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Drug-resistance in *Mycobacterium tuberculosis*: where we stand

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Tuberculosis (TB), an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*), has burdened vulnerable populations in modern day societies for decades. Recently, this global health threat has been heightened by the emergence and propagation of multi drug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mtb* that are resistant to current treatment regimens. The End-TB strategy, launched by the World Health Organization (WHO), aims to reduce TB-related deaths by 90%. This program encourages universal access to drug susceptibility testing, which is not widely available owing to the lack of laboratory capacity or resources in certain under-resourced areas. Clinical assays are further complicated by the slow growth of *Mtb*, resulting in the long turn-around time of tests which severely limits their application in guiding a patient's treatment regimen. This review provides a comprehensive overview of current TB treatments, mechanisms of resistance to anti-tubercular drugs and their diagnosis and the current pipeline of drugs targeting drug-resistant TB (DR-TB) with particular attention paid to ways in which drug-resistance is combated.

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Introduction

Tuberculosis (TB) is a leading cause of death due to it being an infectious disease, resulting in over a million deaths annually.¹ The causative agent of TB, an acid-fast bacillus, *Mycobacterium tuberculosis* (*Mtb*), was first identified by Robert Koch in 1882. However, it was only in the late 1940s to the early 1950s that the first effective anti-TB antibiotics, streptomycin (STR), *para*-aminosalicylic acid (PAS) and isoniazid

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(INH), were discovered and tested in clinical trials.² An upsurge in TB prevalence in recent decades has been caused by the emergence of HIV, which synergistically disarms host immune responses when co-present with *Mtb* infection,³ leading to the declaration of TB as a global public health emergency by the World Health Organization (WHO) in 1993.⁴ In 2017, it was estimated that 10.4 million people developed TB disease while approximately 1.3 million died from it and a further 300 000 died from a co-infection with HIV.¹ Despite substantial efforts by the global health community, the impact of TB in terms of morbidity and mortality rates remains high, especially in developing countries where the disease is complicated by the high incidence of HIV and poorly resourced and inadequate health systems. In addition, a rise in diabetes mellitus prevalence (approximately 422 million people are affected) increases the risk of TB disease by over 3-fold, surpassing the risk due to HIV in certain geographical areas.^{1,5}

Unlike many bacteria which have evolved to disseminate drug resistance in populations through horizontal gene transfer, the majority of *Mtb* drug-resistance is due to mutations in chromosomal genes. Genotypic resistance results mostly from single nucleotide polymorphisms (SNPs), insertions and deletions, and to a certain extent, deletions in genes that encode drug targets or drug metabolizing enzymes within the bacilli.^{6,7} A direct outcome of this acquired resistance is failure to metabolize prodrugs to active forms, poor drug permeability, increased efflux or modifications in the drug-target structure that result in the inhibition of effective drug binding.⁸ There is an increasing need for new drugs with novel mechanisms of action that can bypass these mechanisms of resistance or which could be used as adjunctive therapy to compensate for those that are vulnerable to promoting resistance. The rise in antimicrobial resistant pathogens affects global health control efforts through limiting the efficiency of current treatments, increasing the burden on health care

systems and therefore creating a negative impact on the economy. The sustainability of antibiotic therapies for a number of infectious diseases, including TB, is threatened as more drugs become ineffective and the pharmaceutical industry has drastically reduced antibiotic research over the years. Mortality rates as a result of antimicrobial resistant infections are rising and TB is no exception. Of the nearly 10.4 million people estimated to have been infected with TB in 2017, about 558 000 were cases of rifampicin (RIF) resistant TB (RR-TB) and approximately 82% of these were multidrug-resistant TB (MDR-TB). MDR-TB is defined as the failure to respond to at least RIF and INH, the key drugs in the regimen due to their sterilizing activity.¹ A majority of the above cases were from the African, European, South-East Asian and Western Pacific regions (Fig. 1).¹ Only a quarter of the people estimated to have developed MDR/RR-TB proceeded to the second-line treatment regimen, of which a success rate of only 55% was reported.¹ In addition to MDR-TB, further resistance to at least one of the second line drugs (namely fluoroquinolones and second-line injectables such as amikacin (AMK), kanamycin (KAN) and capreomycin (CAP)) leads to extensively drug-resistant TB (XDR-TB).¹ Approximately 8.5% cases of MDR-TB were estimated to have XDR-TB, a 2% increase from the previous year, 2016.¹ This indicates a huge disparity in treatment coverage and highlights the dismal treatment outcomes that can be expected for resistant forms of TB. In addition, funding for TB drug research, to develop new TB therapies to combat resistance, has declined and fails to meet the global targets,⁹ making the hopes of shifting to individualized therapies even less likely to occur in the near future. In this review we discuss the current TB treatments, mechanisms of resistance and their diagnosis and new drugs and regimens in the pipeline for resistant TB, and further discuss potential interventions to reduce the spread of drug-resistant TB (DR-TB).

Fig. 1 Global distribution of TB drug resistance for rifampicin-resistant/multidrug-resistant (RR/MDR-TB) and extensively drug resistant TB (XDR-TB).¹

Drug resistant tuberculosis: diagnosis, treatment management and monitoring

The World Health Organization (WHO) has mandated *Mtb* drug susceptibility testing (DST) for all TB patients to better guide treatment decisions and improve treatment outcomes, which indirectly will increase drug resistance surveillance. However, DST is still not performed based on the increasing rates of drug resistance, but rather on the availability of resources.^{7,10} This means that under-resourced, high burden, and mostly developing countries remain impacted by unidentified cases of latent MDR or XDR-TB. Concerted efforts in improving diagnosis of resistant TB are required if the global community is to achieve its targets for the End TB strategy.¹⁰ Traditionally, diagnosis of MDR/XDR-TB has relied on phenotypic drug susceptibility testing (Table 1), which can take up to 12 weeks to provide results due to slow growth of *Mtb* clinical isolates in culture media.¹¹ This delay directly impacts the ability to initiate appropriate therapy and achieve effective treatment. In addition, the requirement of specialized laboratories and equipment has a huge impact in low resourced countries. To accelerate this rate determining step, prompt, inexpensive and sufficiently sensitive susceptibility tests to diagnose drug-resistant TB are required.

A first step in this direction came when the WHO recommended a rapid genotypic test, Xpert MTB/RIF (Table 1), a diagnostic test for simultaneous detection of TB and RIF resistance which has radically improved detection and notification of new cases.⁷ Xpert MTB/RIF currently only detects resistance to RIF, and so cannot determine the full extent of a patient's drug susceptibility profile. Moreover, Xpert MTB/RIF only detects resistant RIF strains with common mutations in an 81 bp region of the *rpoB* gene (codon 426 to 452), the RIF-resistance determining region (RRDR), while rare mutant strains such as *rpoB* I491F located outside the RRDR would go undetected.^{12,13} Therefore, a patient may be incorrectly prescribed a treatment plan which may result in sub-optimal treatment and so promote further transmis-

sion. This has replaced the need for culture-based methods for decision making in some settings,¹⁴ expediting treatments and therefore partially improving outcomes. Culture-based methods do however remain the reference standard and are used to monitor treatment progress.¹ A few other WHO recommended molecular diagnostic tests for resistant TB exist, such as first-line rapid line probe assays (LPAs) which test for resistance to INH and RIF and second-line LPAs which test for resistance to fluoroquinolones and injectable second-line TB drugs (Table 1). These assays, however, only target a limited number of resistance variants and lack comparable sensitivity to phenotypic culture assays.¹¹ A single diagnostic test that could determine a complete resistance profile of the infecting *Mtb* strain(s) in a patient, in a similar manner to phenotypic culture-based drug susceptibility tests but with more time efficiency and ease of use, is the ultimate goal required. A first major step in this endeavor has recently been shown utilizing whole genome sequencing (WGS) technology to predict drug susceptibility to first-line anti-TB drugs.¹⁵ Based on knowledge of known mutations that confer resistance to these drugs, the study showed a correlation to the phenotypic standard method with a high degree of specificity and sensitivity sufficient for clinical use. Because of the potential for inclusion of all known mutations associated with all the drugs currently in use or in the drug development pipeline for both susceptible and resistant strains of *Mtb* into this test, there is huge potential for customization of individual therapy to especially improve MDR or XDR-TB treatment outcomes.⁹

With accurate diagnosis and subsequently effective treatment, we gain the ability to improve treatment outcomes by reducing development of resistance. However, DR-TB may also develop as a result of getting infected with a resistant *Mtb* strain. Those with treatment failure remain at risk of transmitting the infection.¹⁶ A number of studies indicate that this is predominantly the case with new MDR or XDR infection cases.^{17,18} Person-to-person transmission accounted for the majority of resistance spread in these areas, meaning MDR and XDR-TB patients were infected initially with an MDR or

Table 1 Current diagnostics used for drug susceptibility testing¹

Diagnostic test	Detection	Time to diagnosis	Recommended by the WHO
Sputum smear microscopy	Ziehl-Neelsen (acid-fast) staining	A few hours	YES, mainly used for treatment monitoring
Culture-based methods	<i>Mtb</i> growth	Up to 12 weeks	YES, current reference standard
Rapid molecular tests:	Rifampicin resistance	Within 2 hours	YES
i. Gene-Xpert MTB/RIF			
ii. Line probe assays first-line LPAs (MTBDRplus)	Isoniazid and rifampicin resistance in acid-fast bacillus smear positive sputum or <i>Mtb</i> cultures		YES
Second-line LPAs (MTBDRs)	Resistance to fluoroquinolones and second-line injectable agents		YES
iii. Lateral-flow lipoarabinomannan assay (LAM)	<i>Mtb</i> in urine samples		YES, for HIV patients with low CD4 counts
Whole-genome sequencing	Changes in <i>Mtb</i> genome		NO, under investigation

XDR-TB strain, rather than slowly acquiring resistance caused by inadequate or irregular treatment. It is not surprising given the fact that TB is predominantly an airborne disease, and by that virtue is predisposed to impact by social determinants such as overcrowding in impoverished social settings.

Currently approved TB drugs and associated modes of resistance

The current TB regimens for drug-susceptible and DR-TB involve a combination therapy of a set of drugs. The standard

regimen, which consists of a 2 month regimen of RIF, INH, pyrazinamide (PZA) and ethambutol (EMB) (intensive phase), followed by a continuous 4 month phase of INH and RIF, remains the recommended treatment for drug-susceptible TB (Table 2).¹⁹ For DR-TB however, given the different levels of resistance in disease, drug combinations may vary from patient to patient. The WHO has issued recommendations on treatment and care of DR-TB patients, with regard to drug combinations to be used, the length of treatment and how patient response should be monitored.²⁰ The MDR/RR-TB regimens can be offered as either i) a standardized shorter

Table 2 TB drugs currently used for treatment of both susceptible and drug-resistant TB as recommended by the WHO

