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Introduction: Novel insights into TB research and drug discovery

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Abstract

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), kills more people than any other bacterium. TB control is threatened by the continued spread of drug resistance; multi-drug and extensively drug resistant *Mtb* require longer, more costly, treatment with multiple drugs causing worse side effects and have a lower likelihood of treatment success. The urgent need for better treatment options for drug resistant *Mtb* has led the World Health Organization to prioritize development of not only new individual antitubercular agents, but also new drug regimens. This introductory chapter of the special issue *Novel insights into TB research and drug discovery* within Progress in Biophysics and Molecular Biology, gives a short overview about the general problems of TB and the treatment of this disease today, and introduces the review topics within this issue.

On 26 September 2018, the United Nations (UN) held its first high-level meeting on tuberculosis (TB) with the title “*United to End TB: An Urgent Global Response to a Global Epidemic*”, highlighting the need for immediate action to accelerate progress towards the goal of ending the TB epidemic. In the months leading up to the UN high-level meeting, major country blocs have issued communiqués on the need for action on TB, including drug-resistant TB in the wider context of antimicrobial resistance. Examples include the G20, the G7, the BRICS group (Brazil, the Russian Federation, India, China and South Africa) and the Asia-Pacific Economic Cooperation (APEC). New commitments were made by ministers from countries in the World Health Organization (WHO) South-East Asia Region at the Delhi End TB Summit in March 2018.

These most recent events and political steps were the results of the alarming statistics of more than 10 million new cases of active TB and 1.6 million deaths per year, with the WHO South-East Asia Region and WHO Western Pacific Region accounting for 47% and 17%, respectively. Of the >10 million new cases, 4.6% were resistant to both the key first-line drugs rifampicin (Rif) and isoniazid (Inh) and are classed as multidrug resistant (MDR). MDR-TB now represents an important emerging disease affecting around 500,000 people annually and causing 110,000 deaths per year. 8.5% of the MDR-TB cases were extensively drug resistant (XDR), where resistance to two of the key second-line drugs is also present. Only 55% of the MDR-TB and 30% of the XDR-TB cases were treated successfully (WHO, 2018).

Standard treatment for drug-sensitive TB comprises 6 months’ treatment with a combination of the first-line drugs Inh, Rif, Pyrazinamid (PZA) and Ethambutol (Emb), each of which may cause side effects. Although cure rates in excess of 95% are the norm in clinical trials, in program settings cure rates may be considerably lower because patients struggle to maintain

adherence throughout long courses of treatment. As well as promoting relapse, poor adherence contributes to the development of drug resistance. The reason why current TB drugs take so long to clear the infection are not entirely clear. However, working models based on bacteriological, animal model and clinical data suggest that the pathogen, *Mycobacterium tuberculosis* (*Mtb*), exists in different physiological subpopulations inside human TB lesions. This unique feature of *Mtb*, termed latency, includes up- and down regulation of genes that enable the survival of the pathogen within host organisms under oxygen- and nutrient-deprived conditions (Rao et al., 2009; Flannagan et al. 2009). Such latent bacteria have been known to be confined in calcified lesions, termed granulomas, which enable the dormant bacteria to resist conventional antibiotics like the bactericidal drug Inh. To achieve shortening of treatment duration, it is therefore necessary to find drugs that work better against these dormant bacilli. This is termed sterilizing activity as clearance of these bacteria is required in order to prevent relapse from clinical TB. A further important aspect of infection is that intracellular mycobacteria interact dynamically with the host cellular machinery, damping down the immune response (Schnappinger et al., 2003; Chao MC, Rubin, 2010). Finding ways of targeting the relevant host factors may provide an alternative approach for the therapeutic management of TB.

After a hiatus of over 40 years in TB drug development, there has been a recent resurgence of activity with a number of new compounds in the TB-drug pipeline (Fig. 1). Most recent developments of the compounds TBI-223, BTZ-043, OPC-167832, and Contezolid, which entered phase 1 (P1), Delpazolid and Sutezolid being in P2, reflect the important target of the pathogen's protein synthesis machinery in particular the ribosome complex with the many regulatory proteins binding to it, emphasizing the adaptive cascades within *Mtb* to address environmental adaptation (Fig. 2). This is in line with the recent promises of respiration by the

