug-discovery efforts where it was used to demonstrate the activity of streptomycin [174]; while less prevalent today, it still sees occasional use [175,176]. This animal is highly susceptible to TB, and upon infection it develops disease with caseation and necrosis that closely resembles the pathology seen in the human lung [177,178]. The majority of compounds that are active in this model are similarly active in humans. In contrast to the guinea pig model, the mouse does not mimic the pathological characteristics of TB in humans, but it remains the best characterized and most economical animal model for modern TB drug discovery and regimen evaluation [179]. Most of the existing data on the bactericidal and sterilizing activity of currently used anti-TB drugs have been acquired through variations of the mouse model. Recently, a novel mouse model has been described that combines the economy of size inherent in the mouse with the human-like disease pathology including necrotic granulomas inherent in the guinea pig [180–182] and larger animal models. This model, the Kramnik mouse model, is currently under validation and development by various investigators for potential use in TB drug development on the premise that bacterial persistence is determined by hypoxia, starvation and low pH, conditions inherent in the necrotic granuloma.

Novel targets

The inherent resilience of Mtb manifests (in part) the current requirement for half a year of treatment with multiple drugs for active DS-TB. The antibiotics that constitute first-line drugs for TB therapy target only a small number of essential functions in the organism: mycolic acid biosynthesis (isoniazid) [183], RNA synthesis (rifampin) [184], disruption of membrane proton motive force [185] or inhibition of FAS I (pyrazinamide) [186], arabinogalactan and lipoarabinomannan biosynthesis (ethambutol) [187], and protein synthesis (streptomycin) [188]. Identification of unique pathways that are required for bacterial growth and persistence should provide additional novel targets for the rational design of effective antibiotics that would shorten therapy and eliminate extant drug-resistant strains. The following discussion will extend the list to include several enzymes and metabolic pathways that have been validated to varying degrees, from the demonstration of essentiality for growth, to the identification of compounds that inhibit bacterial growth *in vitro* and/or during infection. Importantly, novel targets have been identified that are required for the organism's survival in its dormant, nonreplicating state, and inhibitors of such targets could be developed into drugs that have better potential to reduce the duration of therapy. A selection of these pathways/targets is discussed later in the article.

■ The glyoxylate shunt

M. tuberculosis, like all plants and microorganisms, encodes the glyoxylate cycle, an anaplerotic pathway that bypasses the tricarboxylic acid (Krebs) cycle, and replenishes tricarboxylic acid

cycle intermediates during growth on fatty acids. In this organism, the glyoxylate cycle is composed of two enzymes, isocitratelyases (ICL) and a malate synthase [3]. It was shown that the loss of both Icl1 and Icl2 results in Mtb strains that were unable to grow on fatty acids, and unable to establish either chronic or acute infection in mice [189]. Importantly, these studies suggested that Mtb utilizes fatty acids as a carbon source during an infection, making all enzymes required for metabolizing fatty acids ideal candidates for the development of treatment shortening drugs. Although this study demonstrated chemical inhibition of both ICL enzymes with a small molecule (3-nitro-propionate), efforts by various groups to date have failed to identify tractable leads against this promising target. Recently, attention has turned to the other target in the pathway, malate synthase, whose structure is predicted to be more amenable to drug design and chemical inhibition [190].

■ Gluconeogenesis

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the first committed step in gluconeogenesis, which converts oxaloacetic acid into phosphoenol pyruvic acid in a reversible manner [191]. The enzyme from the related *M. smegmatis* has been characterized and has been shown to prefer GTP over ATP as a cofactor, unlike most bacterial enzymes, resembling its mammalian counterpart, and preferentially catalyzes the gluconeogenic direction, whereby phosphoenol pyruvic acid is formed from oxaloacetic acid [192]. Studies utilizing BCG expressing green fluorescent protein from a *pckA* promoter showed that *pckA* was upregulated by growth on fatty acids but downregulated by growth on glucose [193]. The same study reported that a loss of PEPCK resulted in attenuation of virulence both in macrophages and in mice. The data indicated that PEPCK activity is important for a successful infection, suggesting a need for gluconeogenesis *in vivo*. It was recently demonstrated elegantly with the use of *pckA*-knocked down strains of Mtb that PEPCK plays a role in the pathogenesis of TB, as it was necessary both for initiating and for maintaining an infection in mice [194]. These data extended the previous observations that Mtb requires gluconeogenesis for growth and persistence *in vivo*. These studies and others suggest that Mtb PEPCK is a potential target for anti-TB chemotherapy. To date PEPCK has been a highly investigated target for diabetes and obesity in the pharmaceutical industry, and a number of lead molecules that inhibit the human enzyme have been identified [195,196]. Since some of these compounds are based on the GTP cofactor that is shared between these enzymes, they could serve as a starting point for further TB drug discovery.