Treatment of drug susceptible TB (first-line)						
Drug	Route of administration	Treatment duration (phase)		Mechanism of action	Resistance-associated gene(s)	Ref.
Rifampicin	Oral	6 months (2 months for the intensive phase; 4 months for the continuous phase)		Binds to the β-subunit of the RNA polymerase, inhibition of RNA synthesis	<i>rpoB</i>	22
Isoniazid	Oral			A prodrug activated by a catalase-peroxidase, inhibition of mycolic acid synthesis	<i>katG</i> , <i>inhA</i>	32
Pyrazinamide	Oral			A prodrug activated by pyrazinamidase, disruption of membrane energetics; inhibition of coenzyme A biosynthesis	<i>pncA</i> , <i>rpsA</i> , <i>panD</i> , <i>clpC1</i>	38
Ethambutol	Oral			Inhibition of an arabinosyl transferase involved in arabinogalactan synthesis	<i>embB</i> , <i>ubiA</i>	53
Treatment of RR/MDR-TB and XDR-TB (second-line)						
Fluoroquinolones Levofloxacin Moxifloxacin Gatifloxacin		Oral	9–12 months for shorter regimens	Inhibition of DNA gyrase, a topoisomerase II responsible for DNA supercoiling	<i>gyrA</i> , <i>gyrB</i>	59
Second-line injectables (SLIDs) Amikacin		Injectables	Up to 20 months for longer regimens (8 months for the intensive phase)	Binds to 16S rRNA, therefore inhibition of protein synthesis	<i>rrs</i>	66
Kanamycin				Inhibition of acetyltransferase that binds to 16S rRNA, therefore inhibition of protein synthesis	<i>rrs</i> , <i>eis</i>	
Capreomycin				Inhibition of methylation of 16S rRNA & 23S rRNA, therefore inhibition of protein synthesis	<i>tlyA</i>	
Streptomycin				Inhibition of protein synthesis by interaction with 16S rRNA and the ribosomal protein S12	<i>rrs</i> , <i>rpsL</i> , <i>gidB</i>	62, 68
Other core second-line agents Ethionamide/prothionamide		Oral		Prodrugs activated by EthA, interacts with NAD to inhibit synthesis of mycolic acids	<i>inhA</i> , <i>ethA</i>	36
Cycloserine/terizidone				Inhibition of formation of D-alanine and its incorporation for peptidoglycan synthesis	<i>alr</i> , <i>ddl</i> , <i>cycA</i>	71, 72
Clofazimine				A prodrug reduced by NADH oxidoreductase, disruption of energy metabolism	<i>Rv0678</i>	73
Linezolid Add-on agents Bedaquiline		Oral		Inhibits initiation of protein synthesis	<i>rrl</i> , <i>rplC</i>	74
Delamanid and pretomanid				Binds ATP synthase, therefore leading to inhibition of ATP synthesis	<i>atpE</i>	75, 76
<i>p</i> -Aminosalicylic acid				Inhibition of mycolic acid synthesis, therefore a cell-wall synthesis inhibitor	<i>fbiABC</i> , <i>ddn</i> , <i>fgd1</i>	77, 78
				A prodrug that targets dihydrofolate reductase, therefore blocking the folate pathway	<i>folC</i> , <i>thyA</i> , <i>ribD</i>	79
Thioacetazone				Inhibition of mycolic acid synthesis	<i>hadABC</i> , <i>ethA</i>	80
Meropenem/clavulanate				Inhibition of β-lactamase	<i>blaC</i>	81, 82

regimen of 9–12 months (Table 2), where patients with more than one month treatment with second-line drugs or resistance to fluoroquinolones and second-line injectables are excluded; or ii) longer regimens which can last up to 20 months.²⁰ Longer treatment regimens may be built up from all three group A agents (moxifloxacin or levofloxacin, bedaquiline (BDQ) and linezolid (LZD)) and at least one group B (second-line injectables excluding KAN and CAP, clofazimine, cycloserine or terizidone) agent to make up at least a four-drug regimen, or if one or two group A agents are used, both group B should be used. Group C agents are added if a regimen cannot be completed with groups A and B alone. The WHO guidelines strongly recommend that the fluoroquinolones moxifloxacin or levofloxacin and LZD should be used for all patients, and BDQ in patients aged 18 years or older.²⁰ BDQ may be used in patients aged 6–17 years and delamanid may be used from ages of 3 years and above. In addition to KAN and CAP, clavulanate is also excluded in longer regimens. Other drugs such as PZA, EMB, AMK, STR, ethionamide (ETH), PAS, clofazimine, cycloserine or terizidone, meropenem or imipenem–cilastatin may be included in longer regimens on the condition that they either are not used in combinations that contain BDQ, LZD, clofazimine and delamanid, or that susceptibility has been demonstrated and an adverse reaction can be monitored.²⁰ For patients on longer regimens, the standard of treatment monitoring has been recommended as sputum culture and sputum smear microscopy, with sputum culture performed at monthly intervals.²⁰ In Table 2, currently approved drugs and their mode of action are listed. The resistance profiles of anti-tubercular drugs are listed in Table 3.

First-line drugs

Rifampicin (RIF). RIF is a highly bactericidal antibiotic that inhibits RNA synthesis through inhibition of bacterial DNA-dependent RNA polymerase (RNAP). RIF resistance mechanisms mapped to the *rpoB* gene, which encodes the β -subunit of RNAP, were first identified in *Escherichia coli*,²¹ and later shown in *Mtb*.²² These resistance mutations occurring in the 81 bp RRDR of the *rpoB* gene in *Mtb* isolates were found to be mainly amino acid substitutions,^{22–24} which cause conformational changes in RpoB and therefore prevent RIF from binding to its target. Resulting effects are changes in minimum inhibitory concentration (MIC) levels, ranging from low level to high level resistance with up to $\geq 200 \mu\text{g ml}^{-1}$ in some clinical strains.²³ Amino acid substitutions at codons 516, 526 and 531 are the most common mutations associated with RIF-resistance.²⁴ However, a number of deletions and duplications can occur within this region and have been shown in *Salmonella*²⁵ and *Mtb* clinical isolates²⁶ associated with secondary compensatory substitution mutations. Mutations outside the RRDR are also known to occur.^{12,13} RIF binding to the β subunit of RNAP is dependent on the hydroxyl group of the molecule. Enzymatic modifications of the RIF hydroxyl group through phosphorylation, glycosylation

and ADP-ribosylation inactivate RIF, causing resistance.²⁷ Monooxygenases have also been shown to inactivate rifamycins through oxidation of the naphthyl group at position 2, destabilizing their ring structure.²⁸ These mechanisms have been demonstrated in other bacterial species and *M. smegmatis*, but yet to be demonstrated in *Mtb*. Cross-resistance to other rifamycins such as rifabutin (RFB), with mutations in the *rpoB* gene, has been reported; however, some studies have revealed RIF resistant-RFB susceptible clinical isolates suggesting RFB as a potential replacement of RIF for the treatment of RIF-resistant TB in some patients.^{29,30}

Isoniazid (INH). INH is a prodrug, activated by KatG, a catalase-peroxidase enzyme encoded by the *katG* gene in *Mtb* to block synthesis of mycolic acids which form a major component of the *Mtb* cell-wall. Resistance to INH was first observed early on in the 1950s following its use as a monotherapy, which led to the first combination regimen for TB that also included STR and PAS.³¹ However, it was much later that the molecular basis of INH resistance was identified to be mutations in the *katG* gene.³² INH activity requires two key enzymes; KatG, which activates the prodrug INH to form INH-NAD adducts³³ and the second enzyme encoded by the *inhA* gene, is a NADH dependent enoyl-ACP-reductase (a FASII – fatty acid synthase – enzyme) involved in mycolic acid biosynthesis and is inhibited by activated INH.³⁴ Mutations in *katG*, most commonly amino substitution (Ser315Thr),³² cause conformational changes in the INH-binding pocket of KatG and prevent bio-activation of INH. Mutations in the *inhA* promoter region lead to overexpression of the *inhA* target, whereas mutations in the *inhA* coding region cause structural changes which reduce InhA binding affinity to NADH therefore preventing INH from binding to the target, resulting in resistance.³⁵ Cross-resistance to a structural analogue, second-line drug ethionamide (ETH), has also been reported, since they share a common target, *inhA*.³⁶ A combination of mutations in *inhA* and KatG (Ser315Thr) confers a higher level of resistance.³⁷ The impact of this is the requirement of a suitable diagnostic test that determines the INH resistance level to inform INH dosage or discontinuation of INH treatment in such cases, and the development of newer INH derivatives.

Pyrazinamide (PZA). Mutations in the *pncA* gene that encodes for pyrazinamidase (PZase), an enzyme responsible for conversion of inactive prodrug PZA into pyrazinoic acid (POA), drive *Mtb* resistance to PZA.^{38–40} The 561 bp open reading frame of the *pncA* gene houses multiple mutant variations in clinical isolates, meaning no single PZase mutation causes resistance as in *rpoB* for RIF resistance.⁴¹ Therefore, it becomes difficult to identify a genetic hotspot that could be used for molecular diagnostic testing, to inform treatment. Initially thought to be conserved at a few specific amino acid residues such as Asp12 $\rightarrow$ Ala or Asn or Leu85 $\rightarrow$ Pro,⁴² assessments of *pncA* sequences of multiple clinical isolates show high variability across the full *pncA* gene, with mutations that confer PZA resistance ranging from high to very high confidence, some with an unclear role and some with no effect on phenotypic resistance. The crystal

Table 3 Resistance profile of anti-tubercular drugs

Drug	Target gene	Common mutations	Clinical mutation frequency (%)	Resistance MIC range ($\mu\text{g mL}^{-1}$)	Ref.	Future development
Rifampicin	<i>rpoB</i>	Ser531Leu, His526Tyr, His526Asp, His526Pro	100	2–>200	23, 24, 30	Rifamycins, such as rifabutin, with no RIF cross-resistance could be used instead
Isoniazid	<i>katG</i> <i>inhA</i>	Ser315Thr Promoter (c-15 t); Ile21Thr, Ser94Ala	60–95 5–47 11	>19.2 0.8–0.16 3.2–12.8	24, 37	New INH derivatives with more potent activity
Ethambutol	<i>embCAB operon</i> <i>embCAB intergenic region</i>	Met306Ile/Val, Gly406Asp/Ala Gln497Arg/Lys	70 15	10–50	24, 83
Pyrazinamide	<i>pncA</i> <i>panD rpsA</i> <i>clpC1</i>	D8N, Cys138S, Cys138G, IS6110	100	400–>800	24, 39
Ethionamide	<i>ethA</i> <i>ethR</i> <i>inhA</i>	110–138	22–61 37 55	50–>200	24, 36
Moxifloxacin	<i>gyrA</i> <i>gyrB</i>	Asp94His, Asp94Asn, Asp94Gly, Asp94Tyr, Ala90Val Asn533Asp, Asp533Thr	42–88 ≤ 4	0.5–>8	24, 84
Levofloxacin	<i>gyrA</i> <i>gyrB</i>	Asp94His, Asp94Asn, Asp94Gly, Asp94Tyr, Ala90Val T511N	94 64 <2	>10 >1	85
Gatifloxacin	<i>gyrA</i> <i>gyrB</i>	Asp94His, Asp94Asn, Asp94Gly, Asp94Tyr, Ala90Val	42–88 ≤ 4	0.25–>4	84, 61	

Table 3 (continued)

Drug	Target gene	Common mutations	Clinical mutation frequency (%)	Resistance MIC range ($\mu\text{g ml}^{-1}$)	Ref.	Future development
Streptomycin	<i>rpsL</i> <i>rrs</i>	K43R, K88Q A1401G, C1402T, and G1484T	47–90 5		24, 68	New spectinamides
Kanamycin	<i>rrs</i>	A1401G	100%	4	24
Amikacin	<i>rrs</i> <i>tlyA</i>	A1401G	78% 22%	>2.5	24, 65
Capreomycin	<i>rplC</i> <i>rpl</i>	Cys154Arg G2746A, G2270		>1
Linezolid	<i>AtpE</i> <i>Rv0678</i>	Ala63Pro, Asp28Val, Ile66Met
Bedaquiline	<i>ddn</i> <i>fdg1</i> <i>fbiA</i> <i>fbiB</i> <i>fbiC</i>	D49Y G49 ^{fs}	NA	NA
Delamanid						

Table 3 (continued)

Drug	Target gene	Common mutations	Clinical mutation frequency (%)	Resistance MIC range ($\mu\text{g ml}^{-1}$)	Ref.	Future development
Pretomanid	<i>ddn</i> <i>fdg1</i> <i>fbiA</i> <i>fbiB</i> <i>fbiC</i>		NA	NA		

structure of *Mtb* PncA revealed key amino acid residues involved in the catalytic site of PZase (Asp8, Lys96 and Cys138) and for Fe^{2+} coordination (Asp49, His57 and His71) which showed the loss of enzyme activity in mutants.⁴³ Residue Cys138 has been previously implicated in nucleophilic attack of PZA by the PncA active site, and substitutions of Asp8 and Lys96 were hypothesized to decrease its nucleophilicity suggesting the importance of these residues in PncA catalytic activity.⁴⁴ Some of the mutations, however, were associated with changes in protein folding and decreased thermal stability suggesting that these could have significant effects on the three-dimensional structure of PncA and protein abundance.^{41,43}

More recently, mutations in *panD* which encodes aspartate decarboxylase involved in coenzyme-A biosynthesis have been associated with PZA/POA resistance *in vitro* and *in vivo*,^{45,46} and mutations in this gene were also identified in naturally PZA-resistant *M. canettii* and PZA resistant clinical isolates.⁴⁶ POA rather than the prodrug PZA is believed to bind to the target PanD.⁴⁵ Another gene, *clpC1*, which encodes an ATP-dependent ATPase involved in protein degradation mechanisms has been shown to harbor mutations in the N-terminal and D1 domain of the coding region (Gly99Asp, Leu88Val, *etc.*) and another mutation 10 bp upstream of the *clpC1* encoding sequence in the leader mRNA of the transcript and these mutations were associated with PZA/POA resistance in *in vitro* *Mtb* strains.^{47,48} Mutations in the *rpsA* gene, which encodes ribosomal protein S1, were linked to low level PZA-resistance.⁴⁹ A deletion of alanine at 438 bp of *rpsA* was found to impair POA binding to the 30S ribosomal protein S1 causing resistance in strains lacking *pncA* mutations.⁵⁰ However, recent reports present conflicting results on the association between RpsA mutations and PZA resistance^{51,52} suggesting that further evidence is required to ascertain the link between the two.

