EXPERT REVIEWS

New drugs and vaccines for drug-resistant *Mycobacterium tuberculosis* infections

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Tuberculosis remains the most common cause of death due to a single infective organism. Despite the availability of a vaccine and chemotherapeutic options, the global disease burden remains relatively unaffected. The ability of the mycobacterial etiological agents to adopt a semidormant, phenotypically drug-resistant state requires that chemotherapy is both complex and lengthy. The emergence of drug resistance has raised the possibility of virtually untreatable tuberculosis. Furthermore, the currently used bacillus Calmette–Guerin vaccine has had mixed success in protecting susceptible populations. Given this backdrop, the need for novel anti-infectives and more effective vaccines is clearly evident. Recent progress, described herein, has seen the development and entry into clinical trials of several new drugs and vaccine candidates.

KEYWORDS: azole • diarylquinone • isoxyl • *Mycobacterium* • siderophore • tuberculosis

Members of the *Mycobacterium tuberculosis* complex, the causative agents of TB, represent some of the most successful human pathogens affecting both developed and developing countries. It is estimated that a third of the global population is a reservoir for these bacteria and a record 9 million people developed active disease in 2004 [1]. *M. tuberculosis* is transmitted by aerosols and, following inhalation, up to 50% of individuals exposed to the bacillus become infected. Of these, only a fraction (~5%) develop TB, while the rest develop a latent infection and may develop TB later during their lifetime due to reactivation [2].

Current TB chemotherapy

Combination therapies have been used to treat TB since p-aminosalicylic acid (PAS) was successfully coadministered with streptomycin to inhibit the emergence of streptomycin-resistant strains [3]. After the first national survey of UK drug resistance in the mid-1950s showed that most drug-resistant isolates were resistant to only one drug [4], three-agent combination therapies were introduced in which an initial three-drug phase lasting for 2–3 months was followed by a two-drug continuation phase.

The most common recommended standard chemotherapeutic regime for TB treatment consists of an initial 2-month phase of treatment with isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB), followed by a continuation phase of treatment lasting 4 months with INH and RIF [5]. INH and RIF represent the cornerstones of anti-TB therapy; INH is the most powerful mycobactericidal drug available, reducing the bacterial population by approximately 95% over the first 2 days of treatment and normally ensuring early sputum conversion, thereby decreasing TB transmission as a consequence. Despite this superlative early bactericidal activity (EBA), it is no more effective than other drugs after this initial period. In cases where the disease presents as a more chronic infection, the EBA of INH is reduced markedly [6]. Following the initial 2 days of treatment, RIF becomes the most significant bactericidal drug [6] and INH serves to limit the emergence of RIF resistance. The mycobactericidal activity of RIF against sporadically active bacteria is crucial for preventing relapses, and RIF in the continuation phase appreciably improves the outlook for patients infected with an INH-resistant strain [6].

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One of the major problems associated with the treatment of TB is the bacterium's penchant for adopting a nonreplicating persistent state, probably induced by the prevailing environmental conditions, a significant factor being a limiting supply of oxygen [7–10]. Antibiotics are most effective against actively growing *M. tuberculosis* rather than cultures in stationary phase [11]. This antibiotic resistance shown by persistent *M. tuberculosis* is not associated with any genetic changes but with the adoption of a dormant metabolic state by the bacteria, which may require special treatments and passage through liquid culture before they can be cultured on agar plates. The impact of this phenomenon in relation to clinical relapse is well illustrated in the Cornell model of TB infection in mice [12,13]. After treatment with INH and PZA for 3 months, *M. tuberculosis* cannot be detected in tissues of infected mice via growth on agar plates. However, after treatment is withdrawn, a third of the animals relapse within 3 months and, if immunosuppressed using steroids, almost all relapse. The recovered *M. tuberculosis* clones display the same drug susceptibility as the original infecting strain [12,13]. Owing to its apparent ability to kill a subset of bacteria not killed by the other drugs, thought to be sporadically active organisms subject to an hypoxic and possibly acidic environment [14–16], PZA represents an important component of combination therapy; its inclusion has allowed the shortening of the chemotherapeutic regimen to the current 6 months from an initial 9–12-month period.

The structures of the developing tuberculous lesions may effectively define the metabolic status of their bacterial inhabitants and it has been speculated that at least four significant subpopulations of bacteria for which different drugs could be efficacious exist. These might include active growers that may be killed by INH, those with sporadic metabolic bursts that could be killed by RIF, a population with low metabolic activity that are considered likely to experience acidic surroundings and hypoxia that may be susceptible to PZA and, finally, dormant bacilli that are not killed by any current agents [14,17].

Emergence of drug resistance

Poor patient compliance is the major contributing factor in the emergence of drug resistance and this is particularly relevant in TB chemotherapy. The complex nature and length of the treatment certainly contributed to unacceptable levels of noncompliance. Likewise, logistical problems concerning drug supply and the tendency for patients to feel well long before safe completion of the prescribed course also contributed to an unacceptable degree of noncompliance [18] promoting the emergence of drug resistance and prompting the development of directly observed treatment, short-course (DOTS) [19–21]. Initially, this acronym described an intensively managed chemotherapy regime but has since become used to describe a broader public-health strategy that has been adopted in over 150 countries [21,22]. However, over a quarter of the global population have no access to DOTS. A Phase IV clinical trial of single-tablet combination therapy with an initial intensive phase of 8 weeks of daily ethionamide (ETH), INH, RIF and PZA, in a fixed-dose combined tablet, followed by 18 weeks of RIF and INH, in a three-times weekly fixed-dose combined tablet, has recently been completed. It is to be hoped that this simplified regimen may go some way to controlling the emergence of drug resistance.

Strains of *M. tuberculosis* resistant to both INH and RIF, regardless of profiles of sensitivity/resistance to other drugs, have been termed multidrug resistant (MDR). MDR-TB is a major concern due to the associated high risk of death. While resistance to either drug may be managed with other first-line drugs, MDR-TB requires treatment with second-line drugs under DOTS-Plus [5]. These agents often possess limited sterilizing capacity and are not suitable for short-course treatment, necessitating prolonged treatment with drugs that are less effective and more toxic; several require injection [20,23,24]. The WHO currently recommends the use of a regimen including amikacin (AMK), ETH, a fluoroquinolone (FQ; e.g., moxifoxacin [MXF]) and PZA; in a mouse model, 9 months of this therapy are needed to sterilize both lungs and spleen, while only 6 months are needed with the RIF plus INH plus PZA regimen [25]. Recently, the emergence of extensively drug-resistant TB (XDR-TB) strains, defined by the US CDC as those with resistance to at least three of the six classes of second-line drugs (aminoglycosides, polypeptides, FQs, thioamides, cycloserine and PAS), has been reported [23]. In some regions, approaching 20% of MDR-TB cases were classified as XDR-TB, raising concerns over a future epidemic of virtually untreatable TB [23].

