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Priming the tuberculosis drug pipeline: new antimycobacterial targets and agents

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Claiming close to two million lives each year, tuberculosis is now the leading cause of death from an infectious disease. The rise in number of *Mycobacterium tuberculosis* (Mtb) strains resistant to existing TB drugs has underscored the urgent need to develop new antimycobacterials with novel mechanisms of action. To meet this need, a drug pipeline has been established that is populated with new and repurposed drugs. Recent advances in identifying molecules with inhibitory activity against Mtb under conditions modelled on those encountered during infection, and in elucidating their mechanisms of action, have primed the pipeline with promising drug/target couples, hit compounds and new targets. In this review, we highlight recent advances and emerging areas of opportunity in this field.

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Introduction

According to the most recent WHO report, a staggering 10.4 million people developed tuberculosis (TB) in 2016 and 1.7 million lives were lost to this disease; of these, 374 000 deaths occurred among people co-infected with HIV [1]. Having recently surpassed HIV/AIDS in total deaths per year, TB is now the leading killer from a single infectious agent. The steady rise in drug resistance has added an ominous new dimension to this global health crisis with an estimated 490 000 cases of multidrug resistant (MDR) TB, defined as TB that is resistant to isoniazid (INH) and rifampicin (RIF), with or without resistance to other first-line anti-tubercular drugs, having been reported in 2016. Of these cases, 6.2% had extensively drug resistant (XDR) TB, which is classified as being resistant to INH and RIF (i.e. MDR-TB) in addition to any fluoroquinolone and at least one of the injectable second-line drugs, kanamycin, amikacin, or capreomycin [1].

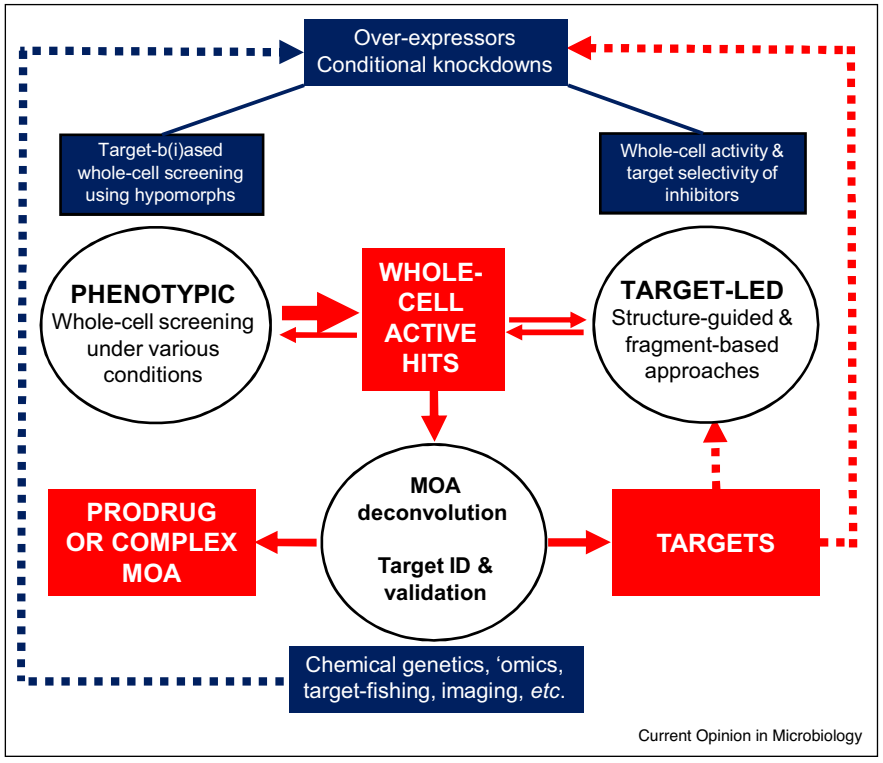
Against this stark background, slow but steady progress has been made in establishing a TB drug pipeline that has delivered two new drugs — bedaquiline (BDQ) and delamanid — which were recently approved for the treatment of MDR-TB [2••]. Given their shorter paths to regulatory approval, there has been considerable interest in drug repurposing for TB. Thus, fluoroquinolones, oxazolidinones, rifamycins and β-lactams feature prominently among the drugs and drugs combinations currently in clinical development (http://www.newtbdrugs.org/pipeline/clinical). However, the development of specific antimycobacterials (i.e. agents with narrow-spectrum activity) remains a major goal. To date, most new antituberculars have been discovered through phenotypic screens to identify inhibitors of growth and/or survival of *Mycobacterium tuberculosis* (Mtb) under various conditions. The availability of molecules with whole-cell activity identified by high-throughput screening (HTS) [3–8], combined with powerful chemical genetic, 'omics' and imaging technologies for use in elucidating mechanisms of action (MOA) [9,10••], and in target identification and validation, have created a rich resource for priming the pipeline with hits and/or new targets (Figure 1).