Ethambutol (EMB). EMB has activity against replicating bacteria, by interfering with arabinogalactan synthesis in the cell-wall. EMB resistance, just like PZA, is due to the wide range of genetic variants. Genetic mutations in the *embCAB* operon which encodes membrane-associated arabinosyl transferases confer EMB resistance.⁵³ Mutations in the *embB* gene, especially codon 306 (Met306Ile and Met306Val), were reported to present the most frequent mutation in resistant isolates of EMB.⁵³ In addition to mutations found in *embB* codon 306, 406 or 497, some mutations were outside the three regions between codons 296 and 426, while more oc-

curred in the *embC-embA* intergenic region of the *embCAB* locus.⁵⁴ *EmbB* mutations in the ERDR (EMB resistance-determining region) spanning a 576 bp region are thought to decrease the hydrophobicity index of the region leading to sup-optimal interaction of the drug with the binding site. A study by Safi *et al.* (2008) reported that codon 306 mutations were rather responsible for predisposition to develop resistance to drugs such as EMB, INH and RIF.⁵⁵ Another study by the same group revealed that a large range of MICs of EMB are observed when mutations occur in the decaprenylphosphoryl-B-D-arabinose (DPA) biosynthetic and utilization pathway genes, *Rv3806c* and *Rv3792*, in conjunction with mutations in *embB* and *embC*.⁵⁶

Second-line drugs

Fluoroquinolones. Fluoroquinolones inhibit *Mtb* through binding of the gyrase-DNA complexes. DNA gyrase is an ATP-dependent enzyme that introduces negative supercoils into the double stranded DNA required for replication, transcription and recombination. Recently classified as group A drugs for treatment of MDR-TB, fluoroquinolones, particularly the newer generation (moxifloxacin, high-dose levofloxacin, gatifloxacin) are currently the best drugs for treatment of MDR-TB with bactericidal and sterilizing activities and are being investigated for potential use as first-line agents.^{57,58} However, the widespread use of these drugs has led to development of resistance at an alarming rate. Mutations in the quinolone resistance determining region (QRDR) of the *gyrA* and *gyrB* genes which encode for the gyrase enzyme are known to confer resistance to fluoroquinolones.⁵⁹ A systematic review of sequencing data of the QRDR from clinical isolates revealed that 64% of 1220 isolates had *gyrA* mutations, whereas of the 534 isolates sequenced for *gyrB* QRDR only 3% has mutations.⁶⁰ Another study in China using ofloxacin-resistant isolates revealed 68/80 isolates with *gyrA* mutations, whereas only three had *gyrB* mutations and a few had either no mutations in the QRDR or had both *gyrA/B* mutations.⁶¹ Some mutations have been found to lie outside the QRDR, which negatively impact the performance of diagnostics such as the MTBDRs_l assay which relies on identification of resistance in the QRDR.¹ This again places an emphasis on the reliability of phenotypic DST use in conjunction with these rapid molecular tests.

Second-line injectables. The use of aminoglycosides against TB began with the discovery of STR inhibitory activity against *Mtb* in the 1940s. Currently classified as group B drugs, KAN, AMK and CAP (aminoglycosides) are currently used for the treatment of MDR-TB, whereas STR is only used when susceptibility is highly likely and the other injectables cannot be used.^{20,57} These continue to be used despite reported adverse side effects, including hearing loss, and invasive administration consisting of intramuscular injection administration over an extended period of 4–8 months, causing serious distress to patients. Aminoglycosides target *Mtb* protein synthesis through inhibition of the 30S subunit of the ribosome and their resistance mechanisms were found to be associated either with mutations in the 16S rRNA (*rrs*) gene or the *rpsL* gene encoding the S12 ribosomal protein *in vitro* and in MDR-TB clinical isolates.^{24,62} Aminoglycosides are chemically diverse; the studies in *E. coli* and other bacterial species have suggested that the different classes bind differently to the A-site on the 16S rRNA.⁶³ The 2-deoxystreptamine ring of aminoglycosides such as KAN and AMK is thought to interact with highly conserved nucleotide bases of the helix 44 of 16S rRNA through hydrogen bonding. This binding results in mistranslation of proteins as a result of incorrect pairing of mRNA and aminoacyl-tRNA, or interference with translocation, with detrimental effects on *Mtb*.⁶⁴ Mutations in the 16S rRNA (*rrs*), most commonly A1401G, hinders this binding and confers cross-resistance to several aminoglycosides.^{65,66} Mutations in the *rrs* gene (A1401G) have been associated with high level resistance to both KAN and AMK.⁶⁷

STR, which lacks a 2-deoxystreptamine ring, is thought to bind to the phosphate backbone of 16S rRNA through salt bridges and hydrogen bonds and makes contact with the ribosomal protein S12.⁶⁴ Mutations in the *rpsL* gene confer high level resistance to STR.⁶² Some of the STR-resistant strains do not possess mutations in the *rrs* or *rpsL* genes; however, they may confer low level resistance with mutations in the *gidB* gene encoding a conserved 7-methylguanosine methyltransferase for 16S rRNA.^{68,65} CAP resistance with mutations in the *tlyA* gene (encoding rRNA methyltransferase) has been observed in strains with or without *rrs* mutations⁶⁵ while CAP resistance mediated by the *rrs* A1401G mutation has been observed as cross-resistance to AMK.⁵⁴ It is hypothesized that TlyA-mediated methylation of rRNA is crucial for CAP binding to 16S rRNA. Acetylation of KAN as a result of mutations which overexpress the enzyme aminoglycoside acetyltransferase (*eis*), modifies the drug structure and alters KAN binding, causing low level resistance.⁶⁹ Mutations in the untranslated region of *whiB7*, a transcriptional activator of *eis*, increased expression of the *eis* and the *tap* efflux gene causing KAN and STR cross-resistance.⁷⁰

New and repurposed drugs for resistant TB in the drug development pipeline

Despite the declaration of TB as a global health emergency in 1993 by the WHO, TB drug discovery has been extremely

slow, with BDQ (TMC207) being the first drug to receive approval (albeit conditional) from the FDA in 2012. Only one more new drug, delamanid, has been introduced into the market since then, and both drugs were approved for the treatment of DR-TB. Some drugs conventionally used in other disease indications have been repurposed for TB to accelerate the incorporation of new drugs into the market.

Bedaquiline (BDQ)

A diarylquinoline with a new mechanism of action, BDQ, was approved for treatment of MDR-TB in 2012 (US) and 2014 (Europe). This drug acts through inhibition of the mycobacterial membrane-bound C-subunit of the adenosine triphosphate (ATP) synthase,⁸⁶ therefore depleting ATP in *Mtb*. BDQ has good activity against *Mtb*, exhibiting various physiological states, growing and non-growing populations and MDR-TB strains.⁷⁵ In non-replicating or dormant bacilli, ATP synthase is down-regulated but the enzyme still remains active and synthesizes minimal ATP to maintain certain cellular processes critical for survival. BDQ has demonstrated the ability to inhibit ATP synthase at such low levels and exerts potent bactericidal activity.⁸⁷ This effect of BDQ activity on dormant bacilli suggests the potential of sterilizing activity against caseum-core resident bacilli. Another study showed a synergistic sterilizing effect of BDQ and PZA (a sterilizing drug) in an infected mouse model.⁸⁸ The major mechanism of resistance identified against BDQ is caused by mutations in the C-subunit of the F1F0 proton ATP synthase encoded by the *atpE* gene.⁷⁵ Computational simulations based on the *E. coli* homology model of AtpE suggest an interaction of BDQ with amino acid residue 61 (Glu) of the C-subunit.⁸⁶ This binding blocks proton transfer between the A- and C-subunits resulting in shutdown of ATP synthase motor activity.⁸⁶ Resistant mutants have shown point mutations in this amino residue (Glu61Asp), and additional amino acid substitutions (Asp28Val/Pro/Gly, Ala63Pro and Ile66Met) which occur in close spatial proximity to position 61 are thought to interfere with BDQ accessing the binding site,^{86,89} therefore maintaining proton transfer and ATP production. Resistant mutants lacking mutations in the *atpE* gene have suggested an alternative off-target mechanism of resistance. Upregulation of the MmpS5–MmpL5 efflux system, through its Mar-like transcriptional regulator, encoded by the Rv0678 gene has recently been shown to cause cross-resistance to BDQ and clofazimine *in vitro* (Fig. 2)⁹⁰ and in clinical isolates.⁹¹

Delamanid & pretomanid

These nitroimidazoles represent the second class of new TB compounds discovered in recent years. Delamanid, also referred to as OPC-67683, was conditionally approved by the European Medicines Agency (EMA) in 2014 for the treatment of MDR-TB, while currently being investigated in phase III trials. Despite the lack of sufficient efficacy and safety data, delamanid also received recommendation from the WHO in

Fig. 2 TB drugs and the frequency of mutations for associated targets. Blue – cell-wall synthesis. Grey – protein synthesis. Black – DNA synthesis. Yellow – RNA synthesis. Pink – cellular metabolism. Orange – ATP synthesis/energy metabolism. Red crosses – high frequency mutations. Green ticks – low frequency mutations.

the same year in efforts to fight the global MDR-TB crisis. Both delamanid and pretomanid have been shown to have activity against both replicating and non-replicating as well as drug-resistant forms of TB, with MIC ranges of 0.006–0.024 and 0.015–0.25 $\mu\text{g ml}^{-1}$, respectively.^{92,93} Evaluation of effects of delamanid on MDR- and XDR-TB showed sputum culture conversion (a predictive measure of treatment outcomes) of patients at 2 months.^{94,95} Pretomanid, currently in phase III clinical development, is mainly being investigated as a component of newer combinations of resistant and susceptible anti-TB regimens.⁹⁶ These nitroimidazoles are thought to have two mechanisms of action: (i) as cell-wall lipid biosynthesis inhibitors through inhibition of mycolic acid synthesis, and (ii) through formation of nitrogen reactive species.^{92,97} Both delamanid and pretomanid are bioactivated by a deazaflavin (cofactor F_{420})-dependent nitroreductase (Ddn). Co-factor F_{420} itself requires a three gene complex or operon of *fbiA*, *fbiB* and *fbiC* for its biosynthesis, and another enzyme glucose-6-phosphate dehydrogenase (Fgd1) converts it to its reduced form through a redox recycle process.⁷⁸ Their activity inhibits mycolic acid biosynthesis, and pretomanid facilitates nitric oxide mediated killing under anaerobic conditions.⁷⁷ Mutations, located within the catalytic domain of enzymes encoded by *ddn*, *fgd1*, *fbiA*, *fbiB* and *fbiC* genes, have

been associated with PA-824 resistance *in vitro*,⁹⁸ and these are likely to inhibit PA-824 activation. A pre-XDR patient treated with a delamanid-containing regimen developed mutations in *fbiA* (D49Y) and *fgd1* (G49^f) emphasizing the clinical relevance of mutations in these genes.⁹⁹

Linezolid (LZD)

A member of the oxazolidinone class of compounds, LZD has long been available in the market for treatment of Gram-positive multi-drug resistant bacterial infections such as streptococcus and methicillin-resistant *Staphylococcus aureus* (MRSA). LZD inhibits bacterial protein synthesis through inhibition of protein translation by binding to the 23S rRNA of the 50S ribosomal subunit. *In vitro*, LZD has been shown to have activity against both MDR and XDR strains of *Mtb*, with MIC values in the range 0.125–0.5 $\mu\text{g ml}^{-1}$. A randomized clinical study on XDR-TB patients with no treatment response for 6 months on the appropriate regimen resulted in 79% sputum conversion after receiving an initial 600 mg LZD for 4 months, and a further 400 or 600 mg dose for 2 months,¹⁰⁰ clearly indicating treatment outcomes comparable to those of MDR-TB and huge potential for use of LZD for treatment of XDR-TB. Of concern are reported toxicity issues related to