New drug 'pipeline' in TB therapy

Given this backdrop, the need for rapid and continued progress in the development of new lead compounds and the identification and characterization of novel drug targets to engage medicinal chemists is clearly evident. A crucial consideration for new agents is their effectiveness in coadministration with antivirals used to treat HIV [19,26,27]; rifamycins, such as RIF, activate human cytochrome P450, which metabolize antivirals, significantly reducing their plasma concentrations [27]. Fortunately, several promising anti-TB agents are currently at various stages of development; some are analogues or derivatives of current first- and second-line anti-TB agents, such as quinolones and EMB, while others have been discovered through extensive screening programs or from screening of focused libraries designed with particular targets in mind.

Classes of agents with representatives in clinical trials

Ethambutol derivative SQ109

The front-line anti-TB agent EMB inhibits cell wall arabinan biosynthesis in both the structural polysaccharide arabinogalactan [28,29] and the wall-associated lipoglycan, lipoarabinomannan [29]. An EMB derivative, *N*-geranyl-*N′*-(2-adamantyl)ethane (SQ109) exhibited improved activity against

M. tuberculosis compared with EMB, their respective minimum inhibition concentrations (MIC) were recorded as 0.5 and 5.0 µg/ml [30,31]. Encouragingly, SQ109 is also more potent than EMB against mycobacteria within macrophages and mice [30]. Analysis of *M. tuberculosis* H37Rv transcriptional profiles after several different drug treatments revealed that, despite many similarities, SQ109 and EMB differentially affected a regulon containing genes implicated in fatty acid modification and in the biosynthesis of the essential cell wall components, the mycolic acids. This apparent mechanistic divergence was confirmed by the observation that, unlike their SQ109-treated counterparts, the wall of *M. tuberculosis* treated with EMB rapidly lost their acid-fast staining properties and contained significantly less arabinose than controls [32].

Despite low bioavailability after oral administration [30], SQ109 was entered into Phase I clinical trials. Recently, both US FDA and EMEA awarded sequella orphan drug and orphan medicinal product status allowing 7 years of market exclusivity for the use of SQ109 in TB treatment. This status provided under the Orphan Drug Act provides economic incentives to encourage the development of drugs for diseases affecting fewer than 200,000 people in the USA.

Quinolones

Quinolones target bacterial type II topoisomerases, DNA gyrase and topoisomerase IV [33,34]. These ATP-dependent enzymes cooperate to facilitate DNA replication and other key DNA transactions [35]. DNA gyrase is involved in catalyzing the negative supercoiling of DNA, is essential for DNA replication, recombination and transcription and appears to be the sole topoisomerase target for quinolones in *M. tuberculosis* [36].

Of the series of developed quinolone derivatives, FQs have shown a broad spectrum of antibacterial activity. FQs have been used sparingly due to the frequent emergence of resistance to the readily available derivatives, ofloxacin and ciprofloxacin [37]. New FQs containing a C-8 methoxy moiety exhibit greater activity and two compounds in particular, MXF and gatifloxacin (GAT) (Figure 1A), showed good activity in murine models of TB [38–41]. MXF is more active than ofloxacin and ciprofloxicin; they require MICs of 0.125, 2 and 4 µg/ml, respectively [40]. In a mouse model, MXF activity against *M. tuberculosis* is comparable to INH [39,40] and, encouragingly, MXF also appeared to kill a subpopulation of RIF persistors [42]. Furthermore, inclusion of MXF in a combination regime greatly reduced time to culture conversion in murine TB. A combination of RIF plus PZA plus MXF was more effective than INH plus RIF plus PZA [43,44], presenting the possibility that MXF might replace INH in a combination regime to shorten TB therapy in humans. However, concerns over the potential toxicity of RIF plus PZA combinations in the absence of INH have been highlighted by the incidence of fatal and severe liver injuries associated with treatment of latent TB infections with RIF plus PZA [45,46].

Several FQs are now in clinical trials. MXF, GAT and high-dose levofloxacin, the potent L-stereoisomer of the ofloxacin racemate, all showed excellent EBA approaching that of INH [47,48]. Encouragingly, a more potent extended EBA, that is, from days 2 to 7, was observed with the FQs than for INH [47]. A recent Phase II trial to investigate whether MXF could replace EMB in INH plus PZA plus RIF plus EMB combinations revealed that, although the MXF regimes showed more frequent sputum conversion at 4 weeks, the success of the treatments provided identical outcomes after 2 months [49]. The 2-month timepoint coincides with the end of the intensive phase of treatment with common regimens, and culture conversion rates at this stage are a well-accepted surrogate marker for the sterilizing activity of anti-tuberculosis drugs. We await the publication of data from a recently completed Phase II trial to determine whether substitution of MXF for INH will improve rates of sputum conversion at this 2-month stage; significant improvement here would support the case for Phase III trials of MXF-containing regimens of under 6 months total duration. A Phase III clinical trial aimed at assessing the success of a 4-month GAT-containing regimen is now recruiting participants. Various trials are also underway to assess the influence of RIF (Phase II)/rifapentine (Phase I) coadminstration upon MXF pharmacokinetics.

Recently, 16 7-substituted GAT derivatives were tested for antimycobacterial activity *in vitro* and *in vivo* against *M. tuberculosis* H37Rv and MDR *M. tuberculosis* [50]. Four compounds were more active (MIC < 0.2 µg/ml) and five compounds were equipotent (MIC: 0.2 µg/ml) to GAT against *M. tuberculosis*. The most active compound, 1-cyclopropyl-6-fluoro-8-methoxy-7-[[[N4-[1′-(5-isatinyl-β-semicarbazo)]methyl]N^1-piperazinyl]4-oxo-1,4-dihydro-3-quinolone carboxylic acid, exhibited an *in vitro* MIC of 0.0125 µg/ml against *M. tuberculosis* and MDR-TB strains. In the intravenously infected mice, the compound decreased the bacterial load in lung and spleen tissues by 3.26 and 3.76 logs, respectively, indicating that increasing the length of the lipophilic side chain at C-7 improves the antimycobacterial activity *in vitro* [50]. With this in mind, appropriate modification of MXF, which is more effective than GAT, could provide more effective inhibitors of DNA gyrase with improved efficacy.

