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ACCOUNTS of chemical research

Article

Cite This: Acc. Chem. Res. 2019, 52, 2340−2348

pubs.acs.org/accounts

Harnessing Biological Insight to Accelerate Tuberculosis Drug Discovery

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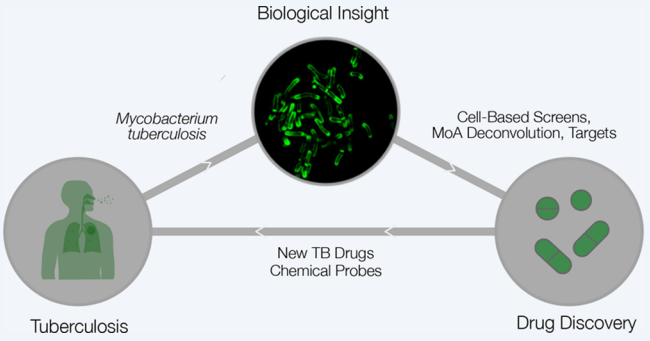
CONSPECTUS: Tuberculosis (TB) is the leading cause of mortality globally resulting from an infectious disease, killing almost 1.6 million people annually and accounting for approximately 30% of deaths attributed to antimicrobial resistance (AMR). This despite the widespread administration of a neonatal vaccine, and the availability of an effective combination drug therapy against the causative agent, *Mycobacterium tuberculosis* (Mtb). Instead, TB prevalence worldwide is characterized by high-burden regions in which co-epidemics, such as HIV, and social and economic factors, undermine efforts to control TB. These elements additionally ensure conditions that favor the emergence of drug-resistant Mtb strains, which further threaten prospects for future TB control.



To address this challenge, significant resources have been invested in developing a TB drug pipeline, an initiative given impetus by the recent regulatory approval of two new anti-TB drugs. However, both drugs have been reserved for drug-resistant disease, and the seeming inevitability of new resistance plus the recognized need to shorten the duration of chemotherapy demands continual replenishment of the pipeline with high-quality "hits" with novel mechanisms of action. This represents a massive challenge, which has been undermined by key gaps in our understanding of Mtb physiology and metabolism, especially during host infection. Whereas drug discovery for other bacterial infections can rely on predictive in vitro assays and animal models, for Mtb, inherent metabolic flexibility and uncertainties about the nutrients available to infecting bacilli in different host (micro)environments instead requires educated predictions or demonstrations of efficacy in animal models of arguable relevance to human disease. Even microbiological methods for enumeration of viable mycobacterial cells are fraught with complication.

Our research has focused on elucidating those aspects of mycobacterial metabolism that contribute to the robustness of the bacillus to host immunological defenses and applied antibiotics and that, possibly, drive the emergence of drug resistance. This work has identified a handful of metabolic pathways that appear vulnerable to antibiotic targeting. Those highlighted, here, include the inter-related functions of pantothenate and coenzyme A biosynthesis and recycling and nucleotide metabolism—the last of which reinforces our view that DNA metabolism constitutes an under-explored area for new TB drug development. Although nonessential functions have traditionally been deprioritized for antibiotic development, a common theme emerging from this work is that these very functions might represent attractive targets because of the potential to cripple mechanisms critical to bacillary survival under stress (for example, the Rel_{Mtb}-dependent stringent response) or to adaptability under unfavorable, potentially lethal, conditions including antibiotic therapy (for example, DnaE2-dependent SOS mutagenesis). The bar, however, is high: demonstrating convincingly the likely efficacy of this strategy will require innovative models of human TB disease.

In the concluding section, we focus on the need for improved techniques to elucidate mycobacterial metabolism during infection and its impact on disease outcomes. Here, we argue that developments in other fields suggest the potential to break through this barrier by harnessing chemical-biology approaches in tandem with the most advanced technologies. As researchers based in a high-burden country, we are impelled to continue participating in this important endeavor.

INTRODUCTION

Few diseases have impacted humanity more than tuberculosis (TB). An airborne, infectious disease, TB traces back thousands of years,[1] and its effects were well documented long before

Received: May 23, 2019
Published: July 30, 2019

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DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

Robert Koch isolated the causative agent, *Mycobacterium tuberculosis* (Mtb), in 1882. Today, socioeconomic upliftment and the availability of a neonatal vaccine and drug therapy have significantly reduced TB incidence in many countries, raising the prospect of its effective elimination in some regions.[2] However, disproportionately high TB rates in a handful of countries ensure that it remains a massive global health challenge: TB was recently declared the leading cause of death from a single infectious agent, with the World Health Organization (WHO) estimating approximately 1.6 million deaths in 2017 and around 1.7 billion latent Mtb infections.[3] The emergence and spread of strains of Mtb that are resistant to first- and second-line TB drugs compounds the problem, with WHO estimates suggesting half a million cases of multidrug resistant (MDR-TB) disease in 2017, almost 40 thousand of which were extensively drug resistant (XDR-TB). In fact, drug-resistant disease accounts for nearly one-third of deaths attributable to antimicrobial resistant (AMR) infections (https://www.tballiance.org/why-new-tb-drugs/antimicrobial-resistance), an often overlooked statistic, which urges a redoubling of efforts to control TB.[4]