LZD use, for example, the clinical study reported that 82% of the participants experienced adverse side effects including peripheral neuropathy, optical neuropathy, hepatic dysfunction, *etc.*, with more pronounced adversity in patients receiving higher dosage.¹⁰⁰ Therefore, recommendations have been made for usage of LZD in patients with very few to no treatment options remaining, as is the case with XDR-TB patients and patients who have had previous treatment failure.⁹ To improve the toxicity profile, newer LZD derivatives¹⁰¹ are currently being tested in phase I and II of clinical development, although LZD continues to be tested in combination regimen and dose-ranging optimization. In addition, caution should be taken for its use in well-functioning programs to prevent its misuse which could further perpetuate spread of resistance.⁹ LZD resistance was first reported in clinical *Mtb* strains by Richter *et al.* (2007), but no mutations were detected in the genes encoding the 23S rRNA gene.¹⁰² LZD blocks the binding of tRNA to the peptidyl-transferase center (PTC) on the 50S bacterial subunit disrupting protein synthesis and therefore inhibiting bacterial cell growth.⁷⁴ An amino acid substitution mutation in the *rplC* gene (T460C), encoding the L3 ribosomal protein, which had been previously reported as a mechanism of resistance to LZD in *S. aureus* has been reported for *Mtb*.^{103,104} A study in China revealed LZD resistance in about only 30% of the clinical isolates with no resistance due to mutations in the 23S rRNA suggesting that there may be other mechanisms of resistance which are yet to be uncovered.¹⁰⁵

SQ109

SQ109 is a drug that was first identified through a combinatorial chemistry and *in vitro* high throughput screening of derivatives of EMB around the diamine pharmacophore. SQ109 was found to have activity against both drug susceptible and resistant *Mtb* strains. Addition of SQ109 to the standard regimen for MDR-TB in a phase IIB/III clinical trial conducted in the Russian Federation reported positive outcome in terms of efficacy, safety profiles and tolerability, with another phase II planned to commence soon in the USA.¹ There have been multiple reported mechanisms of action for SQ109, including inhibition of MmpL3, a membrane transporter protein involved in incorporation of mycolic acid into the *Mtb* cell-wall, and effects on energy production as well as activity on *Mtb* efflux systems.^{106,107} Resistance to SQ109 has been observed to be associated with mutations in the genes encoding the membrane transporter *mmpl3* (ref. 106) (Fig. 2). Although an analogue of EMB, the mechanism of action of SQ109 is distinct from that of EMB and no cross resistance has been observed thus far. In *in vitro* studies, it has been shown to synergize with a number of other TB drugs such as BDQ, INH, sutezolid and to a greater extent RIF.

Meropenem and clavulanate

Meropenem, a β -lactam FDA-approved antibiotic, has been in clinical use for treatment of both Gram positive and negative

bacteria. For *Mtb* and other mycobacterial infections however, β -lactams failed to show potent activity, due to an enzyme, β -lactamase, facilitated mechanism of resistance.⁸² In the *Mtb* genome sequence, the *blaC* gene encodes the β -lactamase enzyme and a knockout of this gene increased *Mtb* susceptibility to β -lactam antibiotics.⁸² Clavulanic acid (clavulanate), also FDA-approved, irreversibly inhibits BlaC,⁸¹ and its combinations with meropenem, a slow substrate for *Mtb* BlaC, were highly effective against XDR-TB clinical isolates, with MIC values comparable to those of drug susceptible strains.¹⁰⁸ Meropenem/clavulanate associated clinical success has been reported in XDR-TB patients.^{109,110}

Building effective regimens for drug-resistant TB

The treatment duration with current regimens for MDR or XDR-TB is lengthy, up to 24 months (Table 2) and with very low success rates for XDR-TB. In addition, some patients may have low tolerability to the drugs or otherwise fail treatment. Therefore, to improve the chances of finding shorter, effective, safe and tolerable regimens for resistant TB or even susceptible TB, a number of combination therapies with both new, old and repurposed drugs are currently in clinical development.¹

BDQ and delamanid, the two newly approved TB drugs, are currently in trial (ACTG5343) in combination with the standard multidrug background treatment (MBT) for MDR-TB. The trial is investigating regimens containing BDQ or delamanid or combinations of both to evaluate the safety, tolerability and pharmacokinetics of adding these drugs in the current regimens (WHO, 2018). An earlier phase II randomized, placebo-controlled trial of BDQ added to optimized background regimens (OBR) showed a reduced time to culture conversion for MDR-TB patients in comparison to OBR alone.¹¹¹ Current regimens with BDQ and LZD in combination with OBR for MDR-TB in the NEX-TB trial are in phase III and proposed to end in 2019.⁹⁶ Another BDQ-containing regimen currently in phase III trials (Nix and ZeNix) consists of BDQ, pretomanid and LZD for the treatment of XDR-TB. While the results have not been published, interim data suggest all patients to have culture conversion at 4 months, with a single case of microbiological relapse. Adverse events were reported due to LZD toxicity; however they were manageable.¹¹²

Pretomanid-containing regimens are currently in phase II and III in different combinations. An earlier 14 day early bactericidal activity (EBA) study (NCT01498419), assessed by measurement of colony forming units (CFU) and sputum culture conversion, showed that the combination of pretomanid-moxifloxacin-PZA (PaMZ) was significantly higher than that of BDQ-PZA, BDQ, or BDQ-pretomanid except for pretomanid-PZA and was comparable with that of the standard treatment regimen.¹¹³ A follow up phase II clinical study assessed the efficiency and safety of PaMZ in drug-susceptible and MDR-TB. For drug susceptible TB, two

regimens receiving 100 mg or 200 mg pretomanid were assessed, while for MDR-TB the combination of 400 mg moxifloxacin (M), 200 mg pretomanid (Pa) and 1500 mg PZA (Z) was assessed; these were tested against the current standard regimen. The 200 mg pretomanid regimen had significantly higher bactericidal activity than the standard regimen. MDR-TB patients showed early bactericidal activity (day 7–14), highly correlated with the day 7–56 bactericidal activity, which suggested that 14 day EBA provides similar information to 2 month bactericidal activity, regardless of known PZA-resistance. The combinations were well tolerated, with adverse effects reported in 31% or less across all groups.¹¹⁴ More recently, BDQ has been added to this regimen (BPamZ), and the clinical trial is still ongoing.

New drug targets for tuberculosis therapy

The increase in prevalence of TB drug-resistance raises challenges, not only to find new therapies but to identify novel drug targets that could be exploited to effectively kill *Mtb*. Development of compounds with novel mechanisms of action against non-replicating bacteria, MDR and XDR-TB could potentially lead to regimens that can shorten the duration of treatment and curb the alarming rate at which resistance is developing against current TB drugs. A few of the new promising compounds in the development and clinical pipeline act on previously unexploited targets (Table 4). To identify targets in drug discovery, two main approaches are typically used. The first, whole-cell screening, has been the traditional method used which relies on screening large compound libraries against the mycobacterium cell without any prior knowledge of their mechanism of action. Whole-cell screening has enjoyed much success since the discovery of STR, resulting in the recent discovery of newly approved BDQ. This method is known to favor promiscuous targets which are inhibited by more than one compound such as DprE1, MmpL3, QcrB, *etc.* The other method is a target-based approach where compounds are screened against a specific tar-

get essential for growth and survival of *Mtb*; however, limited success has come out of this method, owing to failure to deliver compounds with whole-cell antibacterial activity likely due to poor mycobacterial cell penetration amongst other factors. In addition, it is likely that some of the compounds identified in this manner may be found to inhibit another target not initially intended. Despite this, the target-based method has been useful in finding hits and efforts are continued using established targets such as RNA polymerase, malate synthase, Pks13, AtpE, DprE1, menaquinone synthase, inosine monophosphate dehydrogenase (IMPDH) protein GuaB2, InhA, ClpP1P2, LeuRS, *etc.* (<https://www.newtbdrugs.org/pipeline/discovery>). To overcome the limitations with each method, combination of the two methods to form target-based whole-cell screening offers the advantage of delivering cell-permeable compounds with target specificity. A number of new targets, in the mycobacterial membrane, energy metabolism, DNA metabolism amongst many other validated mycobacterial pathways, are currently being exploited in TB drug discovery efforts (Fig. 2).

New cell-wall targets

Mtb has a unique cell-wall which has historically been targeted for anti-TB drug activity. It is composed of a structurally complex peptidoglycan layer attached to arabinogalactan, which in turn connects to the outer mycolic acid layer, and is thought to be essential for viability and virulence of the bacterium. A number of unique enzymes are responsible for assembly and maintenance of this structure, making it attractive as a target for anti-TB drugs such as INH, EMB, ethionamide, cycloserine and thioacetazone. Some previously unknown targets have been uncovered through screening of new chemical scaffolds recently. Currently in the phase I and IIa clinical development are compound BTZ-043 and its improved derivative, PBTZ169, from the chemical class benzothiazinones known to covalently bind to the active site of decaprenylphosphoryl- β -D-ribose 2' epimerase (DprE1).¹¹⁵ Together with its partner enzyme DprE2, DprE1 catalyzes the formation of decaprenylphosphoryl- β -D-arabinose

Table 4 New compounds in the TB drug development pipeline for both drug susceptible and resistant TB^{1,96}

Drug	Chemical class	Mechanism of action	Development phase
Pretomanid	Nitroimidazole	Cell-wall inhibitor, inhibits mycolic acid synthesis	Phase III
Q203	Imidazopyridine amine	Inhibits the ETC, cytochrome bc ₁ complex	Phase I
BTZ-043, PBTZ169	Benzothiazinone	Cell-wall inhibitor, inhibits arabinogalactan biosynthesis	Phase I
SQ109	Ethylene diamine	Targets membrane transport	Phase II
Contezolid	Oxazolidinone	Inhibits protein synthesis	Phase I
Delpazolid	Oxazolidinone		Phase II
GSK-3036656	Oxaborole		Phase I
OPC-167832	Carbostyryl	Inhibitor of the enzyme DprE1, an essential enzyme for cell-wall biosynthesis	Phase I
Sutezolid	Oxazolidinone	Inhibits protein synthesis	Phase II
TBA-7371		Inhibitor of the enzyme DprE1	Phase I
TBI-166	Riminophenazine		Phase I
GSK-286		Inhibits cholesterol catabolism	Preclinical
Spectinamide	Aminoglycoside	Inhibits protein synthesis	Early stage

(DPA) which provides the arabinose sugars that are essential for the arabinogalactan component of the cell-wall in *Mtb* and other *Corynebacterineae* species. These clinical candidates are known to be active against *Mtb* MDR-clinical isolates¹¹⁵ and PBTZ169 has been shown to be safer and more efficacious in animal models, alone and in a combination regimen with BDQ and PZA.¹¹⁶ A phase IIa study of PBTZ169 in a monotherapy for drug-susceptible patients will investigate its safety, pharmacokinetic profiles, and EBA. Another cell-wall target that has generated interest is the previously untargeted membrane transporter MmpL3. This protein functions by transporting the mycolic acid synthesis precursor, trehalose monomycolate. A number of diverse chemical compounds have been reported to inhibit this protein including SQ109, a promising clinical candidate. However, EBA data alone or in combination regimens against drug-susceptible TB have only been moderately promising.^{117,118} Other targets involved in mycolic acid synthesis currently being investigated include FAS-I/II system enzymes, FadD32 and Pks13, identified through whole cell screening. In addition, more targets in the cell wall have resulted in some natural products (such as capuramycin and caprazamycin which target MurX – a translocase enzyme, and WecA – a transferase) as potential starting points for new chemical scaffolds.^{119–121} A capuramycin derivative, SQ641, has rapid bactericidal activity superior to that of most existing drugs including INH and RIF, and is active against MDR-TB clinical strains.¹¹⁹ However, poor PK properties hamper its excellent *in vitro* translation to *in vivo* efficacy. PptT, a phosphopantetheinyl transferase encoded by the *Rv2794c* gene, is a recently identified target involved in biosynthesis of lipids involved in cell-wall structure and virulence. A new compound, 8918, was found to bind to the Ppt binding pockets of PptT through van der Waals interactions and hydrogen bonding to amino acid residues, Tyr160, Phe173, Trp170 and Glu157, partially inhibiting this enzyme. Point mutations involving the binding pocket amino acid residue 170 (Trp170Ser and Trp170Leu) were observed. Most mutations however occurred in another gene adjacent to *pptT*, *Rv2795c*, which encodes Ppt hydrolase (PptH). PptH, co-regulated from the same operon as PptT, cleaves Ppt from the holo-ACP, which undoes PptT activity therefore potentiating the effects of the inhibitor.¹²²