■ ATP synthesis & the electron-transport chain

As mentioned earlier, TMC207 represents the first pharmacological proof that mycobacterial ATP synthesis and the electron-transport chain can be targets of TB therapeutic intervention. Importantly, a recent study has revealed that TMC207 is equally effective against nonreplicating as it is against aerobically grown bacteria [43]. This study showed that even though there was a significant decrease in the transcription of the ATP synthase operon and lower cellular ATP levels in dormant mycobacteria, there was still some residual ATP synthase enzymatic activity, and this activity was highly susceptible to inhibition by TMC207, which had a bactericidal effect. This confirms ATP synthase and, by extension, other members of the electron-transport chain as valid targets against which drugs could be developed that have activity against persistent and dormant bacteria and potentially contribute to treatment shortening. Since ATP synthesis is the culminating step of the electron-transport chain, at least theoretically any agent that interrupts any nonredundant component of this chain could be a potential therapeutic agent for TB [197]. Currently various TB laboratories are actively performing screens against the electron-transport chain in inverted membranes vesicles [198,199] from both Mtb and *M. smegmatis* in an attempt to identify tractable hits against as many of these targets as possible in a search for new compounds with similar attractive therapeutic potential such as TMC207.

■ Cholesterol metabolism

A series of studies from various laboratories have recently focused attention on the idea that endogenous cholesterol is utilized by the invading Mtb organism both as a carbon and energy source. A bioinformatic analysis of the genome of the actinomycete, *Rhodococcus* sp., a relative of Mtb identified a gene cluster for cholesterol metabolism [200]. Some of the genes appeared to be up-regulated in the presence of cholesterol. Most of the identified genes showed homology to known steroid degradation genes from other organisms and were similar to a

cluster present in the Mtb genome. A number of the genes that were affected by cholesterol had previously been shown to be up-regulated and essential for the survival of MTB in activated macrophages [17,201,202]. It was also demonstrated that Mtb can grow on synthetic growth media with cholesterol as the only carbon source and that a loss of the *mce4* gene cluster, which encodes a cholesterol uptake system [203], results in strains that are attenuated for growth on cholesterol [204]. *mce4* has been shown to be required for Mtb to survive during the prolonged infection in mice [205], suggesting that cholesterol is important for Mtb to maintain a chronic infection. It is therefore conceivable that drugs targeted at inhibiting Mtb cholesterol uptake and metabolism during an infection would inhibit persistence and contribute to treatment shortening.

Target identification & validation

While it is not absolutely necessary to know the actual molecular target for every drug under development, knowing the target early during the discovery process can be very helpful in developing specific assays for identifying and validating hits, developing SARs to assist lead optimization, and fully validating the target by demonstrating its essentiality for bacterial growth and persistence both *in vitro* and *in vivo*. A number of methods exist for target identification including isolating and sequencing resistant mutants, demonstrating MIC up-shift for strains that over-express the target, and generating gene knockout strains and demonstrating their reduced fitness. Unfortunately, all these methods are very labor- and resource-intensive and they require a drug molecule/ligand to already exist.

Recently a number of groups have developed expressions systems specifically for controlling gene expression in mycobacteria [206–216]. These systems have been used to identify or validate a number of promising drug targets, but the most useful application of these expression systems for target validation and directing drug discovery has come in the form of conditional knockdown strains. This system is brilliant in that a single, well-behaved knockdown strain can initially be used to establish essentiality of the target *in vitro* under various growth or media conditions, with or without drugs. That same strain can also be used to acquire similar data on the same target *in vivo* by regulating expression at any point in the infection and demonstrating the relevance of the target in either the acute or chronic stages, or both. Examples of such validated targets include *prcBA* [217] and *pckA* [218], both of which are currently being investigated as potential TB drug targets by various research groups.

History & mission of the TB Alliance

The TB Alliance was founded following a meeting in Cape Town, South Africa, in February, 2000, where over 100 representatives from academia, industry, major regulatory agencies, nongovernmental organizations, and philanthropic donors gathered to discuss the problems of current TB treatment. This meeting culminated in the generation of the 'Declaration of Cape Town' [402], a call-to-arms for harmonized global TB drug development, and for the creation of the TB Alliance. The TB Alliance was formally launched in October 2000, at the International Conference on Health Research for Development, in Bangkok, Thailand.