The reasons for the stubborn persistence of TB as a major cause of morbidity and mortality worldwide are manifold: poverty, malnutrition and overcrowding are endemic in high-burden countries;[3] HIV/AIDS remains insufficiently managed, particularly in sub-Saharan Africa;[3] and TB treatment is lengthy, occasionally toxic, and so prone to default and the development of AMR.[2] Perhaps most important, though, is the resilience of the etiologic agent itself. Mtb is an exquisitely host-adapted pathogen that is adept at exploiting social ills and upheavals, deficiencies in host immunity, and vulnerabilities in treatment regimens.

TB chemotherapy depends heavily on a frontline drug combination, comprising isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol, that is effective at treating drug-susceptible disease but requires six months administration.[2] Drug-resistant TB presents an even greater challenge: MDR-TB regimens comprise second-line drugs that are associated with significant toxicity and can require up to two years to complete, while XDR-TB, which is resistant to both first- and second-line drugs, demands bespoke combinations of nonstandard antibiotics for extended periods, often under close clinical management.[5] There is some positive news, though: global efforts to address the urgent need for new antimycobacterial agents have gained momentum through the development of a TB drug pipeline.[6] Excitingly, two new TB drugs, delamanid[7] and bedaquiline,[8] have recently secured regulatory approval for use in the treatment of drug-resistant TB[9] with bedaquiline showing promise in treatment-shortening regimens for MDR-TB.[5] Moreover, there are other new and repurposed drugs and regimens in clinical development that offer some hope,[5,6] but the challenge remains formidable.

Since the release, 21 years ago, of the first complete genome sequence of Mtb,[10] major advances have been made in understanding mycobacterial metabolism and physiology in controlled experimental systems that allow wild-type and mutant strains to be observed in vitro, ex vivo, and in animal models ranging from mice to nonhuman primates. Moreover, by combining powerful genetic tools with developments in 'omics and imaging technologies, a biological platform has been established for early stage TB drug discovery. This platform comprises phenotypic screening for hit molecules with anti-TB activity, assays for elucidating compound mechanisms of action, and tools for the elucidation and validation of new drug targets.[6]

In this Account, we review our contributions to these endeavors, focusing on key insights and highlighting outstanding questions and how they might be tackled. We conclude by arguing that critical gaps in our understanding of the biology of Mtb during human infection and disease[11] must be addressed to develop transformative new therapies for TB.

THE TOOLKIT OF A PROFESSIONAL PATHOGEN

The tubercle bacillus is spread via aerosolized droplets from an infected individual and breathed into the lungs of an exposed individual. Within the alveolus, the organism encounters and is engulfed by tissue-resident macrophages and dendritic cells. In its primary host cell, the macrophage, Mtb resides within a phagosome, preventing fusion with lysosomes and surviving host immune defenses. A period of uncontrolled intracellular replication follows cellular uptake, during which bacillary population expansion and spread to surrounding cells occurs; simultaneously, there is drainage of infected cells to regional lymph nodes and an eventual activation of adaptive immunity. Pro-inflammatory responses lead to the recruitment of additional immune cells, resulting in granuloma formation and control of bacterial replication. While this process can lead to bacillary clearance or even fail completely, in the vast majority of cases, immunity controls, but does not eliminate, the bacilli leading to a state known as latent TB infection (LTBI). During this period of LTBI, which can last for many years, Mtb is thought to persist in a state of metabolic quiescence and slowed or arrested replication. In a minority of hosts, immune failure and reinfection can ensue, which favors bacillary growth and leads to subclinical TB[2], followed by active, symptomatic disease. It is at this end-stage that patients typically present and are prescribed antitubercular therapy (Figure 1).

To complete successive cycles of transmission, infection, and disease, Mtb has evolved mechanisms that enable its survival under variable, often hostile conditions. Between hosts, the organism must endure desiccation, cold shock, and exposure to UV irradiation while retaining the ability for new infection; within hosts, it must withstand nutrient limitation, hypoxia, and