Diarylquinoline TMC207

The antimycobacterial activity of diarylquinolines (DARQs) was identified via screening prototypes of different chemical series for inhibition of the growth of the fast-growing saprophyte *Mycobacterium smegmatis*. Lead optimization generated a series of 20 promising DARQs expressing potent *in vitro* activity against several mycobacteria, including *M. tuberculosis* [51], with three exhibiting encouraging *in vivo* activity. The MIC for the most potent of these, TMC207 (formerly R207910 Figure 1B), was 0.03 µg/ml for *M. tuberculosis* [51] with similar *in vitro* efficacy against *M. tuberculosis* clinical isolates resistant to INH, RIF, streptomycin, EMB, PZA and MXF [51,52]. This

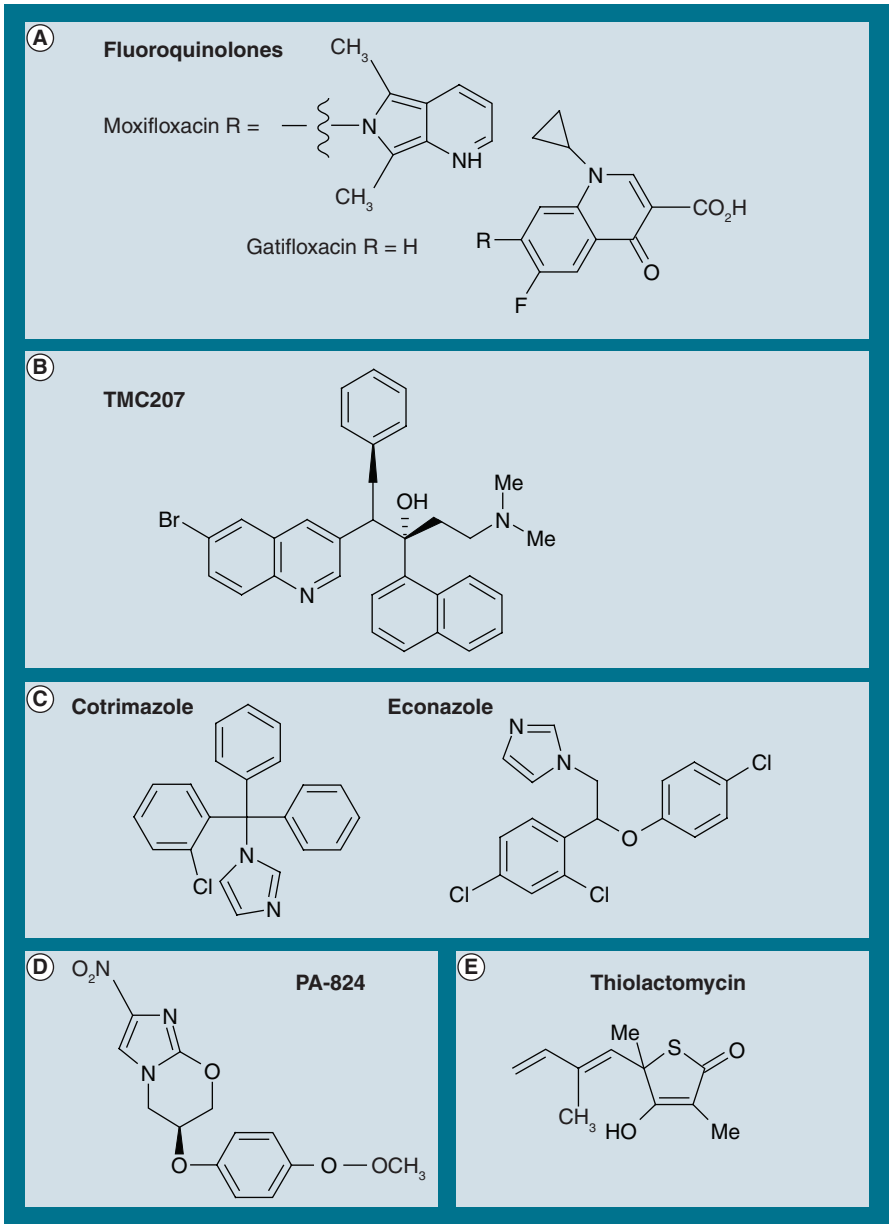

Figure 1. Some promising drug candidates with activity against *Mycobacterium tuberculosis*.

lack of crossresistance with currently used anti-TB agents suggested that TMC207 retains activity against MDR strains and exerts its antimycobacterial effect through a novel cellular target. Consistent with this, TMC207-resistant spontaneous mutants of *M. tuberculosis* had no crossresistance with other anti-TB agents [51]. A role for the product of *atpE,* a component of the mycobacterial F_1F_0 ATP synthase, in resistance to DARQs has been demonstrated [51]. Mutations in its membrane-spanning region confer resistance to TMC207, implying that the *atpE* gene product is the primary target of TMC207 in mycobacteria. Recently, a study of susceptibility of a panel of various mycobacteria including susceptible and resistant TB, representatives of the *Mycobacterium avium* complex and a wide range of other nontuberculous mycobacteria confirmed the drug's broad antimycobacterial spectrum and also correlated the natural resistance of *Mycobacterium xenopi*, *Mycobacterium novocastrense* and *Mycobacterium shimoidei* with a methionine for alanine substitution near a substrate-binding site of ATP synthase [52].

In TMC207 monotherapy, a potent EBA comparable to that of INH was observed in a nonestablished infection murine TB model in which treatment began on the first day of the infection study. Furthermore, a late bactericidal effect exceeding that of RIF was also observed in the established infection murine TB model, in which treatment commenced on the 12th day of infection [51]. However, as TMC207-resistant mutants emerge at a frequency comparable to RIF-resistant mutants *in vitro,* DARQ monotherapy is contraindicated. Despite this, TMC207 appears to be a useful component for combination therapy. Substitution of each of RIF, INH and PZA with TMC207 resulted in a significant increase in potency, leading to complete culture conversion of the lungs in some animals after 1 rather than 2 months, indicating potential for reducing the length of combined therapy [51].

Recently, a study in mice to define the optimal TMC207-containing regimen to treat MDR-TB identified for the first time a regimen without INH and RIF that was able to sterilize the lung and spleen within 2 months [53]. Administered along with AMK plus ETH plus MXF plus PZA, TMC207 appeared to accelerate bacterial clearance from mice, supporting the possibility of the drug shortening treatment duration in humans. However, further studies to assess various combinations of TMC207 with AMK, MXF, ETH and PZA are needed to define their individual contributions to this therapy. In addition, relapse rates with the most promising combinations need to be determined to fully understand the potential of these new regimens.

TMC207 is well tolerated in humans, at least during a limited exposure period, with plasma levels approximately eightfold greater than those associated with potent *in vivo* activity in mice and only mild or moderate adverse effects [51]. Patients with MDR-TB are currently being recruited to a Phase II clinical trial to assess antibacterial activity, safety and tolerability of TMC207 in combination therapy.

