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Future target-based drug discovery for tuberculosis?

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Abstract

New drugs that retain potency against multidrug/extensively drug-resistant strains of *Mycobacterium tuberculosis*, with the additional benefit of a shortened treatment duration and ease of administration, are urgently needed by tuberculosis (TB) control programs. Efforts to develop this new generation of treatment interventions have been plagued with numerous problems, the most significant being our insufficient understanding of mycobacterial metabolism during disease. This, combined with limited chemical diversity and poor entry of small molecules into the cell, has limited the number of new bioactive agents that result from drug screening efforts. The biochemical, target-driven approach to drug development has been largely abandoned in the TB field, to be replaced by whole-cell or target-based whole-cell screening approaches. In this context, the properties of a good drug target are unclear, since these are directly determined by the ability to find compounds, using current screening algorithms, which are able to kill *M. tuberculosis*. In this review, we discuss issues related to the identification and validation of drug targets and highlight some key properties for promising targets. Some of these include essentiality for growth, vulnerability, druggability, reduced propensity to evolve drug resistance and target location to facilitate ready access to drugs during chemotherapy. We present these in the context of recent drugs that have emerged through various approaches with the aim of consolidating the knowledge gained from these experiences to inform future efforts.

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Tuberculosis; Drug targets; Drug development; Target vulnerability; Whole-cell screens

1. Background

Tuberculosis (TB), caused by infection with the slow-growing actinomycete *Mycobacterium tuberculosis*, currently causes 1.4 million deaths every year and 8e10 million new infections [1,2]. TB is treated with a complex treatment regimen, consisting of multiple antibiotics that target diverse cellular processes [3]. Furthermore, TB eradication efforts have included use of the most widely administered vaccine in human history, *Mycobacterium bovis* BCG. Despite this, TB continues to remain a constant source of human suffering, representing a failure on multiple levels in disease prevention, treatment, and health policy implementation. The protracted treatment period currently used for management of active TB disease is logistically complicated in resource-limited settings, which, together with other factors, has resulted in the rapid emergence of progressive drug-resistant TB [4]. There is an urgent medical need to develop new anti-tubercular agents with rapid sterilizing activity and novel modes of action to create a simplified, shorter treatment algorithm that is less onerous on the control programs of endemic countries [5–7].

Current drug development efforts have led to a few new candidate compounds/compound classes, as well as repurposed antibiotics that are in various stages of clinical development [3] (Figure 1). These recent successes provide an opportunity to reflect on current gaps in TB drug development and critical features required for new candidate compounds and their cellular targets. Currently, there is poor consensus on the key properties that constitute the ideal drug target for eliminating *M. tuberculosis*. Moreover, an insufficient understanding of host–pathogen interactions has limited the identification of targets for host-adjunctive therapy [8,9]. In this review, we discuss the recent successes in TB drug development and attempt to highlight some key properties of the targets that have emerged in an attempt to inform further efforts. An emerging approach to target-based drug discovery is the use of strains depleted for essential or conditionally essential targets. These and related concepts will be discussed herein along with issues that emerged from the workshop entitled “Targets for Tomorrow” organized by the Biology/Targets Subgroup of the Stop TB Working Group on New Drugs, held at the Gordon Research Conference for Tuberculosis Drug Development in Lucca, Italy in July 2013.

2. Out with the old – in with the new

The traditional, target-based approaches that utilize biochemical assays, three-dimensional structural information, and demonstrated biological function have worked well in drug discovery for various communicable (in particular viral) and non-communicable diseases. However, these approaches have had no substantive success against bacterial infectious diseases, including TB [10]. Consequently, current screening modalities are dominated by whole-cell screens for bactericidal activity on pathogenic and non-pathogenic mycobacteria. Target identification and validation of vulnerability only occurs once biological potency has

been demonstrated. As such, the useful (or detrimental) properties of any target are not considered during early screening design. The use of hypomorphs in vulnerable metabolic pathways that are essential provides a convenient marriage of the two methods. However, this method is fraught with problems of achieving sufficient gene repression and downstream polar effects in the case of polycistronic genes.