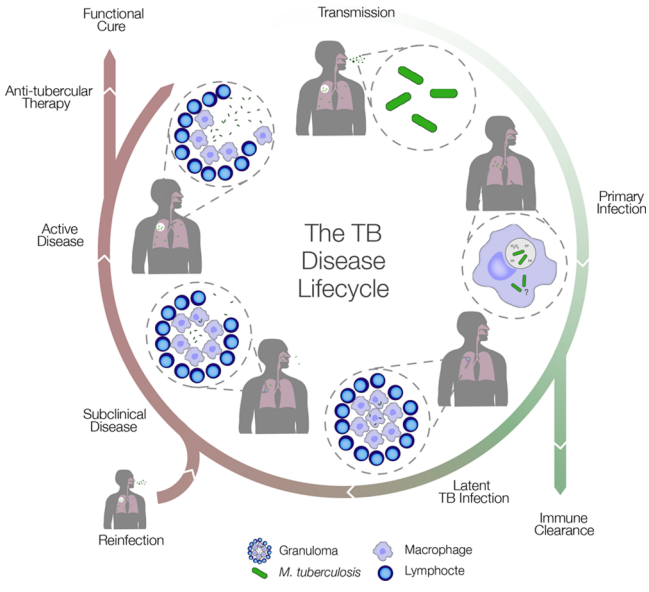

Figure 1. TB disease lifecycle. The schematic is based on information summarized in a number of reviews cited elsewhere in this Account.[2,11,12]

DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

other host-imposed stresses, such as acidification and exposure to reactive oxygen and nitrogen species while retaining pathogenicity.[2] The tempting association between long-term persistence, phenotypic drug tolerance, and the requirement for extended duration chemotherapy, and the ability of the bacillus to enter into a slow- or nonreplicating state under host-imposed stress provided a compelling rationale to investigate stress response mechanisms in Mtb and their implications for drug efficacy.[12]

Complementing the ability to adapt to variable and complex environments is the organism's remarkable metabolic flexibility, a feature evident in the apparent functional redundancy of certain metabolic pathways. For example, Mtb encodes two methionine synthases (MetE and MetH), two thymidylate synthases (ThyA and ThyX), two cytochrome oxidases (QcrCAB and CydAB), five resuscitation-promoting factors (RpfA-E), and an expanded complement of enzymes involved in early steps of molybdopterin biosynthesis.[13] These attributes provided the conceptual framework for our research into the consequences of mycobacterial physiology and metabolism for TB drug efficacy and the identification of potentially exploitable metabolic vulnerabilities in this organism.

Response and Adaptation to Nutritional Stress

Evidence that the hyperphosphorylated guanine nucleotides, ppGpp and pppGpp, mediate a global stress response in many other bacteria identified Rel_{Mtb} as an early focus for investigation. A bifunctional protein, Rel_{Mtb} comprises both RelA and SpoT domains, which catalyze the synthesis and hydrolysis of (p)pGpp, respectively.[14] Consistent with its predicted role in regulating the mycobacterial stringent response, functional inactivation of rel_{Mtb} had pleiotropic consequences for long-term survival under conditions of stress, pathogenicity in animal models of infection, and antibiotic susceptibility.[15,16] In a recent advance, Karakousis and co-workers have demonstrated[17] that functional inactivation of rel_{Mtb} potentiates the activity of the first-line TB drug, INH, under nutrient starvation in vitro and during chronic mouse infection. Under these conditions, the efficacy of this drug—usually bactericidal only against replicating wild-type Mtb owing to its disruption of mycolic acid biosynthesis—is notoriously poor. This validation of Rel_{Mtb} as a target for eliminating replication-restricted persister cells enabled the authors to identify a Rel_{Mtb} synthetase inhibitor from a high-throughput screen of a large compound library. Importantly, the inhibitor shows bactericidal activity against nutrient-starved Mtb and INH-potentiating activity. While promising, the treatment-shortening potential of Rel_{Mtb} inhibition suggested by these findings awaits confirmation in vivo.

Another consequence of prolonged nutritional stress in Mtb is progressive loss of culturability—that is, diminished (or eliminated) capacity to propagate in vitro on agar-solidified growth media. The enigmatic association between nutritional stress, culturability, and remodeling of the cell envelope—especially at the level of the peptidoglycan layer—focused our attention on the mycobacterial Rpfs. The five Rpfs in Mtb comprise a muralytic protein family implicated in cell wall remodeling through hydrolysis of the glycan backbone of peptidoglycan.[18] Rpfs are expressed in a growth-phase-dependent manner and were shown to be essential for resuscitation of Mtb from a state of apparent replicative inertia.[19] These studies yielded an *rpf*-null quintuple deletion mutant of Mtb that was subsequently used to demonstrate the presence in sputum samples from TB patients of both Rpf-dependent and -independent populations of differentially culturable tubercle bacilli.[20] This observation highlighted the limitations inherent in established microbiological techniques for estimating viable bacillary numbers and, by implication, responses to antibiotic treatment.[21]