Nitroimidazopyran PA-824

The nitroimidazopyran PA-824 (Figure 1D) represents a promising new compound for the treatment of TB that is currently undergoing human trials. Similar to its progenitors metronidazole and CGI-17341, PA-824 is a nitroimidazole prodrug, requiring bioreductive activation of an aromatic nitro group to exert its anti-tubercular effect [54]. PA-824 activation requires the bacterial F240-dependent glucose-6-phosphate dehydrogenase (FGD1) and nitroreductase and the protein product of *Rv3547* [55] to inhibit mycolic acid and protein synthesis. PA-824 is highly active with a MIC as low as 0.015–0.025 µg/ml against *M. tuberculosis* and MDR-TB. In preclinical testing against a broad panel of MDR clinical isolates *in vitro*, PA-824 was highly active against all isolates with MICs less than 1 µg/ml [56]. In a short-course mouse infection model, the efficacy of PA-824 was comparable to INH, RIF and MXF. PA-824 also exhibited potent activity during the continuation phase of therapy, during which it targeted bacilli that had persisted through an initial 2-month intensive phase of treatment with RIF, INH and PZA [57]. We await the outcome of a recently completed Phase IIa trial to evaluate the safety, tolerability, extended EBA and pharmacokinetics of PA-824 against newly diagnosed, uncomplicated, adult pulmonary TB in South Africa. The TB Alliance has also initiated an investigation of PA-824 nitroimidazole analogs.

Dihydroimidazo-oxazole OPC-67683

A further azole-based lead compound is currently in Phase II studies [58]. The compound is also a mycolic acid biosynthesis inhibitor possessing potent activity against TB, including MDR-TB, with an exceptionally low MIC in the range of 0.006–0.024 µg/ml *in vitro* and highly effective therapeutic activity at low doses *in vivo* [59]. In addition, a significant post-antibiotic effect, that is, an extended period of inhibition after the plasma concentration is exceeded by MIC, was observed against intracellular *M. tuberculosis* H37Rv when OPC-67683 was removed after 4 h exposure. In this situation, its potency at 0.1 µg/ml was similar to that of the first-line drug RIF at 3 µg/ml. Combination of OPC-67683 with RIF and PZA resulted in a quicker eradication of viable TB bacilli in the lung in comparison with the standard regimen of RIF plus INH plus PZA plus EMB. Furthermore, OPC-67683 was not affected by nor did it affect the activity of liver microsome enzymes, suggesting the possibility for OPC-67683 to be used in combination with drugs, including antiretrovirals, that induce or are metabolized by cytochrome P450 enzymes. As with PA-824 [60], this azole drug also requires reductive activation that appears to be mediated through Rv3547 [59].

We await the publication of the findings of a completed Phase II clinical trial addressing the safety, efficacy and pharmacokinetics of OPC-67683 in patients with uncomplicated pulmonary TB.

Sudoterb (pyrrole LL-4858)

Sudoterb was reported to have potent anti-TB activity *in vitro* and *in vivo* (mice and guinea pig) studies. *In vitro*, sudoterb has bactericidal activity similar to INH and is synergistic with RIF [61]. The combination of sudoterb with INH plus RIF plus PZA led to complete sterilization of sensitive and MDR-TB strains in infected mice within 2 months. In combination with RIF plus PZA, sudoterb also cured TB in all animals after 3 months of treatment, presenting the possibility that it might significantly shorten TB treatment in combination therapy [62]. As for MXF, the safe use of RIF plus PZA combinations without INH must be assessed.

Oxazolidinones

Oxazolidinones inhibit protein synthesis by selectively and uniquely binding to the 23S rRNA of the 50s ribosomal subunit [63]. They possess antimicrobial activity against a variety of Gram-positive bacteria, including *M. tuberculosis*, with a MIC of 2–4 µg/ml and are also active against tubercle bacilli in mice [64–66]. Three clinical candidates, eperezolid, linezolid and PNU-100480, a thiomorpholine analogue of linezolid, were identified as antimycobacterial agents [67]. PNU-100480 exhibited an MIC_{90} of 0.9–2.5 µM against *M. tuberculosis in vitro* [67] and comparable activity *in vivo* to that of INH and RIF in a murine model [68]. More recently, the antimycobacterial activity of 3-(1H-pyrrol-1-yl)-2-oxazolidinones analogues RBx 7644 and RBx 8700 was established [69]. RBx 8700 demonstrated good *in vitro* activity against drug-sensitive, as well as MDR-TB, strains with MIC_{90} values of 0.25 µg/ml (sensitive) and 1.0 µg/ml (MDR strains), respectively. The MIC_{90} for RBx 7644 and linezolid was 16 and 64 µg/ml, respectively [69]. As RBx 8700 showed promising activity and a good correlation between MIC values *in vitro* and in a macrophage system, further study of RBx 8700 in experimental models of *M. tuberculosis* to determine its full potential as a possible anti-TB drug is warranted.

Linezolid has recently been approved by the FDA for the treatment of MDR Gram-positive bacterial infections [63]. However, clinical studies have shown that prolonged use of linezolid in the treatment of MDR-TB caused frequent significant toxicity, including anemia, bone marrow depression and potentially irreversible peripheral neuropathy [70,71]. Recently, the first linezolid-resistant clinical isolates of *M. tuberculosis* have been described [72]. Genetic analyses revealed no mutations in potential target genes, including the 23S rRNA gene, the *rplV* and *rplD* genes, encoding ribosomal proteins L4 and L22, respectively, the *erm-37* gene, encoding a 23S rRNA methyltransferase, and *whiB7*, which encodes a putative regulator. The mechanism of resistance is, therefore, unclear but may be related to the efflux rate [72].

Agents in preclinical development

Encouragingly, many agents are currently showing promise toward potential use in TB chemotherapy. Here, we describe several of these; a more comprehensive list can be found using StopTB [201] and TB Alliance [202].

Azoles

Azoles are commonly used antifungal agents, some of which have been shown to have antimycobacterial activity [73,74]. The recent recognition of a plethora of cytochrome P450s, known cellular targets of azoles in fungi [75,76], encoded in the *M. tuberculosis* genome [77] drove studies to examine the correlation between the presence of P450 and the susceptibility to azole drugs in *M. tuberculosis* [78–80]. Purified MTCYP51, a *M. tuberculosis* cytochrome P450, was found to bind azoles tightly, providing biochemical evidence that azole drugs might also inhibit mycobacterial P450 enzymes. Two azoles, clotrimazole and econazole (Figure 1C), were tested for their antimycobacterial activity against *M. tuberculosis* H37Rv. The MIC_{90} was 0.12 μg/ml, whereas the minimum bactericidal concentration was 0.125 μg/ml for both drugs, demonstrating their excellent bactericidal activities [81]. *Ex vivo* studies on mice splenocytes following exposure to clotrimazole and econazole confirmed the good tolerance and synergistic effects of these drug [82]. Further *in vivo* analyses have determined that econazole is well tolerated in combination therapies and equipotent to RIF in the treatment of murine TB [83]. The crystal structure of MTCYP51 with bound azoles has recently been elucidated and is being probed for development of novel drug therapies against TB [84,85].