Mycobacterial membrane and membrane energetics targets

Mtb bacilli exist in many physiological states within different microenvironments in the lung lesions during host infection. However, one constant crucial component of the bacillus structure is its membrane that is required for survival. *Mtb* bacillus homeostasis requires a functional and physically intact membrane for *Mtb* to maintain its critical processes.¹²³ Disruption of the membrane by potential inhibitors is likely to interfere with these processes such as nutrient uptake, efflux, the electron transport chain (ETC) for ATP synthesis and cell-to-cell signaling. From a resistance point of view, membrane damage or inhibition of crucial membrane proteins

would interfere with mechanisms of resistance for membrane inhibitors.¹²³ Some of the potential inhibitors include antimicrobial peptides, which destabilize the cell envelope causing loss of membrane integrity through pore formation and therefore leakage of the cytosol material and loss of transmembrane potential (inhibition of ATP generation, for example BDQ), xanthenes which are reported to disrupt the potassium ion gradient, and some compounds like verapamil which disrupt membrane energetics therefore potentiating activity of anti-TB drugs like BDQ and CFZ.¹²⁴ Whether intracellularly within macrophages, or extracellularly in the nutrient deficient hypoxic caseum environment, the bacilli always have to adjust energy utilization and production mechanisms to survive the environmental stress. *Mtb* may enter into a state of dormancy or non-replication to save its energy by maintaining metabolic processes at very low rates. Energy metabolism is driven by the ETC, which channels protons across the membrane creating a proton gradient to generate ATP. The discovery that ATP synthase is inhibited by BDQ has generated interest to investigate energy metabolism as a target, particularly for non-replicating bacteria as this phenotype requires ATP production to maintain low metabolic rates. QcrB, cytochrome *b* subunit, is another enzyme in the ETC that has been identified as a target with an inhibitor (Q203) currently in trials^{125–127} and other potential inhibitors under investigation.¹²⁸ Other targets in the ETC, such as NADH dehydrogenases, are currently being investigated. The membrane as a target has low potential for development of resistance, as inhibitors may interact with multiple targets within the membrane. However, one major concern, and probably the reason for the lack of its pursuit as a target, is the potential for its inhibitors to be non-selective to the host membrane therefore causing cytotoxicity.

Revisiting old targets with new or repurposed TB drugs

Spectinomycin (SPC), an antibiotic structurally similar to aminoglycosides, inhibits translocation of peptidyl-tRNA from the A-site through hydrogen bonding between its hydroxyl group and nucleotide bases of helix 34 of the 30S ribosomal subunit.^{64,129} In *Mtb*, limited activity of SPC likely due to the intrinsic resistance mediated by the Tap efflux pump encoded by *Rv1258c* has led to development of new semisynthetic analogues, spectinamides, which have good affinity for mycobacterial ribosomes and evade Tap efflux.^{130,131} This highlights that spectinamides have the potential for MDR and XDR therapy where aminoglycoside resistance is known. Spectinamides are currently in preclinical development after showing more potent activity. MDR-TB isolates are known to upregulate *Rv1258c*, and since spectinamides are not subject to *Rv1258c* efflux, they could be an attractive potent alternative for resistant strains.^{130,131} Another class of ribosomal inhibitors, also in preclinical studies, is novel tRNA synthetase inhibitors, benzoxaboroles (GSK3036656) which target leucyl-tRNA synthetase (LeuRS), an essential enzyme in protein synthesis.¹³² Benzoxaboroles have been shown to inhibit tRNA

synthetase through binding of the boron atom and oxygen atom on the terminal adenosine of tRNA, forming stable adducts and therefore blocking protein synthesis.¹³³ *Mtb* LeuRS inhibitors were designed using targets and cell-based structure–activity relationships and the promising compound GSK3036656 was developed which is in the preclinical development pipeline.¹³⁴ *Streptomyces*-derived griselimycin is being explored for MDR-TB. *DnaN*, a DNA polymerase sliding clamp, was identified as the target for griselimycin derivatives.¹³⁵ Kling *et al.*¹³⁵ also showed that resistance occurs at a very slow frequency, accompanied by severe fitness cost. This observation, in addition to the unique mode of action, not shared by any existing TB drugs, makes griselimycins attractive to contribute in building regimens against both drug-sensitive and resistant TB.

Biochemical pathways

Another strategy in identifying new targets has been through enzymes involved in metabolic pathways such as coenzyme A biosynthesis, fatty acid metabolism, and nucleotide biosynthesis amongst others. Target-based approaches for target identification have enjoyed less success in comparison to phenotypic screening due to the lack of translation of activity in a biochemical assay to bacillus growth inhibition in cell-based assays with regard to target vulnerability, drug penetration, *Mtb* metabolism and susceptibility to efflux mechanisms.¹³⁶ Metabolic pathways are robust in that *Mtb* can rewire its metabolic processes to bypass certain enzymes or an entire pathway under stress conditions, such as in the presence of an inhibitor for a key enzyme.¹²⁰ For example, a validated target *guaB2* involved in guanine biosynthesis was shown to be vulnerable *in vitro*; however it failed to translate the *in vivo* efficacy of the GuaB2 inhibitor likely due to *Mtb*'s ability to supplement guanine through assimilation from the surrounding tissue *in vivo*.^{137,138} However, recently, a benzoxazole based *Mtb* GuaB2 inhibitor was reported which retains activity in the presence of exogenous guanine,¹³⁹ suggesting that GuaB2 may indeed be a vulnerable target in *Mtb*.¹³⁷ Further, recently, a structure–activity relationship study of the original hit from Singh *et al.*¹³⁷ has yielded a novel molecule which retains the activity against the resistant mutant of the original hit in *Mtb*.¹⁴⁰ Condition specific activity of *Mtb* has been cited as a reason for failure of some compounds to translate *in vitro* activity to *in vivo* efficacy. However, some valuable knowledge may come out of such studies. For example, the failure of a pyrimidine inhibitor in *in vivo* efficacy studies due to the glycerol dependent activity of the compound *in vitro* led to the recognition that the carbon source may be crucial for the inhibitor activity.¹⁴¹ Therefore, this has led to pursuit of the glyoxylate cycle to identify targets involved in fatty-acid metabolism.¹²⁰ More novel targets are still needed to complement development of newer drugs especially for resistant TB and thorough validation of each target both *in vitro* and *in vivo* is needed to assess any development of resistance.

Policy on MDR/XDR-TB treatment and management

Given the slow progress in the drug development pipeline over the years, efforts to meet the End-TB goals will rely on concerted involvement from all global health stakeholders. Effective policies at the global and country level on TB management play a significant role in access to quality services that could reduce transmission of MDR and XDR-TB and improve treatment outcomes. The WHO provides guidelines, based on comprehensive data collected globally, which serve as a blueprint from which each country should build their policies on. Increased national surveillance and mandatory notification of resistant TB cases by care providers and the private health sector will improve the underreporting reported for some countries, therefore reducing the gap between estimated and notified cases. In addition, it is thought that a shift from paper-based to electronic surveillance will improve data capture and reporting. Since the roll-out of the WHO recommended Xpert MTB/RIF, there has been an increase in notified cases of MDR-TB in some of the 30 high-burden countries (HBCs).¹ A systematic review and meta-analysis study showed that MDR-TB prevalence in Nigeria is actually higher than reported by the WHO. A shift in government policies regarding the roll-out of molecular DST is thought to likely determine the extent of prevalence in the country.¹⁴² A recent survey in South Africa indicates that DR-TB cases doubled between the period of 2001/2 to 2012/13 and in addition, reports an increased prevalence of INH mono-resistance which is excluded in routine screening tests in the country.¹⁴³ A national prevalence survey conducted in 2017–2018 will provide some insight into how the country's policies on TB reflect on current estimated and notified resistant TB cases and how the country will adjust their policies to treat the notified cases to reduce further spread of resistant strains.¹⁴⁴

Even though the WHO guidelines recommend treatment regimens based on diagnosis outcome, each country's ability to roll-out the recommended drugs is highly dependent on the economic status of that particular country. With the approval of BDQ and delamanid, some countries such as the Russian Federation and South Africa have reported having the highest percentage of patients (79%) on these drugs. Most countries have adopted the shorter MDR-TB regimens with high treatment success rates,¹ suggesting that if more countries adopted the same strategy, MDR-TB prevalence in HBCs could go down even further. Treatment monitoring through more patient-centered models of care is expected to help in improving the outcomes and reduce the risk of transmission. The WHO recommends the use of sputum smear microscopy and culture to monitor MDR-TB treatment progress in a patient-centered directly observed therapy (DOT), which significantly improves rates of treatment success.¹⁴⁵ It is well understood that the adoption of the End-TB strategy goals into government policies will help accelerate the global expansion of tuberculosis resistance control. Perhaps, the

biggest development in 2018 was the special United Nations (UN) high-level meeting held in New York with the agenda to rally a global commitment to accelerating comprehensive response to achieve universal health care through access to treatment, diagnostics, prevention and care to fight to end the TB epidemic. UN member states endorsed a political declaration on TB to achieve specific milestones by 2022, as part of the Sustainable Development Goals' target of ending the TB epidemic by 2030.

Conclusion and outlook

Despite the inherent weaknesses of *Mtb* – a low mutation rate complemented with narrow genetic diversity, lack of horizontal-gene transfer capability, and no benefit from any reservoir other than the host – the development of resistance in *Mtb* against TB-drugs is a horrible reality. Although we gained a lot since the first whole-genome sequence of *Mtb* to the new and economical whole-genome sequencing technologies in the present era, there is a lot to learn to control this menace. Incorporation of new drugs, targeting novel targets, into the current drug regimens is highly needed. Also, patient specific regimens should be encouraged which will require a consideration of cross-resistance, synergies or antagonism for a range of possible combinations. Finally, as we know that mainly mutations are linked to drug-resistance in *Mtb*, finding genetic markers to support the early diagnosis of drug-resistance would be highly beneficial in optimizing the treatment regimen which will also put a check on transmission.

Conflicts of interest

There are no conflicts to declare.

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References

- WHO, *Global Tuberculosis Report World Health Organization*, Geneva, 2018.
- W. Fox, G. A. Ellard and D. A. Mitchison, *Int. J. Tuberc. Lung Dis.*, 1999, 3, S231–S279.
- C. R. Diedrich and J. L. Flynn, *Infect. Immun.*, 2011, 79, 1407–1417.
- WHO, *TB: A global health emergency*, World Health Organization, Geneva, 1994.
- R. H. Al-Rifai, F. Pearson, J. A. Critchley and L. J. Abu-Raddad, *PLoS One*, 2017, 12, e0187967.
- S. Portelli, J. E. Phelan, D. B. Ascher, T. G. Clark and N. Furnham, *Sci. Rep.*, 2018, 8, 15356.
- T. Schon, P. Miotto, C. U. Koser, M. Viveiros, E. Bottger and E. Cambau, *Clin. Microbiol. Infect.*, 2017, 23, 154–160.
- A. Koch, H. Cox and V. Mizrahi, *Curr. Opin. Pharmacol.*, 2018, 42, 7–15.
- H. Cox, J. Hughes, J. Black and M. P. Nicol, *Lancet Infect. Dis.*, 2018, 18, e282–e287.
- WHO, *The End TB Strategy: Global strategy and targets for tuberculosis prevention, care and control after*, 2015, World Health Organization, Geneva, 2014.
- J. Heyckendorf, S. Andres, C. U. Köser, I. D. Olaru, T. Schön, E. Sturegård, P. Beckert, V. Schleusener, T. A. Kohl, D. Hillemann, D. Moradigaravand, J. Parkhill, S. J. Peacock, S. Niemann, C. Lange and M. Merkerb, *Antimicrob. Agents Chemother.*, 2018, 62, e01550.
- E. Sanchez-Padilla, M. Merker, P. Beckert, F. Jochims, T. Dlamini, P. Kahn, M. Bonnet and S. Niemann, *N. Engl. J. Med.*, 2015, 372, 1181–1182.
- E. Andre, L. Goeminne, A. Colmant, P. Beckert, S. Niemann and M. Delmee, *Clin. Microbiol. Infect.*, 2017, 23, e265–e267.
- O. Opota, F. Zakhm, J. Mazza-Stalder, L. Nicod, G. Greub and K. Jaton, *J. Clin. Microbiol.*, 2019, 57, e01717.
- C. R. Consortium, G. P. The, C. Allix-Beguec, I. Arandjelovic, L. Bi, P. Beckert, M. Bonnet, P. Bradley, A. M. Cabibbe, I. Cancino-Munoz, M. J. Caulfield, A. Chaiprasert, D. M. Cirillo, D. A. Clifton, I. Comas, D. W. Crook, M. R. De Filippo, H. de Neeling, R. Diel, F. A. Drobniowski, K. Faksri, M. R. Farhat, J. Fleming, P. Fowler, T. A. Fowler, Q. Gao, J. Gardy, D. Gascoyne-Binzi, A. L. Gibertoni-Cruz, A. Gil-Brusola, T. Golubchik, X. Gonzalo, L. Grandjean, G. He, J. L. Guthrie, S. Hoosdally, M. Hunt, Z. Iqbal, N. Ismail, J. Johnston, F. M. Khanzada, C. C. Khor, T. A. Kohl, C. Kong, S. Lipworth, Q. Liu, G. Maphalala, E. Martinez, V. Mathys, M. Merker, P. Miotto, N. Mistry, D. A. J. Moore, M. Murray, S. Niemann, S. V. Omar, R. T. Ong, T. E. A. Peto, J. E. Posey, T. Prammananan, A. Pym, C. Rodrigues, M. Rodrigues, T. Rodwell, G. M. Rossolini, E. Sanchez Padilla, M. Schito, X. Shen, J. Shendure, V. Sintchenko, A. Sloutsky, E. G. Smith, M. Snyder, K. Soetaert, A. M. Starks, P. Supply, P. Suriyapol, S. Tahseen, P. Tang, Y. Y. Teo, T. N. T. Thuong, G. Thwaites, E. Tortoli, D. van Soolingen, A. S. Walker, T. M. Walker, M. Wilcox, D. J. Wilson, D. Wyllie, Y. Yang, H. Zhang, Y. Zhao and B. Zhu, *N. Engl. J. Med.*, 2018, 379, 1403–1415.
- J. C. Alffenaar, O. W. Akkerman, R. M. Anthony, S. Tiberi, S. Heysell, M. P. Grobusch, F. G. Cobelens and D. Van Soolingen, *Expert Rev. Anti-infect. Ther.*, 2017, 15, 11–21.
- N. S. Shah, S. C. Auld, J. C. Brust, B. Mathema, N. Ismail, P. Moodley, K. Mlisana, S. Allana, A. Campbell, T. Mthiyane, N. Morris, P. Mpangase, H. van der Meulen, S. V. Omar, T. S. Brown, A. Narechania, E. Shaskina, T. Kapwata, B. Kreiswirth and N. R. Gandhi, *N. Engl. J. Med.*, 2017, 376, 243–253.
- H. Lin, S. Shin, J. A. Blaya, Z. Zhang, P. Cegielski, C. Contreras, L. Asencios, C. Bonilla, J. Bayona, C. J. Paciorek and T. Cohen, *Epidemiol. Infect.*, 2011, 139, 1784–1793.
- WHO, *Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017 update*, Geneva, 2017.