Response and Adaptation to Genotoxic Stress

Comparative genomic analyses of clinical isolates have revealed two striking features of Mtb: (i) all drug resistance in this organism is associated with chromosomal mutations in the target or other genes associated with the antibiotic's mechanism of action (e.g., prodrug activators or efflux pumps) and (ii) the microevolution of the bacillus is driven exclusively by chromosomal rearrangements and point mutations.[1,22] These characteristics distinguish Mtb from most other bacterial pathogens and were especially intriguing when viewed in the light of an early bioinformatic analysis of the DNA repair gene repertoire of the H37Rv strain.[23] This analysis unexpectedly revealed that Mtb lacks the canonical systems for mismatch repair and DNA damage-induced (SOS) mutagenesis. Accumulating evidence that Mtb sustains significant DNA damage in vivo[22] heightened the conundrum, prompting our investigations into the potential association between genotoxic stress and chromosomal mutagenesis in mycobacteria. As summarized below, this work has contributed several insights, and the interested reader is referred to recent review articles for more detailed information on this topic.[24,25]

The DNA damage response in mycobacteria has been the subject of intensive investigation, primarily focused on the DNA damage-inducible SOS (*recA*/*lexA*-controlled) regulon. A surprising discovery was that SOS mutagenesis in mycobacteria is mediated by a C-family DNA polymerase IIIα subunit, DnaE2.[26] This finding repudiated the assumption that all C-family DNA polymerases are high-fidelity. Additionally, SOS mutagenesis in mycobacteria did not involve DinB1 or DinB2, the mycobacterial homologues of the Y-family translesion polymerases implicated in SOS mutagenesis in model organisms, such as *Escherichia coli*.[27] DnaE2 was subsequently shown to act together with two other proteins—the cryptic Y-family DNA polymerase, ImuB, and the RecA-like protein, ImuA′—in a mycobacterial "mutasome" which has been implicated in DNA damage tolerance and induced mutagenesis in mycobacteria.[28]

DNA Replication Fidelity: Mycobacteria Pave the Way to a New Paradigm

In a key study, Rock et al. established that the intrinsic 3′−5′-exonuclease within the PHP domain of the replicative DNA polymerase, DnaE1, provides the proofreading component of replication fidelity in mycobacteria.[29] This finding further illustrated the divergence of mycobacteria from classical paradigms of bacterial replication fidelity deduced from studies in *E. coli*, in which the proofreading function is served by the ε subunit of the DNA polymerase III core, a *dnaQ*-encoded 3′−5′-exonuclease.[25] Although Mtb and other mycobacteria contain *dnaQ* homologues, their cellular functions remain obscure. These authors showed that genetic inactivation of the PHP domain proofreader in *Mycobacterium smegmatis* DnaE1 rendered the bacillus susceptible to growth inhibition by ara-A, a chain-terminating adenosine analogue which is inactive against the wild-type strain.[29] This validation of the proofreading domain of DnaE1 as an adjunctive drug target in Mtb coincided with a report identifying DnaE as the target of the

DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

natural product, nargenicin, in *Staphylococcus aureus* and other bacteria.[30] When considered together with the discovery of the β-clamp (DnaN) as the target of griselimycin in mycobacteria,[31] these findings point to DNA replication as an underexplored target space for TB drug discovery[32] (Figure 2).

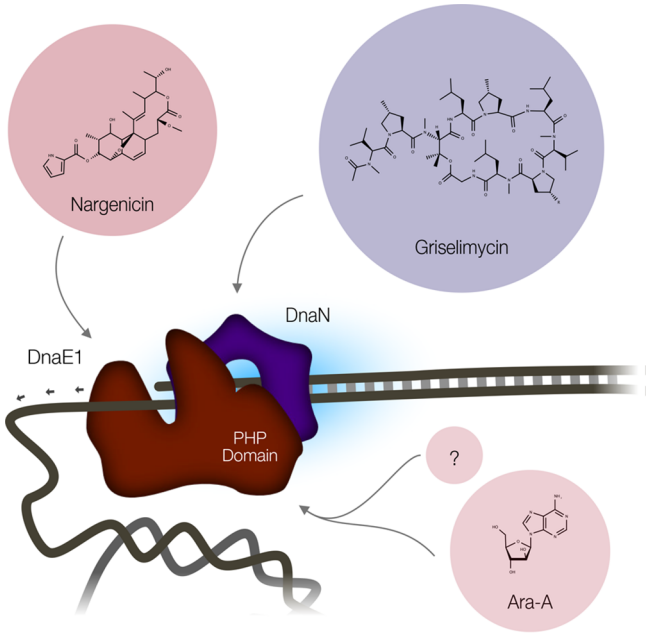

Figure 2. Targeting DNA replication for TB drug development.