In this review, we focus on promising new antimycobacterials and drug targets (Table 1 and Figure 2). We also highlight recent advances in potentiating TB drug efficacy and related treatment-shortening strategies.

Cell envelope biogenesis

The complex, lipid-rich cell envelope of Mtb remains a major source of new TB drug targets. Originally identified as the target of the benzothiazinones, DprE1 has since emerged as a 'promiscuous' target of multiple chemotypes [11•]. Two DprE1 inhibitors are currently in development: the azaindole, TBA-7371 [12] and the benzothiazinone, PBTZ-169 [13]. MmpL3 is another promiscuous cell wall target although recent work has shown that not all molecules for which resistance maps to *mmpL3* are *bona fide* inhibitors of this protein [14]. MmpL3 serves as the flippase for transport of mycolic acids across the inner membrane [14] and has the attractive feature of being a highly vulnerable target *in vivo* [15].

Figure 1



An integrated approach to TB drug discovery. Whole-cell active hits from phenotypic screening or target-led approaches are subjected to MOA deconvolution to distinguish hits with a sole/primary target in Mtb from those with a prodrug or complex MOA. Conditional knockdown mutants (hypomorphs) can be used in target-based or target-biased whole-cell screens to identify target-selective or pathway-selective hits, and/or to confirm the target-selectivity of existing hits [64].

Enzymes involved in mycolic acid (MA) biosynthesis continue to feature prominently as targets for new antimycobacterials. FadD32 is the target of a series of 4,6-diaryl-5,7-dimethyl coumarins which inhibit the fatty acyl ACP synthase activity of this enzyme [16]. This activity transfers the meromycolyl-AMP intermediate to the ACP domain of Pks13, which catalyses the final step in MA biosynthesis. The druggability of Pks13 was confirmed by demonstrating that it is the target of the benzofuran, TAM1 [17], and a series of thiophene (TP) compounds [18]. Optimisation of TAM1 led to the development of TAM16, which inhibits the thioesterase activity of Pks13, is potently bactericidal *in vitro* and *in vivo* and has a low frequency of resistance [19•]. Interestingly, co-treatment with isoniazid (INH) and TP sterilised cultures of Mtb, raising the possibility that simultaneous targeting of two different steps in the MA biosynthesis pathway could eliminate persisters [18]. Yet another enzyme in this pathway, the β-ketoacyl synthase, KasA, was identified as the target of an indazole sulphonamide [20]. Although bacteriostatic *in vitro*, this compound, which binds at a site distinct from other KasA inhibitors, shows cidality in mice. In a further advance, the enzyme WecA, which initiates the biosynthesis of arabinogalactan — another key component of the cell envelope — was genetically validated and shown to be the primary target of tunicamycin [21].

Encouraging results on the early bactericidal activity of meropenem in combination with amoxicillin-clavulanic acid in humans [22] has repositioned β-lactams, and, more broadly, the targeting of peptidoglycan (PG) biosynthesis, centre-stage in TB drug discovery. The non-classical L,D-transpeptidases, Ldt_{Mt1} and Ldt_{Mt2}, which catalyse the formation of 3→3 transpeptide linkages in the PG network, have attracted particular attention as targets of carbapenems [23•]. Faropenem, which is orally bioavailable, approved for use in humans, more resistant to hydrolysis by β-lactamases, and bactericidal against replicating and nonreplicating Mtb, shows considerable promise as an antitubercular [24]. This drug displays synergy with other TB drugs [24], as do combinations of cephalosporins and rifampicin (RIF) [25]. As outlined below, such synergistic interactions may prove important in treatment shortening. In another advance, the first cephalosporins showing selective activity against nonreplicating Mtb have been identified [26]. These compounds are also active in macrophages although their target/s have yet to be identified.