The mission of the TB Alliance is to accelerate the discovery and development of faster-acting and affordable drugs to fight TB. Through innovative TB-focused drug discovery and development with partners around the world, the organization works to ensure access to faster, better TB cures that will improve global health and prosperity. Recognizing that improved TB drugs will only be effective if they are affordable and available, the TB Alliance and its partners work with global, regional and national stakeholders to ensure regulatory approval, adoption by international TB programs, and widespread availability of new TB drug regimens (the 'triple A' mandate).

■ Product-development partnership

A PDP is defined as a specific form of nonprofit, public–private partnership focused on creating and nurturing synergies between the public, private, academic, government and philanthropic sectors to drive the development of new medical products for underserved markets. Such product-related activities can include the development of vaccines, diagnostics, therapeutics and other enabling treatments and associated technologies to address otherwise neglected diseases. Through their global, collaborative efforts, PDPs are uniquely suited for accessing multiple funding sources, and for applying a wide range of tools and knowledge to their specific research and development programs. PDPs maintain direct management oversight of their projects,

although much of the technical activity may be conducted at aligned external research facilities and through a network of contractors/collaborators. In the global health arena, PDPs have been established to fight TB, AIDS, malaria, and a wide range of neglected diseases. PDPs, such as the TB Alliance, have been created for the public good with the specific intention that their products will be affordable to those who need them.

■ Organizational model & major collaborators

The TB Alliance operates as a virtual research and development organization headquartered in New York City. Through collaborations with research institutions and multinational pharmaceutical companies, drug-development risks and financial incentives are apportioned accordingly. While exercising strategic management oversight of its drug-discovery/-development projects (see later discussion), the TB Alliance enlists the expertise and resources of both public and private partners to research and develop novel anti-TB drug candidates. Currently, the TB Alliance has established multi-year collaborations for drug discovery with the Novartis Institute of Tropical Diseases, GSK, AstraZeneca, Johnson & Johnson, Novartis and Bayer.

■ TB Alliance portfolio

The current TB Alliance research and development portfolio is outlined in **Figure 2**. As noted in the figure, the TB Alliance currently oversees approximately 20 discovery programs, two preclinical-development programs and seven novel regimens in late-stage clinical trials. By establishing multiyear collaborations with several major pharmaceutical companies, the TB Alliance is currently interrogating four discovery programs at GSK (Tres Cantos, Spain), six programs at AstraZeneca (Bangalore, India), three at Novartis (Singapore) and one at Johnson & Johnson (Tibotec, in Beerse, Belgium). Efforts in late-stage TB drug development by the TB Alliance are also supported through in-kind contributions from various clinical study co-sponsors (i.e., Bayer, Tibotec and Novartis). The challenge in advancing novel, safe and efficacious regimens for pivotal DS and/or M(X)DR-TB clinical studies is envisioned to be addressed in part through the concerted efforts of the Critical Path to TB Drug Regimens (CPTR) initiative.