The examples of noncanonical proofreading and SOS mutagenesis systems, which together with homologous end-joining (NHEJ), were discovered in mycobacteria, were subsequently shown to be widely distributed across the bacterial domain.[22] Mycobacteria have also served as vehicle for the discovery of a noncanonical system for postreplicative mismatch repair involving, in this case, the mismatch-specific endonuclease, NucS.[33] Notably, subsequent work in the related actinomycete, *Corynebacterium glutamicum*, has shown that the NucS homodimer must interact functionally with the β-clamp.[33] Therefore, assuming that this system operates similarly in mycobacteria, griselimycin would be expected to block mismatch repair, as well as DNA replication and SOS mutagenesis by precluding NucS, the replisome, and the mutasome from binding to the β-clamp (Figure 2). Again, this suggests a polypharmacologic mechanism of action for griselimycin, an interpretation perhaps consistent with the very low frequency of resistance mediated by the high-cost amplification of the *dnaN* chromosomal locus.[31] This notion further supports the prioritization of DNA metabolism as an area for TB drug discovery.

IDENTIFYING METABOLIC VULNERABILITIES IN A METABOLICALLY FLEXIBLE PATHOGEN

Flexibility in Nucleotide Metabolism

DNA replication, repair and mutagenesis are necessarily linked to nucleotide metabolism, another metabolic process that has been studied extensively in mycobacteria.[34] Within the family of enzymes involved in nucleotide metabolism, ribonucleotide reductases (RNRs) represent particularly attractive antibacterial targets given their centrality in enabling DNA synthesis and in determining mutation rates through their essential role in maintaining dNTP pool levels.[35] However, the expanded complement of RNR-encoding genes in Mtb presents a potential confounder to the pursuit of RNRs as TB drug targets.[36]

Our work revealed that the class Ib RNR, NrdEF2, is essential for the growth of Mtb in vitro.[37,38] We also showed that expression of *nrdF2* is regulated by NrdR.[36] Inactivation of this redox-sensing regulator under oxidative stress triggers induction of *nrdF2* as well as *nrdH*, which encodes the glutaredoxin involved in the reduction of NrdF2. This mechanism provides a means of ensuring that dNTP supply is closely linked to the need for DNA synthesis under these conditions. In contrast, the alternative class Ib small subunit, NrdF1, is dispensable for growth and survival of Mtb in vitro and in a mouse infection model even though this subunit forms a biochemically functional class Ib enzyme in association with NrdE.[39] Likewise, the putative vitamin B_{12}-dependent class II RNR, NrdZ,[37] which is part of the so-called DosRST "dormancy" regulon[40] induced in Mtb in response to hypoxia, NO, and CO, is not required for Mtb pathogenicity in mice.[37] However, in this case, the lack of an observable phenotype might result from an insufficiency of the vitamin B_{12} cofactor.[41]

The RNR example highlights key knowledge gaps: for example, do NrdEF1 and NrdZ contribute to maintaining dNTP pools in human infection under conditions, which current in vitro and in vivo models fail to recapitulate? To what extent does vitamin B_{12} availability dictate differential RNR utilization? Here, our work on the biosynthesis and function of molybdenum cofactor in Mtb might be instructive: a *mobA* mutant incapable of producing *bis*-molybdopterin guanine dinucleotide, the cofactor required by NarGHI nitrate reductase and other enzymes in Mtb, was defective for persistence in the lungs of guinea pigs, but not C57BL/6 mice.[42] This perhaps provides another example of the limitations inherent in the standard experimental models used to phenotype mutants in Mtb metabolism, a theme echoed below.

Lessons in Metabolic Vulnerability from De Novo Biosynthesis and Salvage of Purines

Phenotypic screening of a compound library against Mtb identified a reasonably potent molecule belonging to the 1-(5-isoquinolinesulfonyl) homopiperazine (Fasudil) scaffold, a known inhibitor of Rho-associated protein kinase. Elucidation of the mechanism of action of this compound identified its mycobacterial target as GuaB2, one of three members of the inosine 5′-monophosphate dehydrogenase (IMDPH) family encoded in the Mtb genome.[43] IMPDH catalyzes the NAD^+-dependent conversion of inosine 5′-monophosphate to xanthosine monophosphate (XMP), the first committed step in the biosynthesis of guanine nucleotides, which are formed from guanosine monophosphate (GMP), the biosynthetic product of XMP in the de novo purine biosynthesis pathway. Guanosine nucleotides fulfill multiple cellular functions, being involved in nucleic acid synthesis, cell envelope biogenesis, protein synthesis, cofactor biosynthesis, and the stringent response. As such, inhibition of guanosine nucleotide biosynthesis was predicted to be catastrophic for the bacillus. Consistent with this expectation, a *guaB2*-depleted conditional mutant (or hypomorph) was rapidly killed in vitro, in macrophages, and in a mouse model of infection.[43]

On its own, this result appeared to support the identification of GuaB2 as a promising new drug target. However, a potential codicil remained: in addition to being able to synthesize

DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

guanosine nucleotides de novo, Mtb can also salvage purines, thus enabling the production of GMP directly from guanine through the action of hypoxanthine-guanine phosphoribosyltransferase. Since this capacity might render GuaB2 nonessential by metabolic bypass, it was critical to assess the functionality of the predicted salvage pathway in Mtb. By monitoring the ability of exogenous guanine supplement to rescue Mtb from the lethal effects of genetic depletion or chemical inhibition of GuaB2 in vitro, uptake and assimilation of guanine was found to be relatively inefficient in Mtb, with rescue from lethality requiring the provision of a high concentration of this metabolite in the culture medium ($\geq$100 μM). Silencing of *guaB2* at the point of infection, which was enabled by feeding the mice a doxycycline (doxy) inducer prior to infection, completely blocked Mtb replication and led to rapid clearance of infection. This result suggested that Mtb did not have access to enough host-derived guanine to rescue GuaB2 deficiency by salvage, at least in that model, and led to the conclusion that GuaB2 was a "vulnerable and bactericidal" TB drug target (Figure 3).

Contrasting this interpretation, a concurrent study identifying GuaB2 as the target of an indazole sulfonamide series reached a fundamentally different conclusion—namely, that GuaB2 was "essential but not vulnerable".[44] In this study, Park et al.[44] found that treatment of mice infected by wild-type Mtb with an IMPDH inhibitor from this series failed to clear the infection. This result was partially attributed to difficulties in achieving sustained drug exposures in vivo at concentrations required to confer a bactericidal effect. However, further investigation revealed that guanine was present at low millimolar concentrations in normal and diseased tissue from Mtb-infected rabbits and chronically infected TB patients, but was found at levels at least 1 order of magnitude lower in the organs of healthy (uninfected) mice. Since guanine levels detected in human lung tissue were sufficiently high to enable metabolic salvage based on the efficiency of guanine uptake by Mtb, as determined in vitro, these authors concluded that GuaB2 is essential but not vulnerable, regardless of drug efficacy (Figure 3).

On the basis of these results, GuaB2 has been deprioritized as a TB drug target. Furthermore, by highlighting major differences in tissue metabolite levels between host species, this work has wider implications for the validation of other metabolic targets where potential mechanisms for bypass or metabolite scavenging exist. Examples include targets involved in amino acid, vitamin, or cofactor biosynthesis. Critical questions, therefore, remain, which are of profound relevance to new TB drug development. For example, are salvage or scavenging pathways functional in Mtb during infection and disease of a human host? How does the metabolic state of Mtb affect its ability to sense, transport, and assimilate metabolites? Does Mtb enjoy unrestricted access to host-derived metabolites in all host environments or is access (differentially) limited?

Lessons in Target Vulnerability from Pantothenate and Coenzyme A Biosynthesis

Pantothenate (vitamin B5) biosynthesis gained early attention as a potential target for TB drug discovery based on pioneering work by Jacobs and co-workers on the development of pantothenate auxotrophs of Mtb as rationally attenuated vaccine candidates and as tool strains that could be used under biosafety level 2 containment.[45] A key attenuating mutation in these strains is the Δ*panCD* deletion, which renders Mtb auxotrophic for this vitamin and, consequently, incapable of producing the essential cofactor, coenzyme A (CoA). That work laid the foundation for drug discovery programs that were aimed at identifying inhibitors of Mtb pantothenate synthase (PanC) and the essential pantothenate kinase, CoaA (PanK). However, those programs met with very limited success, and provided a timely (and costly) reminder of the serious challenges inherent in target-based approaches to developing potent small-molecule enzyme inhibitors that are also able to traverse the Mtb cell