3. New TB drugs

Among the recent successes in TB drug development is the identification of bedaquiline, which kills *M. tuberculosis* by inhibition of the membrane-bound F_1F_o ATP synthase complex, resulting in depletion of cellular ATP levels and eventual death of the organism [11]. This drug displayed notable bactericidal activity in early bactericidal activity studies and is approved for use under the trade name of Sirturo for treatment of patients with drug-resistant TB [12,13]. Another promising group of compounds is the nitro-dihydro-imidazooxazole derivatives, such as OPC-67683 (delam-anid), which kills *M. tuberculosis* by inhibition of mycolic acid biosynthesis [14–17]. The related bicyclic nitroimidazole, PA-824, has demonstrated promise as a new TB drug with the ability to kill both replicating and non-replicating *M. tuberculosis* bacteria through a multi-faceted mechanism that involves inhibition of mycolic acid biosynthesis and respiratory poisoning through intracellular release of nitric oxide [18–22]. The identification of the benzothiazinones, a new class of candidate compounds that inhibit decaprenyl-phosphoribose epimerase (DprE1) and thereby interrupt the final steps of arabinogalactan biosynthesis, represents another class of antibiotics that target cell wall biogenesis through a distinct, novel mechanism [23,24]. More recent efforts have yielded another novel class of imidazopyridine amide compounds (the lead compound being Q203) that prevent proliferation of *M. tuberculosis* by inhibition of the cytochrome bc_1 complex in the mycobacterial respiratory chain [25–28]. Finally, the diamine SQ109, which is currently in Phase II studies, was shown to target the transporter MmpL3, involved in cell wall biosynthesis and other targets [29].

A striking feature of the abovementioned novel drugs/drug candidates is their ability to either inhibit energy metabolism or cell wall biosynthesis and, in some cases, both of these processes. This would suggest that perhaps these, and related processes represent highly vulnerable areas of mycobacterial metabolism. However, this hypothesis is dependent on the success of Q203 and the benzothiazinones in the clinical setting. Furthermore, the limited diversity in chemistry currently available may preclude the development of drugs that target other processes.

3.1. Repurposed drugs

In addition to novel compounds, there are various clinically validated classes of compounds that have been investigated for biological potency against *M. tuberculosis*. Among these are the oxazolidinones, such as linezolid [30] and, more recently, PNU-100480 (sutezolid), which is more potent than linezolid and synergizes with moxifloxacin and pyrazinamide in the murine model of TB infection [31,32]. Despite this difference in efficacy, the recent demonstration of linezolid's curative activity in patients with XDR-TB validates this class of compounds for further development [30]. The promising activity displayed by the analogue

AZD5847 [33], which is currently in phase IIA trials, is further evidence of this. Clofazimine, a hydrophobic rhiminophenazine used traditionally for treatment of leprosy also features prominently among the repurposed TB drugs for treatment of drug-resistant TB [34]. This drug is postulated to undergo reduction by the mycobacterial type II NADH dehydrogenase and, upon subsequent oxidation, leads to the formation of reactive oxygen species [35]. Clofazimine accumulates inside host macrophages/tissues and is able to crystallize in pulmonary macrophages [36]. Whilst these are undesirable effects in any drug, they may contribute to the efficacy of this compound in treating leprosy and TB. The promising activity of clofazimine in the murine model of TB infection has led to the search for new analogs, where these side effects have been reduced [37].

4. The “ideal” drug target

In considering future drug discovery and development, there are several features inherent from current drug targets that are worth considering. Some of these are detailed below.

4.1.Essentiality and vulnerability

The most obvious, and critical, feature of a target is essentiality for growth and/or survival under the various conditions encountered during *M. tuberculosis* infection in humans. Moreover, the target should be vulnerable to inhibition under these conditions. TB pathogenesis is complex and involves both the innate and acquired arms of the host immune response. A combination of these path-ways leads to cell-mediated killing of infected macrophages. Moreover, *M. tuberculosis* is able to subvert macrophage-killing pathways and can persist in mature phagocytic vacuoles. These events lead to the formation of granulomas wherein nutrient limitation, acid stress, and hypoxia prevail [38–40]. Screening efforts have aimed to replicate these conditions, however our knowledge of the microenvironments encountered by persistent bacilli, which are responsible for the prolonged duration of treatment in the human host, is very limited. It is generally accepted that current *in vitro* and animal models are inadequate in their ability to replicate the complex spectrum of disease that characterizes human TB. Therefore, more accurate predictive preclinical models are urgently needed. In addition to being essential, vulnerability is a key feature for drug targets, where the most ideal targets would be those that cause cell death upon minimal inhibition [41].

An emerging body of evidence points to the fact that many drugs have complex mechanisms for killing bacteria, which involve numerous second-site effects in addition to inhibiting their primary target(s). In this regard, inhibition of the target results in corruptive effects on metabolism, such as accumulation of toxic intermediates, disruption of redox/sulfur or bioenergetic homeo-stasis, all of which result in cell death [42]. In light of this, it is difficult to predict the bactericidal potential of a particular target without considering how the target fits into pathways and general cellular metabolism.