- 20 WHO, *WHO consolidated guidelines on drug-resistant tuberculosis treatment*, Geneva, 2019.
- 21 D. J. Jin and C. A. Gross, *J. Mol. Biol.*, 1988, **202**, 45–58.
- 22 A. Telenti, P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer and T. Bodmer, *Lancet*, 1993, **341**, 647–650.
- 23 H. Taniguchi, H. Aramaki, Y. Nikaido, Y. Mizuguchi, M. Nakamura, T. Koga and S. Yoshida, *FEMS Microbiol. Lett.*, 1996, **144**, 103–108.
- 24 E. Cambau, M. Viveiros, D. Machado, L. Raskine, C. Ritter, E. Tortoli, V. Matthys, S. Hoffner, E. Richter, M. L. Perez Del Molino, D. M. Cirillo, D. van Soolingen and E. C. Bottger, *J. Antimicrob. Chemother.*, 2015, **70**, 686–696.
- 25 G. Brandis and D. Hughes, *Sci. Rep.*, 2018, **8**, 17488.
- 26 J. S. Makadia, A. Jain, S. K. Patra, B. L. Sherwal and A. Khanna, *Indian J. Clin. Biochem.*, 2012, **27**, 370–374.
- 27 S. Quan, H. Venter and E. R. Dabbs, *Antimicrob. Agents Chemother.*, 1997, **41**, 2456–2460.
- 28 K. Koteva, G. Cox, J. K. Kelso, M. D. Surette, H. L. Zubyk, L. Ejim, P. Stogios, A. Savchenko, D. Sorensen and G. D. Wright, *Cell Chem. Biol.*, 2018, **25**, 403–405.
- 29 F. B. Jamieson, J. L. Guthrie, A. Neemuchwala, O. Lastovetska, R. G. Melano and C. Mehaffya, *J. Clin. Microbiol.*, 2014, **52**, 2157–2162.
- 30 F. A. Sirgel, R. M. Warren, E. C. Bottger, M. Klopfer, T. C. Victor and P. D. van Helden, *PLoS One*, 2013, **8**, e59414.
- 31 M. R. Council, *Thorax*, 1954, **9**, 254–259.
- 32 Y. Zhang, B. Heym, B. Allen, D. Young and S. Cole, *Nature*, 1992, **358**, 591–593.
- 33 B. Lei, C. J. Wei and S. C. Tu, *J. Biol. Chem.*, 2000, **275**, 2520–2526.
- 34 C. Vilcheze, H. R. Morbidoni, T. R. Weisbrod, H. Iwamoto, M. Kuo, J. C. Sacchettini and W. R. Jacobs, Jr., *J. Bacteriol.*, 2000, **182**, 4059–4067.
- 35 M. H. Larsen, C. Vilcheze, L. Kremer, G. S. Besra, L. Parsons, M. Salfinger, L. Heifets, M. H. Hazbon, D. Alland, J. C. Sacchettini and W. R. Jacobs, Jr., *Mol. Microbiol.*, 2002, **46**, 453–466.
- 36 G. P. Morlock, B. Metchock, D. Sikes, J. T. Crawford and R. C. Cooksey, *Antimicrob. Agents Chemother.*, 2003, **47**, 3799–3805.
- 37 P. Lempens, C. J. Meehan, K. Vandellanoot, K. Fissette, P. de Rijk, A. Van Deun, L. Rigouts and B. C. de Jong, *Sci. Rep.*, 2018, **8**, 3246.
- 38 A. Scorpio and Y. Zhang, *Nat. Med.*, 1996, **2**, 662–667.
- 39 K. Hirano, M. Takahashi, Y. Kazumi, Y. Fukasawa and C. Abe, *Tuber. Lung Dis.*, 1997, **78**, 117–122.
- 40 A. Scorpio, P. Lindholm-Levy, L. Heifets, R. Gilman, S. Siddiqi, M. Cynamon and Y. Zhang, *Antimicrob. Agents Chemother.*, 1997, **41**, 540–543.
- 41 A. N. Yadon, K. Maharaj, J. H. Adamson, Y. P. Lai, J. C. Sacchettini, T. R. Ioerger, E. J. Rubin and A. S. Pym, *Nat. Commun.*, 2017, **8**, 588.
- 42 A. Scorpio, P. Lindholm-Levy, L. Heifets, R. Gilman, S. Siddiqi, M. Cynamon and Y. Zhang, *Antimicrob. Agents Chemother.*, 1997, **41**, 540–543.
- 43 S. Petrella, N. Gelus-Ziental, A. Maudry, C. Laurans, R. Boudjelloul and W. Sougakoff, *PLoS One*, 2011, **6**, e15785.
- 44 N. Lemaitre, I. Callebaut, F. Frenois, V. Jarlier and W. Sougakoff, *Biochem. J.*, 2001, **353**, 453–458.
- 45 P. Gopal, W. Nartey, P. Ragunathan, J. Sarathy, F. Kaya, M. Yee, C. Setzer, M. S. S. Manimekalai, V. Dartois, G. Gruber and T. Dick, *ACS Infect. Dis.*, 2017, **3**, 807–819.
- 46 S. Zhang, J. Chen, W. Shi, W. Liu, W. Zhang and Y. Zhang, *Emerging Microbes Infect.*, 2013, **2**, e34.
- 47 M. Yee, P. Gopal and T. Dick, *Antimicrob. Agents Chemother.*, 2017, **61**, e02342.
- 48 S. Zhang, J. Chen, W. Shi, P. Cui, J. Zhang, S. Cho, W. Zhang and Y. Zhang, *Emerging Microbes Infect.*, 2017, **6**, e8.
- 49 W. Shi, X. Zhang, X. Jiang, H. Yuan, J. S. Lee, C. E. Barry, 3rd, H. Wang, W. Zhang and Y. Zhang, *Science*, 2011, **333**, 1630–1632.
- 50 W. Shi, P. Cui, H. Niu, S. Zhang, T. Tonjum, B. Zhu and Y. Zhang, *Antimicrob. Agents Chemother.*, 2019, **63**, e02681.
- 51 N. A. Dillon, N. D. Peterson, H. A. Feaga, K. C. Keiler and A. D. Baughn, *Sci. Rep.*, 2017, **7**, 6135.
- 52 D. C. Alexander, J. H. Ma, J. L. Guthrie, J. Blair, P. Chedore and F. B. Jamieson, *J. Clin. Microbiol.*, 2012, **50**, 3726–3728.
- 53 A. Telenti, W. J. Philipp, S. Sreevatsan, C. Bernasconi, K. E. Stockbauer, B. Wiele, J. M. Musser and W. R. Jacobs, Jr., *Nat. Med.*, 1997, **3**, 567–570.
- 54 F. Brossier, C. Sola, C. Bernard, V. Jarlier, N. Veziris and W. Sougakoff, *J. Clin. Microbiol.*, 2015, **53**, 3104.
- 55 H. Safi, B. Sayers, M. H. Hazbon and D. Alland, *Antimicrob. Agents Chemother.*, 2008, **52**, 2027–2034.
- 56 H. Safi, S. Lingaraju, A. Amin, S. Kim, M. Jones, M. Holmes, M. McNeil, S. N. Peterson, D. Chatterjee, R. Fleischmann and D. Alland, *Nat. Genet.*, 2013, **45**, 1190–1197.
- 57 WHO, *WHO treatment guidelines for drug-resistant tuberculosis: 2016 update*, World Health Organization, Geneva, 2016.
- 58 S. Tiberi, A. Scardigli, R. Centis, L. D'Ambrosio, M. Munoz-Torrico, M. A. Salazar-Lezama, A. Spanevello, D. Visca, A. Zumla, G. B. Migliori and J. A. Caminero Luna, *Int. J. Infect. Dis.*, 2017, **56**, 181–184.
- 59 H. E. Takiff, L. Salazar, C. Guerrero, W. Philipp, W. M. Huang, B. Kreiswirth, S. T. Cole, W. R. Jacobs, Jr. and A. Telenti, *Antimicrob. Agents Chemother.*, 1994, **38**, 773–780.
- 60 F. Maruri, T. R. Sterling, A. W. Kaiga, A. Blackman, Y. F. van der Heijden, C. Mayer, E. Cambau and A. Aubry, *J. Antimicrob. Chemother.*, 2012, **67**, 819–831.
- 61 J. Li, X. Gao, T. Luo, J. Wu, G. Sun, Q. Liu, Y. Jiang, Y. Zhang, J. Mei and Q. Gao, *Emerging Microbes Infect.*, 2014, **3**, e19.
- 62 M. Finken, P. Kirschner, A. Meier, A. Wrede and E. C. Bottger, *Mol. Microbiol.*, 1993, **9**, 1239–1246.
- 63 S. Magnet and J. S. Blanchard, *Chem. Rev.*, 2005, **105**, 477–498.
- 64 A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly and V. Ramakrishnan, *Nature*, 2000, **407**, 340–348.

