The open book of infectious diseases

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New classes of chemical compounds along with more efficient methods to identify drug targets have produced exciting developments in antituberculous antibiotics. Will the new drugs now entering clinical trials have an impact on treatment?

Poor William Stewart. This former US Surgeon General is unfairly credited with stating in 1967 that it was "time to close the book on infectious disease." Although it appears that he never said such a thing[1], the sentiment was certainly widely shared. For the past several decades, while microbial populations have been steadily accumulating drug resistance traits, there has been little interest in developing new antibacterial drugs. In fact, most 'new' antibiotics are merely derivatives of old compounds, and the technical innovations that have fueled drug development in other areas have been largely ignored in this arena. However, as reflected in three recent papers, many of these advancements are finally being harnessed to find more effective treatments for tuberculosis.

Why tuberculosis? Although current treatment can be effective if administered correctly, existing drugs must be taken for at least six months to prevent relapsing disease. Low treatment compliance contributes directly to the emergence of multidrug- and extensively drug–resistant (MDR and XDR) strains of *Mycobacterium tuberculosis*, which further limit the efficacy of standard therapy. To ensure compliance, the World Health Organization recommends that observers watch each dose be taken. The small cost of the medications themselves is dwarfed by the logistical expenses associated with maintaining clinics, drug supplies and observers. So treatments that are effective against MDR and XDR strains and shorten the required course of treatment could have an enormous impact on treatment success rates[2].

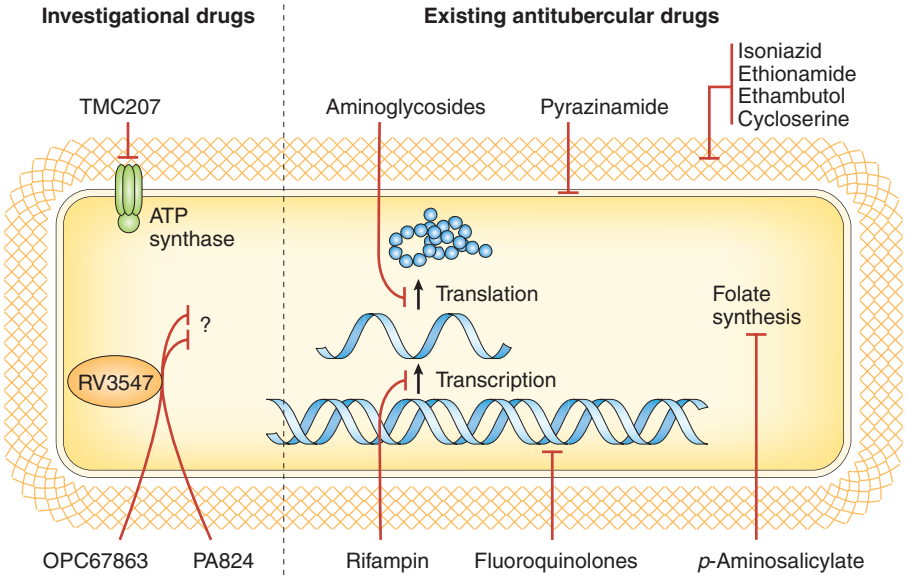

Figure 1 Mechanisms of action for current and investigational tuberculosis drugs. Targets of current drugs include cell wall synthesis (isoniazid, ethionamide, ethambutol and cycloserine), folate synthesis (*p*-aminosalicylate), transcription (rifampin), translation (aminoglycosides), DNA metabolism (fluoroquinolones) and the cell membrane (pyrazinamide). Three new compounds target other bacterial functions. TMC207 seems to inhibit the ATP synthase complex. OPC-67863 and PA-824 are prodrugs, the activation of which depends on the same cellular enzyme (Rv3547). The ultimate targets of these compounds remain unknown.

Why are current drugs not more effective? To paraphrase an old adage, you always get what you screen for, not necessarily what you want. Chemical screens for antimicrobials are generally designed to detect compounds that act quickly to block the growth of bacterial cultures. The resulting drugs kill bacteria rapidly *in vitro*, but are much less effective against infecting organisms that are presumably in a very different metabolic state. In addition, these screens strongly select for drugs that target relatively few bacterial functions, as all existing antibiotics inhibit only a handful of pathways (**Fig. 1**). So if we hope to generate new classes of antibiotics, we need new approaches. Three new potential drugs against tuberculosis may provide a blueprint for advancing this field.

These three newest antitubercular compounds belong to newly exploited chemical classes. This is most striking in the work of Andries and colleagues[3]. They synthesized a library of diarylquinoline compounds that are related to, but functionally distinct from, the quinolones and quinolines that are already used to treat several diseases. Using a traditional screen for compounds that inhibit

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bacterial growth, they isolated an extremely potent diarylquinoline: TMC207. Amazingly, by concentrating on chemicals with 'drug-like' structures, they found a compound that had very favorable pharmacokinetic properties and could be administered to humans without any further chemical modification.