4.2. Low inherent mutability

The rapid emergence of drug-resistant strains suggests that, as with other antibiotics, the selection of spontaneously resistant organisms during infection is a major problem with *M.*

tuberculosis, which is likely to render most of the currently available drugs ineffective over time. Targets that have a low tolerance for mutation would limit the emergence of drug-resistant variants. Alternatively, drugs that target the molecular components of the mutagenic processes that lead to antibiotic resistance are desirable, as these would ensure the fidelity of current and new treatments. Note-worthy in this regard is the fact that pathways contributing to spontaneous and DNA damage-induced mutagenesis have been partially characterized, identifying putative new drug targets. These include the atypical C-Family DNA polymerase, DnaE2, which has been implicated in the emergence of drug resistance *in vitro* and *in vivo* [43], and, more recently, ImuA and ImuB, both of which function to form a complex of proteins that is required for DNA-damage induced mutagenesis [44].

4.3. Chokepoints

Developing drugs for enzymes that are required for multiple, independent processes in cellular metabolism provides the advantage of inhibiting numerous metabolic pathways at once to achieve rapid bacterial death. These targets would serve as ideal chokepoints, inhibition of which would lead to rapid cell death due to the buildup of toxic metabolites. These include amino acid, cofactor biosynthesis, purine/pyrimidine biosynthesis, and cell wall biosynthesis. The mycobacterial cell envelope is one of the most complex bacterial structures identified and serves as a major bar-rier to the entry of drugs. It is noteworthy that most of the new preclinical candidates identified in the last decade target cell wall-related processes, either directly by interfering with cell wall biosynthesis processes, or through a cytosolic effect that leads to inhibition of enzyme complexes in the membrane, such as the generation of nitric oxide by PA-824 [22].

4.4. Essentiality for latency/dormancy

In a significant number of exposed individuals, *M. tuberculosis* establishes latent TB infection (LTBI), which is characterized by the absence of clinical symptoms. Activity of novel TB drugs against LTBI is paramount for global TB eradication efforts, since the 2 billion people latently infected with *M. tuberculosis* represent a vast reservoir for continued reactivation and transmission, especially in the setting of the HIV pandemic [2]. Our knowledge of LTBI is hampered by the lack of adequate research models and molecular tools for studying the condition [45]. Although the physiological state of tubercle bacilli during LTBI is unknown, it is hypothesized that these organisms have reduced or no growth and employ metabolic pathways for growth and survival that are distinct from those utilized by actively replicating organisms [46]. A drug target that is intrinsically essential for the bacteria in both active and latent disease is extremely desirable and would allow for the treatment of both of these clinical presentations. Furthermore, those drug targets or metabolic pathways that are required for pro-longed survival of *M. tuberculosis* during LTBI may be similar to those required for persistence of tubercle bacilli in the face of prolonged TB chemotherapy. Compounds which result in bacterial lysis by targeting cell wall biogenesis or remodeling of the peptidoglycan/arabinogalactan/mycolic acid components would affect both replicating and non-replicating bacteria [47]. Consistent with this, isoniazid preventative treatment has notable beneficial effect in those individuals with LTBI [45]. An alternate, currently emerging approach with LTBI is to “reawaken” persisting organisms, either through inhibition of host immune responses (e.g., steroids, TNF

inhibitors) or by targeting mycobacterial dormancy pathways, thus rendering these organisms more susceptible to killing by conventional antibiotics.

4.5.Real estate – location, location, location

The cellular location of a particular drug target may influence the ability of small molecules to bind and inhibit biological activity. Sequestration of drug targets in multimeric enzyme complexes, nucleoid–protein complexes and stable structural proteins may contribute negatively to the ability to exploit these proteins for drug discovery. However, the efficacy of drugs such as isoniazid, which targets the activity of large enzyme complexes, suggests that this notion may not hold true for all new targets. The mycobacterial cell wall represents a major permeability barrier for the penetration of small molecules and, consistent with this, the overall location of drug targets, that is intracellular or extracellular, will influence druggability dramatically. The high “real estate” value of extracellular drug targets is reflected by the fact that numerous promising TB drug candidates/drugs have targets that are outside of the cytoplasmic compartment.