N-*alkylsulphonylacetamides*

β-ketoacyl-acyl carrier protein (ACP) synthases play an important role in the biosynthesis of fatty acids, the mycolic acid components of the cell wall and various virulence-related polyketides in pathogenic mycobacteria. β-sulphonylcarboxamide compounds were designed as mimics of the reactive acetyl enolate intermediate in the condensation reaction these enzymes catalyze [86]. The most potent antimycobacterial compounds discovered through this strategy were amide derivatives of 3-sulphonyl fatty acids bearing alkyl chains of between eight and ten carbons in length; MICs as low as 0.75 μg/ml are comparable to front-line anti-TB agents. Surprisingly, the activity profile of these compounds was particularly species dependent, they exhibit no significant activity against bacteria other than *M. tuberculosis* and closely related strains, including nonpathogenic mycobacteria [86]. One of these, *N*-octanesulphonylacetamide (OSA), was tested further against several pathogenic and drug-resistant mycobacteria, including MDR-TB, strains [87]. Analysis of lipid biosynthesis after exposure to OSA revealed a marked inhibition of mycolic acid biosynthesis in *Mycobacterium bovis* BCG with no alteration in the panoply of other complex lipids generated by the bacterium; mycolic acid biosynthesis in the relatively insensitive *M. smegmatis* was unaffected [87]. Electron microscopy of treated sensitive bacteria revealed dysfunction in cell wall biosynthesis and incomplete septation [87]. The overproduction in OSA-treated *M. bovis* BCG of the β-subunit of F_1F_0 ATP synthase encoded by *atpF* [88] suggested the involvement of ATP synthase, either directly or indirectly. Consistent with *atpF* overexpression, cellular ATP levels decreased upon treatment with OSA [88]. Reminiscent of TMC207, this OSA-mediated decrease in cellular ATP may signal the inhibition of the F_1F_0 complex or may target other unidentified regulatory components involved in energy production.

Recently, the developers of OSA, FASgen Inc. (MD, USA), announced 3-sulfonyltridecanamide (FAS20013) as their lead proprietary compound for the treatment of TB and MDR-TB [89]. It exhibits potent bactericidal activity for anaerobically adapted *M. bovis* BCG, that is, against anaerobically nonreplicating persistence, and coresistance to established agents has not been reported in clinical isolates [89].

Phenothiazines

The antimycobacterial activity of phenothiazines has been reported sporadically over the past 40 years [90–92]. One such phenothiazine, trifluoroperazine (TPZ), exhibited a significant effect on *in vitro* ATP synthesis in *Mycobacterium leprae* [93]. An insight into the mode of action of TPZ was gained through the discovery that cellular ATP levels in *M. leprae* were dramatically reduced in the presence of the drug, suggesting a target in the mycobacterial oxidative phosphorylation system. A thorough biochemical analysis implicated type II NADH: menaquinone oxidoreductase (NDH-2) as the target of TPZ in *M. tuberculosis* [94]. The bacterium possesses a branched respiratory chain with specific termini induced under aerobic or microaerobic conditions [94,95] but both chains are initiated by NDH-2. The essential role of NDH-2 in *M. tuberculosis* is supported by biochemical studies, transcriptional studies, gene-deletion analysis, investigations of bacterial growth in various culture conditions and animal experiments [32,94]. TPZ and its analogue chlorpromazine are effective against *M. tuberculosis* H37Rv in a macrophage model of infection, and are synergistic with both INH and RIF [96,97]. The poor pharmacokinetics for TPZ result in a large (>100-fold) disparity between the minimal bactericidal concentration *in vitro* and concentrations achievable *in vivo* [97], thus 50 analogues were screened for greater potency. Three showed potential, with the most active having a MIC of 1.11 μg/ml in comparison with those for TPZ (19.2 μg/ml), RIF (0.5 μg/ml) and INH (0.15 μg/ml). Encouraging activity in a mouse model of acute infection reducing the bacterial load within the lungs and spleen established it as a potential lead for drug development [94].

Peptide deformylase inhibitor BB-3497

Bacterial peptide deformylase (PDF) is a metalloprotease that removes the N-terminal formyl group from newly synthesized proteins. Various PDF inhibitors have activity against several pathogens, including *Escherichia coli* and *Staphylococcus aureus*, *in vitro* [98–100]. Six PDF inhibitors were screened against two isolates of *M. tuberculosis* and initial testing showed that three compounds, BB-3497, BB-84518 and BB-83698, exhibited MICs in the range of 0.06–2 μg/ml [101]. These were further tested against 17 isolates of *M. tuberculosis* and BB-3497 was the most active with a median MIC of 0.25 μg/ml. Further

in vivo evaluation is required to fully determine the potency of BB-3497 and clinical tests must be carried out that address whether the drug is toxic in humans. A recent study suggested that PDF inhibitors had no detectable effect on two different human cell lines *in vitro* [102].

Acetohydroxyacid synthase inhibitor KHG20612

Acetohydroxyacid synthase (AHAS) catalyses the first common step in the biosynthesis of branched amino acids leucine, isoleucine and valine [103,104]. The AHAS encoded by *ilvB* and *ilvN* in *M. tuberculosis* was overproduced in *E. coli* and purified to homogeneity [105]. A microplate-based enzyme assay was developed to screen small molecules as potential inhibitors of the catalytic subunits of AHAS from a chemical library composed of 5600 compounds. Library screening identified four structurally related hit compounds that inhibited AHAS activity by more than 90% at 40 µM. These compounds were functionally related, bearing a disulfide bond and containing a phenyl or 1-substituted triazolyl groups. A range of IC_{50} values of 1.8–2.6 µM was reported for these four compounds. KHG20612 also inhibited growth of various drug-resistant strains of *M. tuberculosis* [105].

Thiolactomycin

Thiolactomycin (TLM) is a thiolactone antibiotic (Figure 1E) isolated from a soil *Nocardia* spp. [106]. TLM exhibits potent *in vitro* activity against many pathogenic bacteria, including Gram-negative and Gram-positive bacteria and *M. tuberculosis*. TLM inhibits the β-ketoacyl-ACP synthase condensing enzymes mtFabH and KasA of the FAS-II system in *M. tuberculosis* [107,108], which are involved in the synthesis of the essential mycolic acids of the cell wall. *In vitro* and *in vivo* inhibition of mtFabH and KasA leads to the inhibition of mycolic acid biosynthesis. The total synthesis of TLM was first reported by Wang and Salvino [109] and was improved to yield the active 5R stereoisomer [110]. Several analogues have since been designed and tested against *M. tuberculosis* [60,111–115]. Preliminary studies showed that modifications to the thiolactone core in the C-5 position with a 5-tetrahydrogeranyl substituent gave an MIC_{90} of 29 µM and 92% inhibition in extracts of *M. smegmatis*, compared with 125 µM and 54%, respectively, for TLM [115]. As a continuation to these studies, a series of C-5 substituted biphenyl and acetylene analogues were developed and two compounds showed a marked increase in an *in vitro* assay against mtFabH [112,113]. 5-[3-(4-acetyl-phenyl)-propyl-2-ynyl]-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one and 5-(4′-benzyloxy-biphenyl-4-yl-methyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one gave an 18-fold and fourfold increase in activity with an IC_{50} value of 17 and 4 µM, respectively, compared with 74.9 µM for TLM. By contrast, others have suggested that C5 substitutions of the TLM core renders the analogues inactive against *M. tuberculosis* [60]; this matter awaits clarification. However, the recent determination of the crystal structure of *M. tuberculosis* KasB and subsequent homology modeling of KasA, using the KasB structure as a template, supports the potential for C5-derivatization of the TLM scaffold towards the design of improved antimycobacterial KAS inhibitors [116].