The two other compounds, PA-824 and OPC-67863, are related nitroimidazoles. Compounds of this class act as prodrugs: they are inert until activated by cellular enzymes. Metronidazole is a nitroimidazole antibiotic that is only activated under low-oxygen conditions, and is therefore useful in treating anaerobic infections. Several other compounds of this class are activated in a similar manner and have been investigated for the imaging or treatment of tumors that create a similar hypoxic environment. In fact, the parent compound of PA-824 started as an investigational anticancer drug. What distinguishes PA-824 and OPC-67863 from other nitroimidazoles is their activity against aerobically growing *M. tuberculosis*.

Each of these three compounds was isolated using a different strategy. The parent compound of PA-824 was directly tested for activity against *M. tuberculosis*[4]. The diarylquinoline was identified in a high-throughput screen for its ability to kill the nonpathogenic, rapidly growing species *M. smegmatis*. Matsumoto *et al.* undertook the most complicated strategy[5]. They decided to focus on one of the unique properties of *Mycobacterium*, the requirement for mycolic acid synthesis to make an intact cell wall. In an amazing technical tour de force, this group spent twenty years searching for specific inhibitors of the synthesis of this lipid, ultimately identifying OPC-67683.

Clearly, the identification of an active compound is only the first step in drug development. The subsequent steps that are required to refine a lead compound into a drug critically rely on defining its mechanism of action, which is not a trivial endeavor. Consider the examples of isoniazid and *p*-aminosalicylate, two of the first drugs used to treat tuberculosis. Both were introduced in the 1950s, but the mechanisms by which they act were not elucidated until the past decade[6,7] and are still the subject of lively debate.

In the case of the new compounds, to rapidly identify their mechanism of action, two groups isolated antibiotic-resistant mutants and performed whole genome sequence analysis looking for resistance-associated polymorphisms. A mere 10 years ago, the *de novo* sequencing of a bacterial genome required hundreds of thousands of dollars and several years of work. Taking advantage of the ever increasing efficiency of sequencing technology, Andries *et al.* completely sequenced the genomes of four independent TMC207-resistant isolates and found common mutations in a single subunit of an ATP synthase. Further genetic experiments proved that an identified mutation was responsible for resistance to the drug. ATP synthase is certainly a plausible target, although further structural and biochemical work is required to prove this point.

Similarly, Manjunatha *et al.*[8] used a microarray-based resequencing method to rapidly identify polymorphisms associated with resistance to PA-824. In this case, however, the identified mutations did not define the target but, instead, identified an enzyme that is likely to be required for activation of the prodrug into the active molecule. Somewhat surprisingly, this same enzyme seems to be involved in the activation of both PA-824 and OPC-67863. It is therefore possible that both of these promising new drugs possess similar or even identical activities, although their precise targets remain elusive.

We won't know for sure if these new approaches produced better drugs until they are tested in humans, but with the existing evidence it seems likely that this will be the case. As these compounds have novel mechanisms of action, they kill MDR strains as efficiently as strains susceptible to existing drugs. Perhaps more significantly, the use of any of these three drugs in combination with standard antitubercular drugs results in significantly faster bacterial clearance in mouse models, indicating that they may be useful for shortening tuberculosis treatment by several weeks. So, if even one of these compounds succeeds in human trials, it could represent a significant breakthrough in tuberculosis treatment.

It is, however, too early to conclude that we will be closing the book on tuberculosis any time soon. Although a shorter course of therapy is desirable, only a marked shortening will have a real impact on how we provide care for those with the disease. In addition, we know that drug resistance to the new agents can be easily derived *in vitro*, and that evolution of new resistant strains is virtually inevitable once these drugs are used clinically. So any claim that tuberculosis will soon be vanquished is destined to reside alongside the famous quote that was never uttered by poor William Stewart.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Defining the 'survivasome' of *Mycobacterium tuberculosis*

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Identification of drug targets in *M. tuberculosis* is a challenge for bench science. High-throughput mutagenesis with transposons together with microarray-based genome and transcriptome profiling has begun to meet this challenge.

The worldwide mortality and morbidity due to tuberculosis remains excessive despite the availability of drugs that can stably cure it. Multiple factors underlie this paradox. One is treatment duration: regimens of multiple drugs that need to be taken for at least six months without interruption means low rates of treatment completion, particularly in poor nations. Another one is the emergence of multiply and extensively drug resistant (MDR and XDR) strains that are difficult, time-consuming and expensive to treat. The swift lethality of XDR tuberculosis reinforces the necessity of new drugs as global health priority[1].

The solution lies in the development of shortened and simplified tuberculosis drug regimens. Ideally, new drugs will inhibit new molecular targets, assuring activity against the growing burden of resistant microbes; act synergistically in multidrug cocktails; offer compatibility with highly active antiretroviral

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