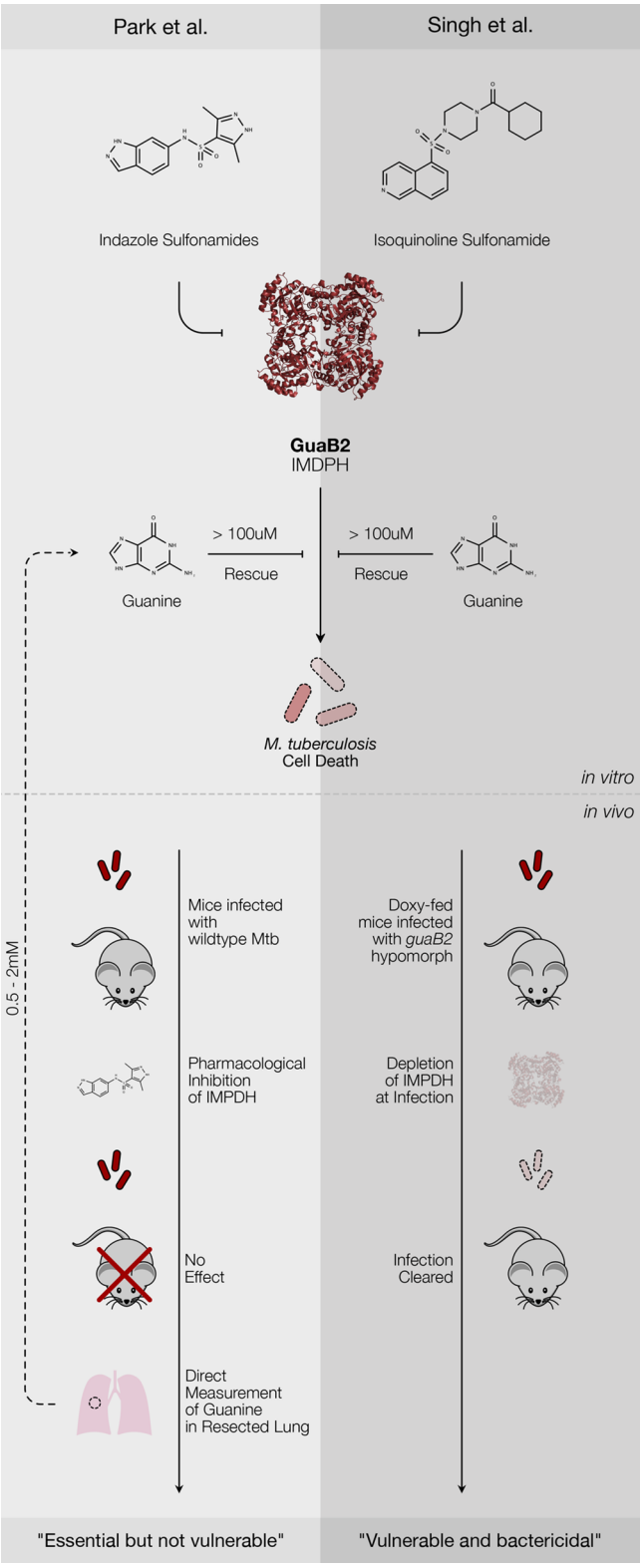

Figure 3. A lesson in metabolic vulnerability.

DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

envelope, evade metabolism and efflux, engage their target in whole mycobacterial cells, and inhibit bacillary growth. Encouraged by advances in other areas of antimicrobial drug discovery, and by the development of tools to construct conditional knockdown mutants of Mtb, we developed a *panC* hypomorph for use in target-based whole-cell screens for PanC inhibitors.[46] By enabling growth inhibition to be monitored as a function of the degree of target knockdown, hypomorphs simultaneously provide a means of addressing another major confounder of target-led approaches to drug discovery, namely, target vulnerability—that is, the extent to which a target's function must be blocked to inhibit growth. Using this approach, PanC was found to be relatively invulnerable and, hence, an unattractive target, requiring >95% knockdown for complete suppression of Mtb growth.[47]

By applying this approach at a pathway level, we discovered that the consequences of silencing *coaBC* distinguished this gene from others involved in pantothenate and CoA biosynthesis: whereas silencing of *panB*, *panC*, and *coaE* was bacteriostatic, *coaBC* silencing was bactericidal in Mtb in vitro for reasons that remain unclear.[47] Furthermore, whether initiated at the time of infection or during acute or chronic stage disease in vivo, *coaBC* silencing was likewise bactericidal for Mtb in C57BL/6 mice. This result confirmed that, in this model, Mtb does not have access to sufficient pantetheine to render CoaBC nonessential by metabolic bypass.[47] On the basis of this observation, subsequent drug discovery efforts have been directed toward CoaBC as a preferred target in the pathway.

In a parallel development, aspartate decarboxylase (PanD) has emerged as another target of interest based on studies on the mechanism of action of PZA. There, PanD was identified as the target of pyrazinoic acid (POA)[48]—the active metabolite of PZA—with results indicating that POA binding to PanD inhibits CoA biosynthesis.[49] Although more work is required, the picture emerging is of a potential association between stress physiology, the inhibition of CoA biosynthesis and the mechanism of action of PZA/POA in Mtb. This possibility warrants further investigation given the critical, albeit poorly understood, role of PZA in shortening treatment.[50]

The pleiotropic consequences of CoA depletion in Mtb are likely to include impaired transfer of the 4′-phosphopantetheine (Ppt) group of CoA to acyl carrier proteins (ACPs) by the two essential Ppt transferases, PptT and AcpS. In an exciting new advance, Ballinger et al. have identified an amidino-urea inhibitor of PptT, the phosphopantetheinyl transferase responsible for the generation of >20 holo-ACPs involved in the production of mycolic acids, virulence lipids, and mycobactins, thereby crippling multiple essential cellular functions.[51] In a fascinating twist, the authors identified a hydrolase, PptH, which reverses the action of PptT in Mtb, effectively synergizing with the amidino-urea inhibitor. The discovery raises important questions about the role of this nonessential hydrolase in Mtb: does PptH regulate the synthesis of lipids or polyketides by controlling holo-ACP levels, or is its primary function in CoA metabolism?[52] These questions notwithstanding, this work has strongly reinforced the view of CoA metabolism as a promising target space for TB drug discovery.