electron transport chain (ETC) and ATP synthesis (oxydative phosphorylation) as a new target space for drug development in bacterial pathogens, since the generation of ATP is essential for the bacterial pathogen physiology, persistence and pathogenicity (Fig. 2). For example, Clofazimine (CFZ) is an antimycobacterial agent (Barry et al., 1957) with good *in vitro* activity. CFZ shuttles electrons from the ETC enzyme type 2 NADH dehydrogenase (NDH2) to O₂, generating bactericidal reactive oxygen species (ROS) (Yano et al., 2011). Telacebec (Q203), a cytochrome *bc₁-aa3* terminal oxidase complex inhibitor is a bacteriostatic drug against *Mtb* and entered P2 (Fig. 1) (Pethe et al., 2013). The concept that drugs that inhibit oxidative phosphorylation could shorten the time of therapy for drug-resistant tuberculosis is supported by the clinical development of Sirturo® (bedaquiline, BDQ), an anti-TB drug targeting the *Mtb* F₁F_O ATP synthase (Andries et al., 2005). Despite a very good efficacy for the treatment of MDR- and XDR-TB, clinical resistance has already been observed after three years after market approval (Andries et al., 2014; Hartkoorn et al., 2014). Furthermore, an unfavourable toxicity profile, such as phospholipidosis and cardiovascular risks prevent a wider use of the drug, which is restricted to the most difficult to treat cases. Toxicity of BDQ is linked to its high lipophilicity (logP = 7.52) and cationic, amphiphilic properties resulting in tissue accumulation. Recent discoveries of 3,5-dialkoxypyridine analogues (DARQ) of BDQ including the molecules TBAJ-876 and TBAJ-587 resulted in molecules which are less lipophilic, have higher clearance and display lower cardio-toxic potential (Tong et al., 2017; Choi et al., 2017; Sutherland et al., 2018; Blaser et al., 2019; Sutherland et al., 2019) (Fig. 1). Although the outcome of most recent compounds within the TB drug pipeline increase, MDR- and XDR-TB emerges as an important threat to TB control and the extensive resistance profile makes it difficult to construct an effective treatment regimen. On 14th August 2019, the U.S. Food and Drug Administration

approved pretomanid (PA-824) in combination with BDQ and linezolid for treating a limited and specific population of adult patients with extensively drug resistant, treatment-intolerant or nonresponsive multidrug resistant pulmonary TB.

This issue of Progress in Biophysics and Molecular Biology, entitled *Novel insights into TB research and drug discovery* brings together renowned scientists, leaders and advisers of world-wide leading institutions in TB, which presented at the 1st Annual Symposium *From crisis to cures: New approaches to TB Drug discovery* of TOPNet in Singapore their novel insights about TB research and drug discovery, to discuss tomorrow new strategies and avenues to tackle TB and to connect teams in the US, Europe, Southeast Asia, and New Zealand. The issue begins with a recent patient-pathway analysis of the Bill & Melinda Gates Foundation (BMGF), described and interpreted by the former Senior Adviser of the BMGF, G. Kaplan. This chapter addresses the questions like why is the decline in TB incidence so slow and what is needed to fix this failure?

Mtb has evolved specialized secretion complexes to secrete virulence factors in their extracellular environment or directly to the host. Its success largely depends on the secretory virulence factors to exploit nutrient resources and to escape the host immune response (Ates et al., 2015). This secretion system has been termed as a novel type VII secretion system (T7SS) or ESAT-6 secretion system (ESX) (Siegrist et al., 2014) (Fig. 2). Because of its importance in pathogen-host interaction, this system became a candidate for future drug and vaccine development. Previously, a negative stained electron microscopy (EM) structure of the T7SS core complex from *M. xenopi* was reported by the teams of M. Wilmanns and Houben (Beckham et al., 2017). The structure revealed the molecular architecture of T7SS for the first time. In this issue, the Wilmann's team displays the importance of the activity of the AAA⁺-ATPase domains

within the T7SS core complex for the translocation of proteins and virulence factors across the membrane.

In order to translocate proteins, they have to be synthesized in the macromolecular ribosome complex. This engine undergoes conformational transitions during protein synthesis to translate the genetic code into the nascent polypeptide chains. As described here by the team of S. Bhusan, cryo-EM structures of the *M. smegmatis*- and *Mtb* ribosome complex have provided significant understanding of the translational mechanism as well as to species specific variation/adaptation in the mycobacterial ribosome structures, a key point in species specific TB-drug development. These structures unraveled the two additional ribosomal proteins *bS22* and *bL37*, a number of unique ribosomal-proteins and rRNA extensions (Mishra et al., 2018), and revealed with the Hibernation Promoting Factor (HPF) bound ribosome structure of *M. smegmatis* (called hibernating ribosome), a possibly new mechanism of ribosome protection during dormancy. These data provide an opportunity for further drug-discovery purposes.

As described for the DARQ's of BDQ or the molecule TBI-166 (Phase 1; Fig. 1), which mirrors CFZ's potential, continuous optimization of compounds, which often goes hand in hand with more insights into the mechanism of action, are essential to improve potency and improved physico-chemical properties of the molecules. This is highlighted by T. Dick's team in their present article about new aspects of the mechanism of action of the first line TB-drug PZA. The inclusion of PZA in the regimen of drug-susceptible patients in the 1970s resulted in remarkable shortening of therapy duration from 12 to 6 months although the antibacterial mechanism of action of the drug remained elusive for decades (Mitchison, 1985). It was uncovered that the drug inhibits bacterial Coenzyme A biosynthesis (Gopal et al., 2017). PZA blocks this pathway by triggering degradation of its target aspartate decarboxylase (Gopal et al., 2019). This new

mechanistic knowledge will pave the way for the discovery of next generation PZA. Furthermore, the question will be addressed, whether targeted protein degradation could be generalized as an approach to anti-bacterial drug discovery.