- 65 C. E. Maus, B. B. Plikaytis and T. M. Shinnick, *Antimicrob. Agents Chemother.*, 2005, **49**, 3192–3197.
- 66 G. J. Alangaden, B. N. Kreiswirth, A. Aouad, M. Khetarpal, F. R. Igno, S. L. Moghazeh, E. K. Manavathu and S. A. Lerner, *Antimicrob. Agents Chemother.*, 1998, **42**, 1295–1297.
- 67 F. Brossier, A. Pham, C. Bernard, A. Aubry, V. Jarlier, N. Veziris, W. Sougakoff and C. N. R. MyRMA, *Antimicrob. Agents Chemother.*, 2017, **61**, e01299.
- 68 S. Okamoto, A. Tamaru, C. Nakajima, K. Nishimura, Y. Tanaka, S. Tokuyama, Y. Suzuki and K. Ochi, *Mol. Microbiol.*, 2007, **63**, 1096–1106.
- 69 M. A. Zaunbrecher, R. D. Sikes, Jr., B. Metchock, T. M. Shinnick and J. E. Posey, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 20004–20009.
- 70 A. Z. Reeves, P. J. Campbell, R. Sultana, S. Malik, M. Murray, B. B. Plikaytis, T. M. Shinnick and J. E. Posey, *Antimicrob. Agents Chemother.*, 2013, **57**, 1857–1865.
- 71 J. M. Chen, S. Uplekar, S. V. Gordon and S. T. Cole, *PLoS One*, 2012, **7**, e43467.
- 72 C. A. Desjardins, K. A. Cohen, V. Munsamy, T. Abeel, K. Maharaj, B. J. Walker, T. P. Shea, D. V. Almeida, A. L. Manson and A. Salazar, *Nat. Genet.*, 2016, **48**, 544.
- 73 S. Zhang, J. Chen, P. Cui, W. Shi, W. Zhang and Y. Zhang, *J. Antimicrob. Chemother.*, 2015, **70**, 2507–2510.
- 74 K. L. Leach, S. M. Swaney, J. R. Colca, W. G. McDonald, J. R. Blinn, L. M. Thomasco, R. C. Gadwood, D. Shinabarger, L. Xiong and A. S. Mankin, *Mol. Cell*, 2007, **26**, 393–402.
- 75 K. Andries, P. Verhasselt, J. Guillemont, H. W. Gohlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis and V. Jarlier, *Science*, 2005, **307**, 223–227.
- 76 A. Koul, N. Dendouga, K. Vergauwen, B. Molenberghs, L. Vranckx, R. Willebrords, Z. Ristic, H. Lill, I. Dorange, J. Guillemont, D. Bald and K. Andries, *Nat. Chem. Biol.*, 2007, **3**, 323–324.
- 77 U. Manjunatha, H. I. Boshoff and C. E. Barry, *Commun. Integr. Biol.*, 2009, **2**, 215–218.
- 78 U. H. Manjunatha, H. Boshoff, C. S. Dowd, L. Zhang, T. J. Albert, J. E. Norton, L. Daniels, T. Dick, S. S. Pang and C. E. Barry, 3rd, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 431–436.
- 79 F. Zhao, X. D. Wang, L. N. Erber, M. Luo, A. Z. Guo, S. S. Yang, J. Gu, B. J. Turman, Y. R. Gao, D. F. Li, Z. Q. Cui, Z. P. Zhang, L. J. Bi, A. D. Baughn, X. E. Zhang and J. Y. Deng, *Antimicrob. Agents Chemother.*, 2014, **58**, 1479–1487.
- 80 A. E. Grzegorzewicz, J. Kordulakova, V. Jones, S. E. Born, J. M. Belardinelli, A. Vaquie, V. A. Gundi, J. Madacki, N. Slama, F. Laval, J. Vaubourgeix, R. M. Crew, B. Gicquel, M. Daffe, H. R. Morbidoni, P. J. Brennan, A. Quemard, M. R. McNeil and M. Jackson, *J. Biol. Chem.*, 2012, **287**, 38434–38441.
- 81 J. E. Hugonnet and J. S. Blanchard, *Biochemistry*, 2007, **46**, 11998–12004.
- 82 A. R. Flores, L. M. Parsons and M. S. Pavelka, Jr., *Microbiology*, 2005, **151**, 521–532.
- 83 F. Brossier, W. Sougakoff, C. Bernard, M. Petrou, K. Adeyema, A. Pham, D. Amy de la Breteque, M. Vallet, V. Jarlier, C. Sola and N. Veziris, *Antimicrob. Agents Chemother.*, 2015, **59**, 4800–4808.
- 84 A. Von Groll, A. Martin, P. Jureen, S. Hoffner, P. Vandamme, F. Portaels, J. C. Palomino and P. A. da Silva, *Antimicrob. Agents Chemother.*, 2009, **53**, 4498–4500.
- 85 X. Yin and Z. Yu, *J. Infect.*, 2010, **61**, 150–154.
- 86 M. R. de Jonge, L. H. Koymans, J. E. Guillemont, A. Koul and K. Andries, *Proteins*, 2007, **67**, 971–980.
- 87 A. Koul, L. Vranckx, N. Dendouga, W. Balemans, I. Van den Wyngaert, K. Vergauwen, H. W. Gohlmann, R. Willebrords, A. Poncelet, J. Guillemont, D. Bald and K. Andries, *J. Biol. Chem.*, 2008, **283**, 25273–25280.
- 88 R. Tasneen, S. Y. Li, C. A. Peloquin, D. Taylor, K. N. Williams, K. Andries, K. E. Mdluli and E. L. Nuermberger, *Antimicrob. Agents Chemother.*, 2011, **55**, 5485–5492.
- 89 E. Segala, W. Sougakoff, A. Nevejans-Chauffour, V. Jarlier and S. Petrella, *Antimicrob. Agents Chemother.*, 2012, **56**, 2326–2334.
- 90 R. C. Hartkoorn, S. Uplekar and S. T. Cole, *Antimicrob. Agents Chemother.*, 2014, **58**, 2979–2981.
- 91 J. Xu, B. Wang, M. Hu, F. Huo, S. Guo, W. Jing, E. Nuermberger and Y. Lu, *Antimicrob. Agents Chemother.*, 2017, **61**, 150–154.
- 92 Y. Zhang and W. W. Yew, *Int. J. Tuberc. Lung Dis.*, 2015, **19**, 1276–1289.
- 93 M. Matsumoto, H. Hashizume, T. Tomishige, M. Kawasaki, H. Tsubouchi, H. Sasaki, Y. Shimokawa and M. Komatsu, *PLoS Med.*, 2006, **3**, e466.
- 94 M. T. Gler, V. Skripconoka, E. Sanchez-Garavito, H. Xiao, J. L. Cabrera-Rivero, D. E. Vargas-Vasquez, M. Gao, M. Awad, S. Park, T. S. Shim, G. Y. Suh, M. Danilovits, H. Ogata, A. Kurve, J. Chang, K. Suzuki, T. Tupasi, W. Koh, B. Seaworth, L. J. Geiter and C. D. Wells, *N. Engl. J. Med.*, 2012, **366**, 2151–2160.
- 95 R. Gupta, L. J. Geiter, C. D. Wells, M. Gao, A. Cirule and H. Xiao, *N. Engl. J. Med.*, 2015, **373**, 291–292.
- 96 W. G. O. N. T. Drugs, *Clinical Pipeline*, <https://www.newtbdrugs.org/pipeline/clinical>, (accessed January 17, 2019).
- 97 D. Rifat, S.-Y. Li, T. Iorger, J.-P. Lanoix, J. Lee, G. Bashiri, J. Sacchettini and E. Nuermberger, 2018, bioRxiv 457754, DOI: 10.1101/457754.
- 98 H. L. Haver, A. Chua, P. Ghode, S. B. Lakshminarayana, A. Singhal, B. Mathema, R. Wintjens and P. Bifani, *Antimicrob. Agents Chemother.*, 2015, **59**, 5316–5323.
- 99 G. V. Bloemberg, P. M. Keller, D. Stucki, A. Trauner, S. Borrell, T. Latshang, M. Coscolla, T. Rothe, R. Homke, C. Ritter, J. Feldmann, B. Schulthess, S. Gagneux and E. C. Bottger, *N. Engl. J. Med.*, 2015, **373**, 1986–1988.
- 100 M. Lee, J. Lee, M. W. Carroll, H. Choi, S. Min, T. Song, L. E. Via, L. C. Goldfeder, E. Kang, B. Jin, H. Park, H. Kwak, H. Kim, H. S. Jeon, I. Jeong, J. S. Joh, R. Y. Chen, K. N. Olivier, P. A. Shaw, D. Follmann, S. D. Song, J. K. Lee, D. Lee, C. T. Kim, V. Dartois, S. K. Park, S. N. Cho and C. E. Barry, 3rd, *N. Engl. J. Med.*, 2012, **367**, 1508–1518.

- 101 J. W. Alffenaar, T. van der Laan, S. Simons, T. S. van der Werf, P. J. van de Kastele, H. de Neeling and D. van Soolingen, *Antimicrob. Agents Chemother.*, 2011, **55**, 1287–1289.
- 102 E. Richter, S. Rusch-Gerdes and D. Hillemann, *Antimicrob. Agents Chemother.*, 2007, **51**, 1534–1536.
- 103 P. Beckert, D. Hillemann, T. A. Kohl, J. Kalinowski, E. Richter, S. Niemann and S. Feuerriegel, *Antimicrob. Agents Chemother.*, 2012, **56**, 2743–2745.
- 104 D. Hillemann, S. Rusch-Gerdes and E. Richter, *Antimicrob. Agents Chemother.*, 2008, **52**, 800–801.
- 105 L. Zhang, Y. Pang, X. Yu, Y. Wang, M. Gao, H. Huang and Y. Zhao, *Infection*, 2014, **42**, 705–711.
- 106 K. Tahlan, R. Wilson, D. B. Kastrinsky, K. Arora, V. Nair, E. Fischer, S. W. Barnes, J. R. Walker, D. Alland, C. E. Barry, 3rd and H. I. Boshoff, *Antimicrob. Agents Chemother.*, 2012, **56**, 1797–1809.
- 107 W. Li, A. Upadhyay, F. L. Fontes, E. J. North, Y. Wang, D. C. Crans, A. E. Grzegorzewicz, V. Jones, S. G. Franzblau, R. E. Lee, D. C. Crick and M. Jackson, *Antimicrob. Agents Chemother.*, 2014, **58**, 6413–6423.
- 108 J. E. Hugonnet, L. W. Tremblay, H. I. Boshoff, C. E. Barry, 3rd and J. S. Blanchard, *Science*, 2009, **323**, 1215–1218.
- 109 M. C. Payen, I. Muylle, O. Vandenberg, V. Mathys, M. Delforge, S. Van den Wijngaert, N. Clumeck and S. De Wit, *Int. J. Tuberc. Lung Dis.*, 2018, **22**, 34–39.
- 110 N. Dauby, I. Muylle, F. Mouchet, R. Sergysels and M. C. Payen, *Pediatr. Infect. Dis. J.*, 2011, **30**, 812–813.
- 111 A. H. Diacon, A. Pym, M. Grobusch, R. Patientia, R. Rustomjee, L. Page-Shipp, C. Pistorius, R. Krause, M. Bogoshi, G. Churchyard, A. Venter, J. Allen, J. C. Palomino, T. De Marez, R. P. van Heeswijk, N. Lounis, P. Meyvisch, J. Verbeeck, W. Parys, K. de Beule, K. Andries and D. F. Mc Neeley, *N. Engl. J. Med.*, 2009, **360**, 2397–2405.
- 112 C. o. R. a. O. Infections, The NIX-TB Trial Of Pretomanid, Bedaquiline And Linezolid To Treat XDR-TB, <http://www.croiconference.org/sessions/nix-tb-trial-pretomanid-bedaquiline-and-linezolid-treat-xdr-tb>, (accessed January 28, 2019).
- 113 A. H. Diacon, R. Dawson, F. von Groote-Bidlingmaier, G. Symons, A. Venter, P. R. Donald, C. van Niekerk, D. Everitt, H. Winter, P. Becker, C. M. Mendel and M. K. Spigelman, *Lancet*, 2012, **380**, 986–993.
- 114 R. Dawson, A. H. Diacon, D. Everitt, C. Niekerk, P. R. Donald and D. A. Burger, *Lancet*, 2015, **385**, 1738–1747.
- 115 V. Makarov, G. Manina, K. Mikusova, U. Mollmann, O. Ryabova, B. Saint-Joanis, N. Dhar, M. R. Pasca, S. Buroni, A. P. Lucarelli, A. Milano, E. De Rossi, M. Belanova, A. Bobovska, P. Dianiskova, J. Kordulakova, C. Sala, E. Fullam, P. Schneider, J. D. McKinney, P. Brodin, T. Christophe, S. Waddell, P. Butcher, J. Albrethsen, I. Rosenkrands, R. Brosch, V. Nandi, S. Bharath, S. Gaonkar, R. K. Shandil, V. Balasubramanian, T. Balganes, S. Tyagi, J. Grosset, G. Riccardi and S. T. Cole, *Science*, 2009, **324**, 801–804.
- 116 V. Makarov, B. Lechartier, M. Zhang, J. Neres, A. M. van der Sar, S. A. Raadsen, R. C. Hartkoorn, O. B. Ryabova, A. Vocat, L. A. Decosterd, N. Widmer, T. Buclin, W. Bitter, K. Andries, F. Pojer, P. J. Dyson and S. T. Cole, *EMBO Mol. Med.*, 2014, **6**, 372–383.
- 117 M. J. Boeree, N. Heinrich, R. Aarnoutse, A. H. Diacon, R. Dawson, S. Rehal, G. S. Kibiki, G. Churchyard, I. Sanne, N. E. Ntinginya, L. T. Minja, R. D. Hunt, S. Charalambous, M. Hanekom, H. H. Semvua, S. G. Mpagama, C. Manyama, B. Mtafya, K. Reither, R. S. Wallis, A. Venter, K. Narunsky, A. Mekota, S. Henne, A. Colbers, G. P. van Balen, S. H. Gillespie, P. P. J. Phillips and M. Hoelscher, *Lancet Infect. Dis.*, 2017, **17**, 39–49.
- 118 N. Heinrich, R. Dawson, J. du Bois, K. Narunsky, G. Horwith, A. J. Phipps, C. A. Nacy, R. E. Aarnoutse, M. J. Boeree, S. H. Gillespie, A. Venter, S. Henne, A. Rachow, P. P. Phillips, M. Hoelscher and A. H. Diacon, A. Pan African Consortium for the Evaluation of Antituberculosis and A. Pan African Consortium for the Evaluation of Antituberculosis Antibiotics Pan, *J. Antimicrob. Chemother.*, 2015, **70**, 1558–1566.
- 119 B. Nikonenko, V. M. Reddy, E. Bogatcheva, M. Protopopova, L. Einck and C. A. Nacy, *Antimicrob. Agents Chemother.*, 2014, **58**, 587–589.
- 120 S. Wellington and D. T. Hung, *ACS Infect. Dis.*, 2018, **4**, 696–714.
- 121 S. Huszar, V. Singh, A. Polcicova, P. Barath, M. B. Barrio, S. Lagrange, V. Leblanc, C. A. Nacy, V. Mizrahi and K. Mikusova, *Antimicrob. Agents Chemother.*, 2017, **61**, e01310.
- 122 E. Ballinger, J. Mosior, T. Hartman, K. Burns-Huang, B. Gold, R. Morris, L. Goullieux, I. Blanc, J. Vaubourgeix, S. Lagrange, L. Fraisse, S. Sans, C. Couturier, E. Bacque, K. Rhee, S. M. Scarry, J. Aube, G. Yang, O. Ouerfelli, D. Schnappinger, T. R. Ioerger, C. A. Engelhart, J. A. McConnell, K. McAulay, A. Fay, C. Roubert, J. Sacchettini and C. Nathan, *Science*, 2019, **363**, eaau8959.
- 123 H. Chen, S. A. Nyantakyi, M. Li, P. Gopal, D. B. Aziz, T. Yang, W. Moreira, M. Gengenbacher, T. Dick and M. L. Go, *Front. Microbiol.*, 2018, **9**, 1627.
- 124 C. Chen, S. Gardete, R. S. Jansen, A. Shetty, T. Dick, K. Y. Rhee and V. Dartois, *Antimicrob. Agents Chemother.*, 2018, **62**, e02107.
- 125 K. Pethe, P. Bifani, J. Jang, S. Kang, S. Park, S. Ahn, J. Jiricek, J. Jung, H. K. Jeon, J. Cechetto, T. Christophe, H. Lee, M. Kempf, M. Jackson, A. J. Lenaerts, H. Pham, V. Jones, M. J. Seo, Y. M. Kim, M. Seo, J. J. Seo, D. Park, Y. Ko, I. Choi, R. Kim, S. Y. Kim, S. Lim, S. A. Yim, J. Nam, H. Kang, H. Kwon, C. T. Oh, Y. Cho, Y. Jang, J. Kim, A. Chua, B. H. Tan, M. B. Nanjundappa, S. P. Rao, W. S. Barnes, R. Wintjens, J. R. Walker, S. Alonso, S. Lee, J. Kim, S. Oh, T. Oh, U. Nehrbass, S. J. Han, Z. No, J. Lee, P. Brodin, S. N. Cho, K. Nam and J. Kim, *Nat. Med.*, 2013, **19**, 1157–1160.
- 126 B. S. Lee, N. P. Kalia, X. E. F. Jin, E. J. Hasenoehrl, M. Berney and K. Pethe, *J. Biol. Chem.*, 2019, **294**, 1936–1943.
- 127 N. P. Kalia, E. J. Hasenoehrl, N. B. Ab Rahman, V. H. Koh, M. L. T. Ang, D. R. Sajorda, K. Hards, G. Gruber, S. Alonso, G. M. Cook, M. Berney and K. Pethe, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 7426–7431.