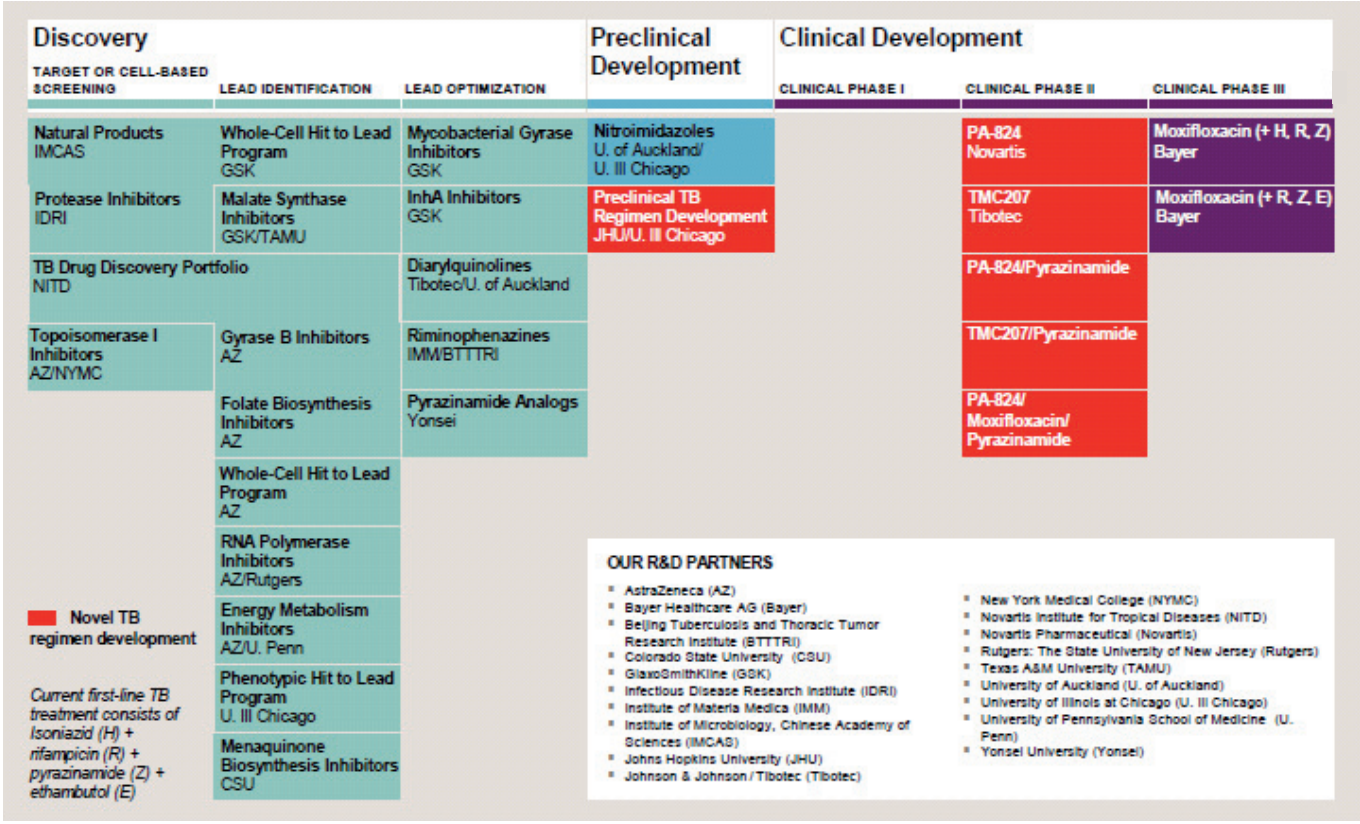


Figure 2. Current TB Alliance portfolio.

■ Critical Path to TB Drug Regimens

On 18 March 2010, the CPTR Initiative was formally announced and commended by the US FDA in Washington, DC, USA. The CPTR initiative represents a diverse collaboration of pharmaceutical companies, government, regulatory, and multilateral agencies, academic institutions, TB advocates and nongovernment organizations, which aim to accelerate the development of new, safe and highly effective TB treatment regimens with shorter courses of therapy. The initiative was founded and is managed by a joint partnership of the Critical Path Institute, the BMGF and the TB Alliance. The CPTR operates under three divisions: Regulatory Science Consortium led by the Critical Path Institute, Research Resources Group led by the BMGF and Drugs Coalition led by the TB Alliance **(Figure 3)**. At the time of writing, the following organizations are the participant member organizations for the CPTR initiative: Anacor, AstraZeneca, Bayer, CDISC, Celgene, Cepheid, Colorado State University, Doctors Without Borders, European and Developing Countries Clinical Trials Partnership, Family Health International, Faster Cures, FIND, GSK, Johns Hopkins University, Johnson & Johnson, Mc Laughlin & Rotman Center for Global Health, Merck & Co., NIH/NIAID, Novartis, Otsuka, Pfizer, Reagan Udall Foundation, Resist-TB/Boston University, Aanofi-Aventis, Sequella, Treatment Action Group, University of California, San Francisco, the FDA and the WHO. While this initiative is still evolving, the ongoing, collaborative activities among the participant organizations holds great promise for a more efficient and expeditious TB drug regimen development process for the future. More specifically, this arrangement should encourage collaborations among the participating pharmaceutical or biotechnology companies to combine candidate drugs in novel regimens, closer communications between the regulatory agencies and drug developers, and coordination between the funders and recipients for TB drug development.