FUTURE OUTLOOK

Many of the questions raised in this review highlight our poor understanding of Mtb metabolism within the human host. Laboratory investigations have provided numerous insights into the metabolic flexibility of the bacillus,[13] yet pathway function, metabolite accessibility and regulation of assimilation during host disease remain opaque. Information on target vulnerability has been gleaned primarily from studying the impact of genetic inhibition (conditional knockdown) of a target on Mtb survival in vitro, in macrophages, and in mouse models. While relative vulnerabilities inferred in this way can be useful for target prioritization, target depletion is an imperfect surrogate for chemical inhibition, particularly for inhibitors that bind irreversibly or induce conformational changes in the target. Differences in target vulnerability, when transitioning from in vitro to in vivo conditions or between model organisms, also highlight the limitations of these approximations of human disease. Together, these limitations strongly reinforce the need for tractable approaches to study Mtb metabolism within the host—an advance critical to the development of improved models and, ultimately, therapeutics.

The inaccessibility of the human lung necessarily limits direct studies of in situ infection to autopsy studies, biopsies, or lung resections (a treatment modality in extreme cases of intransigent disease). While clinical samples are limited—both in their availability and their capacity to provide temporal insight into disease progression—comprehensive descriptions of metabolite profiles in these materials or from nonhuman primate samples[53] would be invaluable in the development of improved models to assess the attractiveness of metabolism-targeting antitubercular agents. Improved models should reflect the metabolite milieu of infection, as well as the different metabolic states that Mtb adopts. Currently, however, simultaneous, absolute and non-targeted quantification of metabolites through mass spectrometry poses a significant technical challenge.[54] That said, targeted approaches to metabolite quantitation have found some utility, primarily in the quest for diagnostic biomarkers,[55] and their systematic application presents a feasible opportunity to construct higher-resolution metabolic landscapes of TB disease pathology.

Though distal to the disease locus, bacilli isolated from sputum or even aerosols represent a more accessible alternative to tissue resection and so offer the possibility to study microbial physiology directly.[56] To gain insight into microbial state within the host, however, analytic techniques must be rendered culture-independent, a critical development that requires near-single-cell sensitivity. The application of single-cell transcriptomic approaches to mycobacteria appears imminent[57] and promises to provide important insights into mycobacterial state during infection, and the interaction between the pathogen and the host. A caveat here is that the correlation between transcriptional profile and metabolic state is often limited. More promising, therefore, is the potential to adapt single-cell metabolomic techniques used for studying bacterial heterogeneity[58] to sputum-derived bacilli, with the aim of providing information about metabolic status, albeit qualitative and for a small number of metabolites.

The development of chemical probes based on synthetic, functionalized derivatives of known metabolites to target and label specific pathways offers an exciting new tool-set for studying microbial physiology, with potential application to both disease biology and drug discovery.[59,60] When combined with either fluorescent or radio labels, these probes might be utilized for direct visualization of metabolite incorporation and bacillary metabolic activity in sputum or even in the human host. Here, the potential to complement existing approaches to imaging disease biology that have utilized radiolabeled glucose[61] (and therefore reflect host metabolism) with novel, specific

DOI: 10.1021/acs.accounts.9b00275
Acc. Chem. Res. 2019, 52, 2340−2348

bacillary probes[62] appears compelling. It also hints at the exciting convergence of in situ measures of metabolism—imaging, metabolomics, and sequencing—not a fanciful leap given recent examples from cancer biology that have demonstrated the power of multimodal analytics to gain direct insight into disease biology.[63]

In conclusion, the TB field seems poised to harness multiple complementary approaches to obtain key insights into the mycobacterial metabolic pathways active in different lesions, their impact on disease outcomes and, critically, how they might be exploited for improved anti-TB drugs and regimens. As an infectious agent, Mtb has evolved to cope with complex and hostile environments—our challenge remains to understand this complexity to develop simple interventions.

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ACKNOWLEDGMENTS

Research in the Molecular Mycobacteriology Research Unit has been enabled by generous support from the South African Medical Research Council, the Department of Science and Technology and National Research Foundation of South Africa, a Senior International Research Scholars grant from the HHMI (V.M.), the University of Cape Town, the University of the Witwatersrand, the National Health Laboratory Service, the Bill and Melinda Gates Foundation (via subaward from the FNIH), the European Community's Seventh Framework program (grant 260872), the US National Institute for Child Health and Human Development (NICHD, UO1HD085531-02), the US National Institute for Allergy and Infectious Diseases (NIAID, R21AI115993), and the Research Council of Norway. We thank all members of the MMRU, past and present, along with our many collaborators, for their enormous contributions, and apologize to those authors whose work was not cited owing to space limitations.

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