Siderophore biosynthesis

Siderophores are ferric ion-specific chelating agents elaborated by various aerobic bacteria to assimilate iron [117]. Although the metal might be plentiful, as in the tissues of pathogen-infected host organisms, it is sequestered and hence not readily available to them [117]. Siderophores can remove iron from insoluble inorganic polymers or from eukaryotic proteins and are commonly recognized as virulence factors of important bacterial pathogens, although some pathogens use alternate acquisition mechanisms [117]. *M. tuberculosis* produces two biosynthetically related siderophores: carboxymycobactins that operate in the normal extracellular mode, returning chelated iron to the bacterium from its environment; and mycobactins that are unique among bacterial siderophores in remaining cell bound [117]. Both types of siderophores have identical iron-binding centers and differ only in their long acyl chains, the water soluble carboxymycobactins bear a terminal carboxylic acid group absent in the cell-bound mycobactins [117]. Several of the genes associated with the synthesis of the mycobactin core have been identified in the ten-gene *mbt* cluster [118] and a further cluster recently designated *mbt2* [119,120]. Component genes of both clusters are essential for the *in vivo* growth of *M. tuberculosis* [121–123] and expression is regulated by iron availability.

The first committed steps towards the biosynthesis of the iron-binding center of the (carboxy)mycobactins is the MbtA-catalyzed adenylation of salicylic acid, which activates the aryl acid for its subsequent transfer to a phosphopantetheinyl-serine residue of MbtB for its subsequent ligation with serine or threonine [118]. Recently, two groups designed analogues of the salicyloyl-AMP intermediate incorporating stable bioisosteres to replace the labile phosphate moiety. These showed good activity against *M. tuberculosis* grown under iron-limiting conditions [124,125] at low micromolar concentrations. Especially impressive was an analogue in which the phosphoryl moiety was replaced with a sulphamide that exhibited an MIC for *M. tuberculosis* of 0.19 µM [124].

Purine analogues

Recently, the screening of novel compounds has highlighted modified purines as potential antimycobacterial drugs [126–129]. Screening of a library of 6-substituted and 2,6-di-substituted 9-benzyl-purines revealed several compounds with encouraging activity against *M. tuberculosis*, with a general trend suggesting that 6-substituted varieties were more effective than their disubstituted counterparts, although chlorination at the 2 position did tend to increase activity [126]. 2-monosubstituted library members possessed no anti-TB activity. High inhibitory activity was found for those carrying phenylethynyl-, *trans*-styryl or aryl substituents in the 6 position; the most active bears a furyl

substituent with MICs as low as 0.78 μg/ml, comparable to that for RIF, and representing a good lead. In order to optimize the *N*-9 substituent, a library of 6-aryl, 9-alkyl-purines was synthesized and screened, confirming that the *N*-9 benzyl substituent used in the previous study showed greatest activity [128]. More extensive testing revealed that the 6-furyl derivative exhibited low cytotoxicity for Vero cells, retained activity against several singly drug-resistant strains of *M. tuberculosis* and was active (MIC: 8.46 μg/ml) against *M. tuberculosis* Erdman in bone marrow macrophages [128].

Further active 6,9-di-substituted purines with significant activity against *M. tuberculosis* were generated by reacting 6-mercaptopurine with sulphonyl/sulphenyl halides [127]. Several compounds had low MICs between 0.39 and 3.9 μg/ml for *M. tuberculosis* H37Rv. Screening of two classes of derivative against *M. tuberculosis* strains resistant to nine of the main clinically used antimycobacterial agents revealed that extensive crossresistance with several of these agents, one showed little crossresistance and thus was identified as having great potential as a lead compound [127].

Screening of another library of 6-thiopurines incorporating 6-thioaryl/alkyl-urines, 2-thioaryl/alkyl-pyrimidines and 2- and 4-thioaryl/alkyl-pyridines identified highly active compounds 9-(ethylcarboxymethyl)-6-(decylthio)-9H-purine and 9-(ethylcarboxymethyl)-6-(dodecylthio)-9H-purine with MICs of 1.56 and 0.78 μg/ml, respectively, against *M. tuberculosis* H37Rv with the former showing good activity against the Erdman strain in bone marrow macrophages [129]. Together, these various studies demonstrate that further development of these purine derivatives may generate interesting novel anti-TB agents.

M. tuberculosis immunology & vaccines

Following entry into the respiratory tract, the TB bacillus is phagocytosed by alveolar macrophages. Once inside the macrophage phagosome, the bacterium evades the usual post-phagocytic defense mechanisms by using a range of evasive tactics, which include altering the endosomal pH (inhibiting phagolysosome acidification) [130,131], detoxification of harmful radicals and inhibition of apoptosis [131]. This is followed by a stage of rapid intracellular mycobacterial replication. The bacteria are then taken up by dendritic cells, which transport them to the adjacent lymph nodes. Antigen presentation causes a Th1-type immune response and this results in the formation of granulomas at the primary site of infection in the lung. The granuloma is not only thought to prevent dissemination but to also facilitate a microenvironment for immune cell communication that may result in bactericidal activity in the lesion. Crucial to this complex response is the synergy between the cytokines IFN-γ and TNF-α [132] that results in killing of *M. tuberculosis* by macrophage activation and by cytolysis. A complex set of T cells ($CD4^+$, $CD8^+$, γδ and CD1-restricted αβ T cells) are involved in containing the infection [133]. However, in many cases, infection is not contained, resulting in caseous necrosis, active clinical disease and the infected individual becoming capable of transmission. On the other hand, when the tubercle bacillus is contained within a granuloma, the bacteria can remain in a dormant state for decades, resulting in latent TB. Reactivation may occur at a later stage due to a weakened or compromised immune system.

Despite the demonstration of the etiological agent of TB more than a century ago, there is no effective prophylactic vaccine available. Bacille Calmette–Guerin (BCG), an attenuated strain of the bovine TB bacillus *M. bovis* used globally, has had mixed success, ranging from excellent protection to none at all [134]. Variations in protection depend on different factors, such as age and geographical location, and the protective effect of BCG vaccination has been known to wane over time [135]. For a while, an emphasis for new candidate vaccines has been for a prophylactic (pre-exposure) vaccine; a better understanding of the pathogenesis of *M. tuberculosis*, latency and the immune response to TB has led to the idea of a post-exposure, therapeutic vaccine that would be able to not only induce a protective response in exposed individuals but also target dormant bacteria, thus preventing latency [136]. This is particularly relevant in the light of recent immunological studies on antigens expressed by the dormancy (DosR) regulon: tuberculin test-positive individuals recognized more latency antigens and with a stronger IFN-γ response than TB patients [137]. Furthermore, BCG vaccination failed to induce significant responses to latency antigens [138].