5. Drug targets and screening

With the exception of a few cases that aim to develop inhibitors against clinically-validated drug targets, the majority of current screening efforts are utilizing whole-cell determinations of com-pound potency for lead finding and optimization. The disadvantage of such approaches lies in the lack of drug target knowledge hampering rational improvement of active compounds by structure-based design. Target identification approaches are being used, but these are not always successful.

The development of hypomorphs, which allow for depletion of candidate drug targets to identify inhibitors for specific pathways, is a notable advance in the area of phenotypic screening. The ability to fine tune target dosage in these screens is essential for success and the technology to do this is constantly being revised, as evidenced by the development of new, dual repression systems that deplete transcription and translation of the target in question. This approach has been used successfully to validate the *panC*-encoded pantothenate synthetase as a novel drug target [48–50]. The ability to accurately modulate target dosage, without untoward secondsite effects is critical to success in these ventures and those targets that do not lend themselves to these methods cannot be effectively validated using this approach. In this regard, modulating the dosage of vulnerable drug targets with very low basal levels of expression is difficult to undertake using available technology. Currently, there is no evidence that depleting a target of interest has the same biological effect as chemical inhibition/modulation of the same target. This is an important consideration in rational drug discovery initiatives, since bacillary death resulting from specific gene depletion may differ significantly from chemical modulation of the corresponding gene product. Another, more target-based, approach is pathway-based whole cell screening, which uses reporter genes that are transcriptionally upregulated upon interference with certain pathways. An example of this is the recent identification of thiophenes targeting Pks13. These compounds were identified as inducers of the *iniBAC* promoter, known to be upregulated by a range of cell wall inhibitors [51]. An alternate

approach is to use strains that have increased expression of putative drug targets which may be useful to identify new hits, mode of action and off-target effects.

5.1. Corruption versus inhibition of drug targets

An alternative to reversible inhibition of a drug target, for those proteins that lend themselves to corruption through binding of a small molecule, may prove particularly useful. In addition to inhibiting a specific function, corrupt proteins can have general toxicity effects and also affect the function of other essential proteins. Corruption may also have unwanted consequences, such as enhanced mutation rates, a possibility with molecules such as fluoroquinolones, which corrupt DNA gyrase and, through this ac-tivity, lead to the induction of a mutagenic SOS response [52,53].

5.2. Druggable properties: from promising inhibitors to clinical trials

One of the more important properties of a good target is the ability to find drugs that inhibit its function. Complex secondary structure, oligomeric protein complexes and membrane association may contribute to poor access to drug targets/active sites by small molecules. There are various algorithms to predict target drugg-ability, the simplest being an assessment of whether the putative drug target is part of a larger family of proteins that have a proven track record of clinically validated targets. Alternative modalities to screen for druggability involve assessment of the three-dimensional structure of a protein to determine the diversity in structural space to bind small molecules. This allows for elucidation of important mitigating factors, such as the relative depth of binding pockets for insertion of small molecules, steric hindrance effects, and localized structural rearrangement. However, in the case of *M. tuberculosis*, such approaches are limited during screening due to the poor diffusion of molecules across the cell wall. Another feature of druggable targets is the ability to develop a biochemical or whole-cell based assay to assess catalytic activity and inhibition thereof during high-throughput screening. Multi-step enzymatic mechanisms with unstable substrates/products make for poor assay development and limit the ability to thoroughly assess inhibition of a target. Hence, consideration of physico-chemical properties of compounds during lead finding is critical. Often compounds with high lipophilicity are generated to improve *in vitro* potency resulting in *poor in vivo* pharmacokinetic properties, loss of selectivity and, consequently, attrition during development [54].

Following chemical validation of a target and the identification of a lead, the lead optimization phase aims to identify new analogs with enhanced potency, good tolerability, drug-like physicochemical properties, and favorable pharmacokinetic profile. This process is accomplished by modifications of the scaffold of interest based on knowledge of the structure-activity (SAR) and structure-property (SPR) relationship. Numerous reasons contribute to the high attrition rate in the discovery of new antimicrobial agents, including toxicity, poor drug entry into the cell, complex synthesis process, and poor pharmacokinetic/dynamic properties. Efforts to address this earlier in the process include the development of *in vitro* assays based on purified enzyme and mammalian cell lines to identify potential liabilities. For example, the Enhanced Cross Screen Panel (eXP) consists of more than fifty *in vitro* assays designed to measure the effects of test compounds on receptor sites, ion channels, transporters, and critical enzymes activity [55]. The data obtained from these