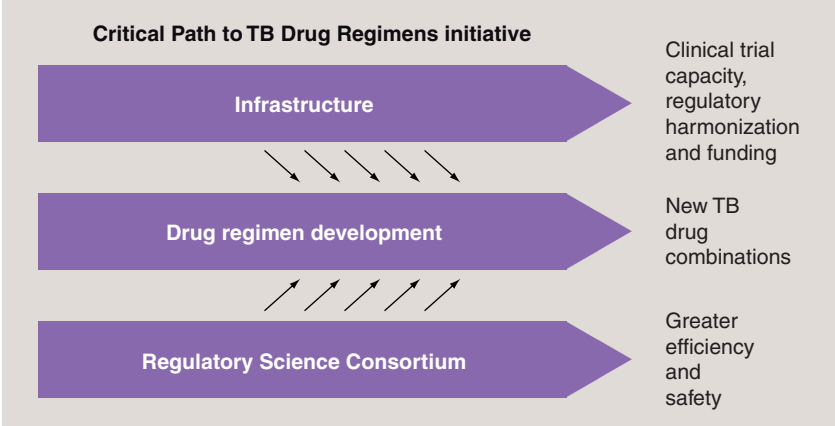


Figure 3. Major components of Critical Path to TB Drug Regimen initiative operations.

Future perspective

Although it was mentioned at the outset, it is unlikely (if not unfeasible, because of the pathophysiology of the disease) that we could ever identify a single agent that would cure TB. One can certainly strive, however, 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. We need 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. The challenge is that the need is urgent but the discovery and development process takes an exorbitant amount of time and resources. The nature of the disease and its geopolitical spread makes it apparent that that we need to consolidate our efforts in the face of limited resources from available research funds, the number of committed TB research laboratories, and the breadth of global organizations involved in TB drug development. In this regard, the recent move by major multinational pharmaceutical companies to actively join in the fight against TB is very encouraging. Even then, we will still require improved mechanisms whereby newly discovered, critical biochemical TB targets can be more routinely interrogated for the drug-discovery purposes. Since most of the basic discovery is expected to be carried out at academic institutions, we need a robust process that helps translate basic TB science into drug-discovery practice, including the development of assays, the collection and/or design of chemicals, HTS, hit-to-lead and lead optimization medicinal chemistry activities. The global TB community must reach a consensus on the establishment of a robust drug-development mechanism, which can then become the standard for the future. For this we need to formalize the dialogue among academic institutions, biotechnology companies, pharmaceutical companies and nonprofit organizations. As mentioned earlier, the anticipated long development period for each combination therapy can in principle be

reduced through the CPTR initiative. In addition, since TB is a disease of a complex interaction between the human host immune responses and the pathogen Mtb, future treatments may need to include agents that can favorably affect components of the human immune system. Although it is beyond the scope of this review, there are emerging research activities in this regard [219,220]. Clearly, research for more efficacious vaccines should continue in order to provide long-term solutions to TB. In parallel, drug and regimen development should be accelerated with sharper focus and increased collaborations.

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Executive summary

- One of the most urgent goals in TB drug development is to shorten treatment duration from the current 6 months to 4 months (or shorter) for drug-sensitive TB patients.
- New drugs are needed to achieve this and to overcome drug resistance. In addition, it should be possible to use new drugs for HIV/AIDS co-infection patients.
- Drugs currently in clinical trials include moxifloxacin, gatifloxacin, TMC-207, OPC-67683, PA-824, high-dose rifampicin and rifapentine, linezolid, PNU-100480, AZD5847 and SQ-109.
- Compounds currently in preclinical stages include BZT043, caprazmycins, β-lactam and β-lactamase inhibitors and DC-159a.
- Potential sources of new leads could be: beyond 'the rule-of-five' compounds, prodrugs designed to be activated by Mtb-specific mechanisms, novel antiparasitic-active compounds, compounds based on the structural information of the targets, and natural products.
- Novel drug targets include biochemical mechanisms from: the glyoxylate shunt, gluconeogenesis, ATP synthesis and the electron-transport chain, and cholesterol metabolism.
- The TB Alliance is a New York-based not-for-profit organization established in 2000 to accelerate drug development of TB drugs by collaborating with academic laboratories and pharmaceutical companies.
- The TB Alliance in a partnership with Critical Path Institute and the Bill and Melinda Gates Foundation established in 2010 the Critical Path to TB Drug Regimens to shorten the time necessary for developing novel treatment regimens for TB. The participating organizations include the WHO, the US FDA, the European and Developing Countries Clinical Trials Partnership, major academic institutions, representative pharmaceutical companies and foundations involved in development of TB treatments.

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