This part of the review will deal with the newer approaches toward development of effective TB vaccines. There are currently three main strategies employed by researchers:

- Improving the existing BCG strain as a vaccine
- Development of live-attenuated *M. tuberculosis* strains
- The use of 'nonbacterial' vaccines, such as viral delivery vectors, DNA and subunit vaccines

Improving BCG vaccination

A number of suggestions have been made to explain the varying efficacy of BCG as a vaccine, including the loss of protective antigens from BCG and an inadequate T-cell response against BCG [139]. To overcome the former, a number of research groups have overexpressed immunodominant antigens of *M. tuberculosis* into existing BCG vaccine strains [139]. These include recombinant BCG strains expressing antigen 85B (a highly immunogenic, secreted mycobacterial protein involved in cell wall biosynthesis) or early secreted antigenic target 6-kDa protein (ESAT-6), which gave better protection than BCG in animal challenge models [140–142].

Class I-restricted CD8 T cells play an important role in containing TB infection. Apoptosis is one of the main inducers of cell-mediated immunity and antigens contained in apoptotic vesicles play an important role in MHC class I restriction of CD8 T cells [143,144]. As a part of its immune evasion strategy,

the TB bacillus causes inhibition of apoptosis of infected host cells, thereby reducing class I presentation. A similar inhibition of apoptosis was also observed for BCG-infected cells [145], suggesting that this property of BCG may be one of the factors responsible for the poor efficacy of the strain as a vaccine. Recombinant BCG strains that induce apoptosis have also been evaluated as vaccines (reviewed in [139]): strains expressing a recombinant listeriolysin (pore-forming cytolysin from *Listeria monocytogenes*) led to an increased presence of cytoplasmic BCG (due to pore formation in the phagosome) and a resultant induction of apoptosis [146]. Recently, Hinchey *et al.* demonstrated that inactivation of *secA2*, a secretion system component, resulted in enhanced apoptosis of infected macrophages and increased priming of specific $CD8^+$ T cells *in vivo* [147]. Mice and guinea pigs vaccinated with the mutant strain showed increased resistance to *M. tuberculosis* challenge than those infected with BCG [147]. These studies demonstrated that both recombinant, apoptosis-inducing BCG strains and avirulent *M. tuberculosis* mutants that have lost an apoptosis-inhibiting component have potential use as live vaccines due to their ability to cause enhanced MHC class I presentation.

Attenuated *M. tuberculosis* strains as live vaccines

The motivation behind using attenuated *M. tuberculosis* strains is that these strains are based on the actual causative agent rather than a related bovine strain and thus would be closest to mimicking the immune response to TB. Furthermore, there are an additional 120 *M. tuberculosis* genes that are absent in BCG [148,149]. Rather than using extended serial passage to achieve attenuation, as was the case for BCG, the rationale is to use mutants of *M. tuberculosis* that contain deletions in genes essential for virulence. The key to the success of such a vaccine is achieving the right balance between loss of virulence and generating an effective immune response against the attenuated strain (i.e., achieving a 'safe' level of attenuation without much loss of immunogenicity).

However, there are obstacles to using *M. tuberculosis*-attenuated mutant strains as vaccines. First, there is a risk of reversion; however, this risk can be greatly reduced by generating strains that have at least two independent deletions in known virulence genes. Furthermore, these strains would need to be unmarked, that is, any antibiotic resistance markers used for selection of mutants must be subsequently removed. Finally, the safety of these strains would need testing in immune compromised animal models.

One promising candidate is a *phoP* mutant (PhoP is a transcription factor); although attenuated in a mouse infection model, it induced an immune response identical to virulent TB [150,151]. Researchers at the Albert Einstein College of Medicine (NY, USA) have generated two triple mutants, $mc^2$6020 and $mc^2$6030, that were highly attenuated and safer than BCG in severe combined immunodeficiency mice [152–155]. While both strains are panthothenate auxotrophs, the former is also a lysine auxotroph, while the latter has a deletion in the RD1 locus implicated in the attenuation of BCG. Both strains are currently undergoing Phase I clinical trials. Recently, a mutant with an unusual persistence phenotype has been identified: a *kasB* mutant that synthesized shorter mycolic acids was shown to persist in immunocompetent mice for up to 1 year without causing disease and the pathology associated with it [156], suggesting that *kasB* would be a good candidate for deletion in a future live TB vaccine strain.

Nonbacterial vaccines

Direct immunization with immunogenic *M. tuberculosis* polypeptides has been tested as an alternative: antigen 85A, ESAT-6 protein from the *RD1* locus and the PPE family proteins Mtb39a-e and Mtb41 have all been assessed as candidates [139]. A 'hybrid' approach has also been used, wherein recombinant fusions of these polypeptides were used to assess protection in animal models [157,158]. Many of these are currently under Phase I clinical trials. Another strategy is injection of naked (plasmid) DNA encoding immunogenic *M. tuberculosis* antigens, which has been used to elicit a response against the bacterium in mouse and guinea pig models of infection [159]. Recombinant polypeptides and DNA vaccines have also been used in a 'prime–boost' strategy that involves vaccination with BCG or recombinant BCG, followed by a booster of selected antigen(s) that are also common to the 'prime' [139]. A novel approach has been used by researchers at Oxford University (UK) making use of the boosting potential of a modified vaccinia Ankara virus. The recombinant virus expressing Ag85A when used as a boost provided greater protection than BCG or recombinant virus alone [160,161].

Lipid antigens

Similarities to MHC class I molecules and expression on antigen-presenting cells suggested a role for CD1 molecules in T-cell recognition. CD1 isoforms, CD1a and CD1c, were established as targets for recognition by αβ- and γδ-cytotoxic T-lymphocyte lines, respectively [162]. Studies of the proliferative and cytotoxic responses of an $\alpha\beta$-TCR^+ $CD4^-CD8^-$ T-cell line specific for an undefined *M. tuberculosis* antigen indicated recognition was absolutely dependent on the presence of CD1b on the presenting cell, providing direct evidence for CD1-mediated antigen presentation and suggesting a role for CD1 in antimicrobial immunity [163].