- 128 C. S. Foo, A. Lupien, M. Kienle, A. Vocat, A. Benjak, R. Sommer, D. A. Lamprecht, A. J. C. Steyn, K. Pethe, J. Piton, K. H. Altmann and S. T. Cole, *mBio*, 2018, 9.
- 129 M. A. Borovinskaya, S. Shoji, J. M. Holton, K. Fredrick and J. H. D. Cate, *ACS Chem. Biol.*, 2007, 2, 545–552.
- 130 R. E. Lee, J. G. Hurdle, J. Liu, D. F. Bruhn, T. Matt, M. S. Scherman, P. K. Vaddady, Z. Zheng, J. Qi, R. Akbergenov, S. Das, D. B. Madhura, C. Rath, A. Trivedi, C. Villellas, R. B. Lee, Rakesh, S. L. Waidyarachchi, D. Sun, M. R. McNeil, J. A. Ainsa, H. I. Boshoff, M. Gonzalez-Juarrero, B. Meibohm, E. C. Bottger and A. J. Lenaerts, *Nat. Med.*, 2014, 20, 152–158.
- 131 J. Liu, D. F. Bruhn, R. B. Lee, Z. Zheng, T. Janusic, D. Scherbakov, M. S. Scherman, H. I. Boshoff, S. Das, Rakesh, S. L. Waidyarachchi, T. A. Brewer, B. Gracia, L. Yang, J. Bollinger, G. T. Robertson, B. Meibohm, A. J. Lenaerts, J. Ainsa, E. C. Bottger and R. E. Lee, *ACS Infect. Dis.*, 2017, 3, 72–88.
- 132 A. Palencia, X. Li, W. Bu, W. Choi, C. Z. Ding, E. E. Easom, L. Feng, V. Hernandez, P. Houston, L. Liu, M. Meewan, M. Mohan, F. L. Rock, H. Sexton, S. Zhang, Y. Zhou, B. Wan, Y. Wang, S. G. Franzblau, L. Woolhiser, V. Gruppo, A. J. Lenaerts, T. O'Malley, T. Parish, C. B. Cooper, M. G. Waters, Z. Ma, T. R. Ioerger, J. C. Sacchettini, J. Rullas, I. Angulo-Barturen, E. Perez-Herran, A. Mendoza, D. Barros, S. Cusack, J. J. Plattner and M. R. Alley, *Antimicrob. Agents Chemother.*, 2016, 60, 6271–6280.
- 133 F. L. Rock, W. Mao, A. Yaremchuk, M. Tukalo, T. Crepin, H. Zhou, Y. K. Zhang, V. Hernandez, T. Akama, S. J. Baker, J. J. Plattner, L. Shapiro, S. A. Martinis, S. J. Benkovic, S. Cusack and M. R. Alley, *Science*, 2007, 316, 1759–1761.
- 134 X. Li, V. Hernandez, F. L. Rock, W. Choi, Y. S. L. Mak, M. Mohan, W. Mao, Y. Zhou, E. E. Easom, J. J. Plattner, W. Zou, E. Perez-Herran, I. Giordano, A. Mendoza-Losana, C. Alemparte, J. Rullas, I. Angulo-Barturen, S. Crouch, F. Ortega, D. Barros and M. R. K. Alley, *J. Med. Chem.*, 2017, 60, 8011–8026.
- 135 A. Kling, P. Lukat, D. V. Almeida, A. Bauer, E. Fontaine, S. Sordello, N. Zaburannyi, J. Herrmann, S. C. Wenzel, C. Konig, N. C. Ammerman, M. B. Barrio, K. Borchers, F. Bordon-Pallier, M. Bronstrup, G. Courtemanche, M. Gerlitz, M. Geslin, P. Hammann, D. W. Heinz, H. Hoffmann, S. Klieber, M. Kohlmann, M. Kurz, C. Lair, H. Matter, E. Nuermberger, S. Tyagi, L. Fraisse, J. H. Grosset, S. Lagrange and R. Muller, *Science*, 2015, 348, 1106–1112.
- 136 V. Singh and V. Mizrahi, *Drug Discovery Today*, 2017, 22, 503–509.
- 137 V. Singh, S. Donini, A. Pacitto, C. Sala, R. C. Hartkoorn, N. Dhar, G. Keri, D. B. Ascher, G. Mondesert, A. Vocat, A. Lupien, R. Sommer, H. Vermet, S. Lagrange, J. Buechler, D. F. Warner, J. D. McKinney, J. Pato, S. T. Cole, T. L. Blundell, M. Rizzi and V. Mizrahi, *ACS Infect. Dis.*, 2017, 3, 5–17.
- 138 Y. Park, A. Pacitto, T. Bayliss, L. A. Cleghorn, Z. Wang, T. Hartman, K. Arora, T. R. Ioerger, J. Sacchettini, M. Rizzi, S. Donini, T. L. Blundell, D. B. Ascher, K. Rhee, A. Breda, N. Zhou, V. Dartois, S. R. Jonnala, L. E. Via, V. Mizrahi, O. Epemolu, L. Stojanovski, F. Simeons, M. Osuna-Cabello, L. Ellis, C. J. MacKenzie, A. R. Smith, S. H. Davis, D. Murugesan, K. I. Buchanan, P. A. Turner, M. Huggett, F. Zuccotto, M. J. Rebollo-Lopez, M. J. Lafuente-Monasterio, O. Sanz, G. S. Diaz, J. Lelievre, L. Ballell, C. Selenski, M. Axtman, S. Ghidelli-Disse, H. Pflaumer, M. Bosche, G. Drewes, G. M. Freiberg, M. D. Kurnick, M. Srikumaran, D. J. Kempf, S. R. Green, P. C. Ray, K. Read, P. Wyatt, C. E. Barry, 3rd and H. I. Boshoff, *ACS Infect. Dis.*, 2017, 3, 18–33.
- 139 S. Chacko, H. I. M. Boshoff, V. Singh, D. M. Ferraris, D. R. Gollapalli, M. Zhang, A. P. Lawson, M. J. Pepi, A. Joachimiak, M. Rizzi, V. Mizrahi, G. D. Cuny and L. Hedstrom, *J. Med. Chem.*, 2018, 61, 4739–4756.
- 140 V. Singh, A. Pacitto, S. Donini, D. M. Ferraris, S. Boros, E. Illyés, B. Szokol, M. Rizzi, T. L. Blundell, D. B. Ascher, J. Pato and V. Mizrahi, *Eur. J. Med. Chem.*, 2019, 174, 309–329.
- 141 K. Pethe, P. C. Sequeira, S. Agarwalla, K. Rhee, K. Kuhen, W. Y. Phong, V. Patel, D. Beer, J. R. Walker, J. Duraiswamy, J. Jiricek, T. H. Keller, A. Chatterjee, M. P. Tan, M. Ujjini, S. P. Rao, L. Camacho, P. Bifani, P. A. Mak, I. Ma, S. W. Barnes, Z. Chen, D. Plouffe, P. Thayalan, S. H. Ng, M. Au, B. H. Lee, B. H. Tan, S. Ravindran, M. Nanjundappa, X. Lin, A. Goh, S. B. Lakshminarayana, C. Shoen, M. Cynamon, B. Kreiswirth, V. Dartois, E. C. Peters, R. Glynn, S. Brenner and T. Dick, *Nat. Commun.*, 2010, 1, 57.
- 142 C. C. Onyedum, I. Alobu and K. N. Ukwaja, *PLoS One*, 2017, 12, e0180996.
- 143 N. A. Ismail, L. Mvusi, A. Nanoo, A. Dreyer, S. V. Omar, S. Babatunde, T. Molebatsi, M. van der Walt, A. Adelekan, V. Deyde, C. Ihekweazu and S. A. Madhi, *Lancet Infect. Dis.*, 2018, 18, 779–787.
- 144 WHO, South Africa conducts its first National TB Prevalence Survey 2017–2018, <https://afro.who.int/news/south-africa-conducts-its-first-national-tb-prevalence-survey-2017-2018>, (accessed January 17, 2019).
- 145 E. W. Orenstein, S. Basu, N. S. Shah, J. R. Andrews, G. H. Friedland, A. P. Moll, N. R. Gandhi and A. P. Galvani, *Lancet Infect. Dis.*, 2009, 9, 153–161.