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BMCL Digest

A medicinal chemists' guide to the unique difficulties of lead optimization for tuberculosis

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ABSTRACT

Tuberculosis is a bacterial disease that predominantly affects the lungs and results in extensive tissue pathology. This pathology contributes to the complexity of drug development as it presents discrete microenvironments within which the bacterium resides, often under conditions where replication is limited and intrinsic drug susceptibility is low. This consolidated pathology also results in impaired vascularization that limits access of potential lead molecules to the site of infection. Translating these considerations into a target-product profile to guide lead optimization programs involves implementing unique in vitro and in vivo assays to maximize the likelihood of developing clinically meaningful candidates.

Globally tuberculosis (TB) ranks as the tenth most common cause of death in the world, killing 18 of every 100,000 humans annually, placing it second only to HIV/AIDS among lethal infectious diseases.[1] An astounding 2 million people perish every year despite the existence of curative chemotherapy. TB is widely viewed as a disease of the developing world and death rates are often attributed to failure of overburdened public health systems to deliver appropriate care to infected individuals.[2] While this is certainly a contributing factor, it is worth noting that standard TB treatment for simple, uncomplicated disease consists of combination chemotherapy with four agents (isoniazid, rifampicin, pyrazinamide and ethambutol) for two months followed by an additional four months of isoniazid and rifampicin.[3] The logistics of delivering such complex regimens, and ensuring patient compliance with the full course of chemotherapy, challenge the resources of the public health sector even in most developed countries.[4] Inevitably as this complex therapy is administered under less than ideal conditions, drug-resistant forms of the disease have become increasingly common, leading the World Health Organization to declare multidrug-resistant (MDR) TB a global threat.[5] While it is easy to ascribe this to a failure of governments to mobilize sufficient resources to deliver curative treatment, it is equally reasonable to blame the lead optimization programs that resulted in candidates that require such extended durations of treatment.

The current investment in TB drug discovery is unprecedented and last year saw the approval of the first new agent for the treatment of TB in three decades.[6] Many other agents are in preclinical development and clinical trial results are beginning to emerge for agents that were developed using current thinking about criteria adopted in lead optimization. How successful has our current paradigm been in predicting the results emerging from these clinical trials? How might we amend our strategies to improve our chances of actually shortening therapy? In this *Digest* we will review current assays used in lead optimization, how contemporary agents fare in these and other more exploratory assays, and how these results square with the emerging clinical trial data. We will focus this manuscript on TB-specific considerations and not attempt to cover all aspects of lead optimization.

The current paradigm: According to a recent survey of 25 institutions involved in preclinical drug development for TB, most current strategies largely center on assessing minimal inhibitory concentrations (MIC), which is typically defined as the concentration of an agent that inhibits growth of 99% of a standardized inoculum of a laboratory strain of *Mycobacterium tuberculosis* (*Mtb*).[7] Most laboratories use standard rich liquid growth media containing glucose, glycerol, a detergent (Tween-80) and bovine serum albumin, and score growth visually. Most investigators utilize minimum bactericidal activity (MBC) measurements to assess hit compounds, where 'cidal' activity is enumerated by measuring the drug concentration required to effect a 1 or 2 log reduction in bacterial counts after a defined exposure period. Other assays such as activity of compounds against TB-infected macrophages or activity under non-replicating conditions are rarely employed and seldom used to prioritize leads.

Potent compounds are often developed based solely on MIC/MBC criteria and subjected to optimization for traditional in vitro

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ADME parameters with the goal of achieving proof of concept in an animal model. Although historically other species such as Guinea pigs were employed, currently nearly all groups working in this area utilize mouse models of disease and then progress directly to clinical development. While this simple and linear progression strategy—often referred to as the 'three Ms' for MIC, Mouse and Man (Fig. 1)—is attractive in its simplicity, it ignores many of the important subtleties of the disease and promises to deliver only more of the same kind of agents we've unearthed in the past. Moving beyond this to a regimen that would potentially really shorten treatment duration and have a large public health impact requires a more deliberate and thoughtful approach. We'll consider the TB specific subtleties from two viewpoints; first from the perspective of the physiology of the pathogen and its impact on the intrinsic susceptibility of the bacterium to drug killing, and second from the perspective of the complex pathology generated by the host that is required to contain the infection within discrete lesions.

Factors associated with the pathogen's physiology and metabolism—The lifecycle of an infection-variable microenvironments: Even under conditions where the causative agent is maintained in a non-replicating state in vitro, a greater than 90% kill can be achieved by standard drugs in less than a week.[8] Why then does it require six months of multiple antibiotics to sterilize a patient's lungs? One potential explanation lies in the variety of microenvironments within which the bacterium resides during the infectious cycle. Initial infection results when Mtb is engulfed by pulmonary macrophages residing on the airway surface.[9] The bacilli arrest development of the phagosome within which they arrive, blocking the normal acidification of these degradative vacuoles at pH 6.3. For this initial round of replication, lung macrophages are largely unactivated and mount only a weak burst of reactive oxygen and nitrogen species, but as the innate immune system engages during subsequent rounds of infection and replication, these defensive host molecules become increasingly abundant.[10] During this initial period, the bacteria have access to glucose, probably triacylglycerides, and normal lung concentrations of oxygen to drive respiration. As immune activation proceeds, bacterial replication slows in response to increasing stress from the host resulting in a phenotypic form of drug tolerance where specific processes such as cell wall synthesis become much less sensitive to drug action.[11]