The pathogen *Mtb* is an obligate aerobe that is strictly dependent on oxygen to meet its energetics demand during growth. In the absence of effective fermentative processes, the oxidative phosphorylation pathway, is crucial to maintain redox homeostasis and synthesis of sufficient amount of ATP (Cook et al., 2014). The NADH-dehydrogenase (NDH) and the succinate dehydrogenase (SDH) accept electrons from the donors NADH and succinate, with the latter also being a substrate within the Krebs cycle (Fig. 2). Succinate is required for generating a membrane potential under hypoxia and maintaining the balance of menaquinone:menaquinol. *Mtb* encodes two SDH enzymes (SDH1 and -2), as well as a separate fumarate reductase (Frd) with possible bidirectional behavior (Cook et al., 2014). As described by the group of G. Cook, SDH1 is essential for optimal growth under aerobic conditions and SDH2 plays a pivotal role in the transition of *Mtb* from aerobic to hypoxic conditions. In comparison, the Frd has been shown to be non-essential for growth in various studies. Like for the SDH complex, *Mtb* possess two terminal oxidases: a proton-pumping cytochrome-*bc₁:aa₃* oxidase supercomplex (cyt-*bc₁:aa₃*) and a non-proton pumping, less energetically efficient, cytochrome-*bd* oxidase (cyt-*bd*) (Bald et al., 2017). Pethe and colleagues will describe the discovery of several small-molecules including Telacebec (Q203) targeting the cyt-*bc₁*, which validated this respiratory branch for drug development for TB. Furthermore, the synthetic lethality between the cyt-*bc₁:aa₃* and the cyt-*bd* highlights the promise of targeting the *bd*-type terminal oxidase to rapidly eradicate *Mtb* infection (Kalia et al., 2017; Lamprecht et al., 2016) The value of the cyt-*bd* as drug target is further reinforced by the observation that deletion of the cyt-*bd*-encoding genes sensitizes

mycobacteria to BDQ and CFZ (Berney et al. 2014; Kalia et al., 2017), two agents interfering with the oxidative phosphorylation pathway (Lu et al., 2015; Hards et al., 2015). Therefore, knowledge about the role of cytochrome *bd* during infection of the mammalian host and in the defense against antibacterials is essential as highlighted by Bald and his team. They explain, why implementation of specific drug-mediated inhibition of the *cyt-bd* oxidase could increase efficacy and possibly decrease treatment time for a rational drug combination for tuberculosis. The enzyme, which uses the energy provided by the complexes of the ETC described, is the F_1F_0 ATP synthase, by catalyzing the condensation of adenosine-diphosphate (ADP) and inorganic phosphate (P_i) to ATP. This engine is essential for *Mtb* growth (Sasseti et al., 2003). A special feature of the mycobacterial F-ATP synthase is its inability to establish a significant proton gradient during ATP hydrolysis, and its latent ATPase activity, to prevent energy waste and to control the membrane potential (Haagsma et al., 2010; Hotra et al., 2016). The Grüber lab describes that unique epitopes of mycobacterial F_1F_0 -ATP synthase subunits contribute to the regulation of the low ATPase activity and shed light to the understanding of mechanisms, regulation and structural differences of the mycobacterial F_1F_0 -ATP synthase compared to other bacterial and eukaryotic engines. These novel insights provide the basis for the design of new compounds targeting this engine and even novel regimens for multidrug resistant TB.

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Figure legends

Figure 1. The TB drug development pipeline based on the information provided by the Working Group on New TB Drugs (www.newtbdrugs.org).

Figure 2. Key players and TB drug targets within the mycobacterial cell. The ETC comprises the NDH- and SDH complexes accepting the electrons from the donors NADH and succinate, respectively. Together with the *cyt- bc_1 :aa₃* and the *cyt-bd* complexes, they generate the electrochemical gradient, essential for the synthesis of ATP within the F-ATP synthase. The hexameric shape (*green*) displays the mycobacterial type VII secretion system (T7SS) from *M. xenopi* (Beckham et al., 2017). The ribosome machinery (*blue-red*) translates the genetic code into the nascent polypeptide chains and became an important TB-drug in recent years. A so-called old TB-drug is PZA, which inhibits bacterial coenzyme A biosynthesis. PZA blocks this pathway by triggering degradation of its target aspartate decarboxylase, encoded by the gene *panD*. The recombinant aspartate decarboxylase (*yellow-pink*) exists as an octamer in solution (Gopal et al., 2017).

Figure 1

Figure 2