assays combined with *in vitro* pharmacokinetic assays, which measure metabolic stability, solubility and membrane permeability, provide useful tools for improving the drug-like properties of new compounds, while eliminating drugs with toxic properties. In this example, compounds with a 'promiscuity index' (the target hit rate, which is defined as the percentage of at least 50 off-site targets giving greater than 50% inhibition at 10 μM) of greater than 20% are considered to be 'promiscuous', and are eliminated from further testing. Early *in vitro* toxicity assays include cyto-, cardio-, geno-, and photo-toxicity determinations, in addition to determining binding to panels of human receptors. Considering that current TB treatment involves protracted drug administration, the abovementioned toxicological analyses may provide limited information. Hence, detection of toxicity remains a serious bottleneck in drug discovery and, in some cases, toxic properties are often only uncovered for advanced compounds with an acute toxicity study in mice or more detailed studies in a 2-week rat model [54].

Ideally, novel anti-TB compounds would be inexpensive, and administered orally no more than once a day to ensure widespread accessibility and medical adherence. Special considerations in the case of TB include drug penetration into eukaryotic cells such as macrophages, dendritic cells, foamy macrophages (where bacilli can persist or replicate) and within arrested phagolysosomes. Moreover, drugs need to enter into cavitary lesions teeming with actively multiplying organisms during active TB, and into the poorly vascularized, necrotic cores of granulomas, where persistent bacilli responsible for clinical relapse may reside. In addition, given the dramatic convergence of the TB and HIV pandemics, TB drugs lacking drugedrug interactions with antiretroviral agents are ur-gently needed.

Historically, TB drug development efforts have focused on pre-clinical screening and development of individual drugs. However, such an approach has been associated with long delays in the development of novel regimens, especially for the treatment of drug-resistant TB. Therefore, the Critical Path Institute (CPTR) and the Global Alliance for TB Drug Development (TB Alliance) recently have espoused a novel paradigm, in which an entirely new multidrug regimen is tested in combination in animal models, with the goal of reducing the time and cost associated with developing novel regimens for drug-sensitive and drug-resistant TB.

6. Concluding remarks

As recently described by Lechartier et al., [56] whole cell approaches have been quite successful over the past few years and delivered a series of lead and development compounds, some drug candidates and one new, approved drug, bedaquiline. This approach also delivered a series of novel, chemically validated targets, which can now be exploited for target-based optimization of compounds. Interestingly, these novel antimycobacterial drugs appear to largely affect energy metabolism and cell envelope biosynthesis, suggesting that those metabolic areas are particularly vulnerable or accessible. Although target-or pathway-based whole cell screens have not been employed in a major way yet, these approaches might be useful to increase efficiency of the drug discovery process for TB and reduce the attrition in the lead compound isolation and optimization steps.

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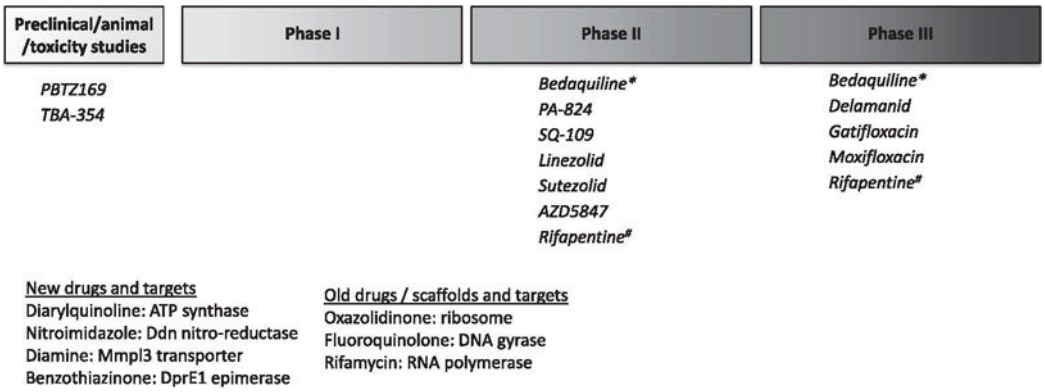

Figure 1.

Tuberculosis drug development pipeline. Shown are the drugs in various stages of preclinical and clinical development with their corresponding drug targets. Information provided courtesy of the Working Group on New TB Drugs (www.newtbdrugs.org, updated June 2014). *Bedaquiline is currently in Phase III for MDR-TB and Phase II for drug susceptible TB. #Rifapentine Phase II for drug susceptible TB and Phase III for LTBI.