Immune activation triggers signaling pathways that recruit lymphocytes and more macrophages to the infection site that ultimately assemble into the complex structure known as a granuloma (vide supra). This multicellular structure serves to wall off and contain the bacteria, slowing their replication further and reducing their vulnerability to drug-mediated killing even further. The interior of the granuloma ultimately becomes necrotic (often referred to as 'caseous') and severely hypoxic with pO_2 values down to less than 2 mm Hg.[12] Under these conditions, normal aerobic respiration becomes impossible for the bacterium which reverses its

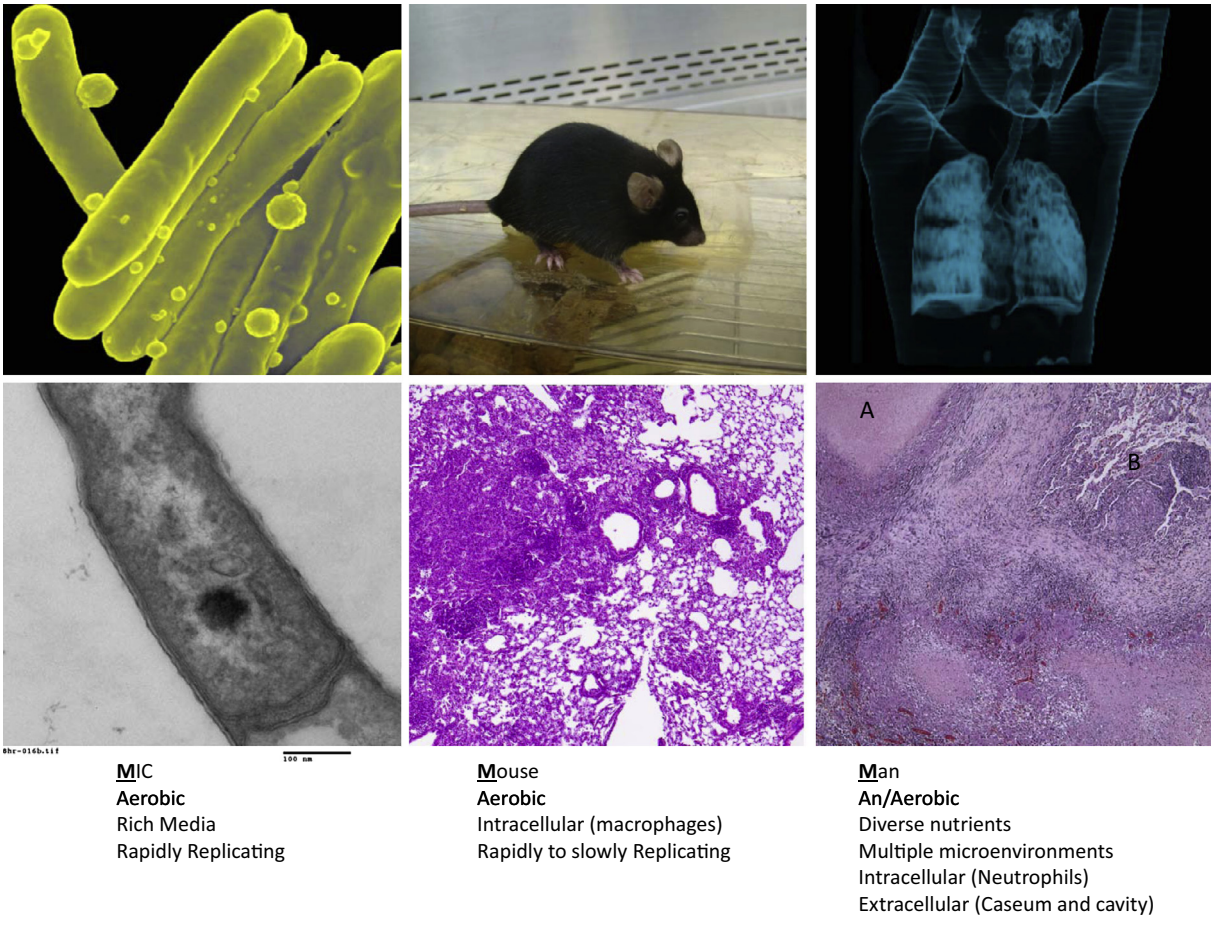

Figure 1. The traditional methodology for lead optimization is driven by the 'three Ms'. Left represents determination of the Minimum Inhibitory Concentration (MIC) with the top panel showing a scanning electron micrograph of *Mtb* and the bottom showing a transmission electron micrograph of the same culture. The center panels show the second M, mouse efficacy is typically assessed as both single agents and as combinations of agents. The lower panel shows a Hematoxylin and Eosin (H&E) stain of murine TB lesions which are typically not well organized discrete structures but rather loose aggregates of infected cells. The right panels show the third M, man, with the top figure being a 3-dimensional reconstruction of Computed Tomography (CT) scans of a patient with extensive excess high-density lesions in the lungs. The bottom shows an H&E stain from a human lung resection surgery in which both acellular, caseous lesions (A) and necrotic lesions in the process of cavitation are present (B).