The first evidence that CD1-presented antigens differed from their MHC-presented counterparts was provided through studies on CD1b-restricted $\alpha\beta$-TCR^+ T-cell lines specific for *M. tuberculosis*; CD1b-restricted recognition was insensitive to prior protease treatment of the *M. tuberculosis* antigen. Fractionation of organic solvent extracts of mycobacteria revealed that the CD1b-restricted antigen was mycolic acid [164], subsequent studies have defined other microbial lipids as antigens

presented by the group 1 molecules CD1b [165–167], CD1c [168] and CD1a [169] and, more recently, by the group 2 molecule CD1d [170–175], and support the idea that CD1 molecules predominantly present lipid antigens. Mycobacteria, including *M. tuberculosis*, have an especially rich repertoire of complex bioactive lipids and the group 1 CD1 isotypes are upregulated by exposure to these, including mannosyl-β-1-phosphomycoketides [168], diacylated sulphoglycolipid [167] and glucose monomycolate [176], while downregulation of CD1d is apparent on mycobacterial infection [177]. There is little evidence for an important role for CD1d presentation of lipid antigens in mycobacterial infection in mice [178]; these animals only possess a single CD1 isoform most closely related to human CD1d and thus their utility to understanding the role of CD1-presented antigens in the development of protective immunity is somewhat limited. The use of animal models that possess analogues of the other human CD1 isoforms, such as guinea pigs [179], may prove very useful in understanding the role of lipid antigens in the development of protective immunity [178]. Hiromatsu *et al.* demonstrated that CD1-restricted responses could be generated *in vivo* by immunization of guinea pigs with mycobacterial glycolipids, providing support for their use as a relevant small animal model for the study of CD1-restricted immune responses to mycobacterial pathogens [180]. Furthermore, these authors provided evidence that guinea pigs vaccinated with a protein-free heterogeneous mycobacterial lipid extract showed reduced bacterial burdens in the lung and spleen at 4 weeks after infection by an aerosol challenge and also had significantly less necrotic pathology [181]. These limited data support an important role for lipid antigens in the immune response to *M. tuberculosis* infection and their further study to determine whether their inclusion in subunit vaccines might enhance their efficacy.

Conclusion

The recent increase in TB cases forced the WHO to declare TB a global emergency. The emergence of multidrug resistance, now superseded by extensive drug resistance, has compounded the healthcare problem. Fears regarding the effective redundancy of several antimycobacterial drugs stresses the need for identification of new drug targets and development of new drugs, several of which are now under clinical evaluation. In addition, given that the current BCG vaccine is highly variable in its efficacy, control of TB would be helped with a newer, more effective vaccine. Significant research efforts have been applied to the development of efficient agents to be used in both immunoprophylaxis and chemotherapy. A number of new drug candidates outlined in this review show promise against not just existing targets but also new ones. Also, although a multipronged approach is being used to develop new vaccines, all candidates are currently in different stages of clinical trials and there is not yet a clear candidate for replacing BCG.

Expert commentary

Despite current therapies being based on drugs that have been available for decades with few additions, the recent and increased research effort into TB chemotherapeutics has produced a stream of new molecules in clinical and preclinical assessment that might at least be useful in the treatment of drug-resistant TB. Several have excellent extended EBA and may ultimately improve the global healthcare outlook by allowing the shortening of anti-TB chemotherapy. Several of these new drugs represent extremely good candidates as they act against novel targets and thus avoid issues of crossresistance with others drugs. We are of the opinion that there is great utility in continuing to define the modes of action of several preexisting drugs. Although the recent determination of the mode of action of ETH was disappointing in that it was revealed to be identical to that for INH, save for a different enzymic route to prodrug activation [182], other agents, such as isoxyl and thiacetazone, are still to have their modes of action completely defined [183], although these probably both target the meromycolic acid-elongation processes in the biosynthesis of mycolic acid and their cyclopropanation, respectively [184]. Progress here, and elsewhere, is likely to highlight novel enzymes for development of possibly better lead compounds. Controversy surrounds the search for new drugs that inhibit *M. tuberculosis* cell wall synthesis. It is felt that adding new drugs that act on actively growing *M. tuberculosis* will only help with treating MDR organisms (admittedly an increasing problem) but may do little to address the main problem, the length of time of treatment, which is essentially defined by persistent populations. Some argue that cell wall synthesis may be already defective or unnecessary in these organisms as they are not stainable or otherwise detectable microscopically [185]. We, however, would strongly argue in support of the need for new drug targets and inhibitors that target the mycobacterial cell wall. Since their discovery in the 1950s, inhibitors of cell wall synthesis have been the mainstay of many chemotherapeutic regimens. Agents, such as INH, an inhibitor of mycolic acid biosynthesis, when implemented into directly observed therapies have proved highly successful in combating drug-sensitive TB. However, a key issue is the spread of MDR-TB. As a result, the search for new drug targets and cheap alternative agents is greatly needed. While drugs that target nonreplicating forms appear desirable on theoretical grounds, we cannot predict how new drugs will impact on time to cure and we need to await clinical trial data before drawing any such conclusions. Also, the challenge of drug resistance and adverse drug reactions means that, in a significant number of cases, we are searching for cheap yet effective alternative agents. As a result, there will always be a place for new drugs in these areas, with cell wall inhibitors being highly profitable in the past.

The variable efficacy of BCG requires that several avenues of vaccine research must be pursued. T-cell responses specific for foreign lipid antigens presented by CD1 molecules are clearly

important components of the host response to microbial infection, especially for pathogens such as *M. tuberculosis* that have adapted to an intracellular existence in the endosomal compartment and are therefore poor targets for humoral immunity. Current evidence suggests that CD1-restricted responses may make significant contributions to protective immunity in guinea pigs and eventually inclusion of lipid antigens might contribute to the formulation of improved vaccines [180,181]. Further studies in animal models that closely reflect the profile of human CD1 isoforms will probably clarify their importance. Establishing the structures of such CD1-restricted lipid antigens and the molecular and immunological basis of their generation and recognition may become key to the development of novel therapeutic approaches.

Five-year view

The anti-TB 'pipeline' is currently awash with new clinical and preclinical candidate drugs. Several of these will have utility at least in the management of MDR/XDR-TB and some may even have sufficient potency to allow the shortening and simplification of current regimens. In the coming years, as we increase our knowledge regarding the metabolism of *M. tuberculosis* and, more specifically, of the problematic persistent populations, key enzymes worthy of future investigation as novel drug targets will probably come to light. Pursuing their characterization and designing and developing inhibitors might afford us new tools with which to combat these long-standing adversaries.

The great structural diversity of microbial lipids and the limited number of defined antigens to date suggests we are only at the very beginning of a journey towards understanding the structural basis for CD1-restricted lipid antigen presentation. As more antigens are defined, an improved understanding of their relative immunodominance during infection in appropriate models, as well as the kinetics of lipid-specific T-cell responses and their correlation with susceptibility to disease, will become critical to their candidacy as components of developmental vaccines.

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Key issues

- TB remains a major global healthcare concern.
- Latent infections can last decades before active disease develops.
- Latent infection represents a huge global reservoir of infection.
- Extensive drug resistance is emerging.
- Several new drugs are in development, many of which disrupt ATP synthesis.
- The current bacillus Calmette–Guerin vaccine is, at best, variable in its efficacy.
- Preventative and prophylactic vaccines are under development, particularly focused towards improved clearance of latent infection.
- Lipid antigens represent an interesting avenue for future vaccine development.

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