Drug discovery for tuberculosis: Bottlenecks and path forward

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The drugs used in the present day six-month combination therapy for treating tuberculosis were discovered 40 years ago. The choice of the combination was derived based on results obtained following clinical trials with different permutations and combinations of the individual compounds. Unique features of tuberculosis as a disease and MTB as a microbe were discovered during these trials. The need for combination therapy and the problem of relapse were two major findings during these studies. Detailed scientific understanding of these aspects form the basis of current efforts in designing and developing a more efficient therapeutic regimen for the treatment of tuberculosis. Advances in genome biology and medicinal chemistry have influenced the new paradigm in drug discovery research. The mechanism driven modern process uses the knowledge of the target as the starting point for drug discovery. The basis of this approach is in identifying and validating MTB targets essential for survival in different environmental niches and physiological states. Several lacunae in our understanding of the biology of MTB present challenges in advancing chemical compounds through the lead identification and lead optimization stages of drug discovery.

The drug discovery path

THE success of a drug discovery programme for combating infectious diseases rests on three major factors, viz. identification of key elements contributing to the pathogenicity of the microbe, an understanding of the interplay of responses between the host and the microbe and most importantly the properties of the candidate chemical compound. The challenge to scientific research is in the identification of metabolic pathways in the pathogen, inhibition of which interferes with the survival of the microbe. Identifying and inhibiting specific pathway(s) that influence the survival of the microbe in different environments within the host represents the final hurdle. The fundamental aspect of this 'process' is the interdisciplinary nature of the research.

The drug discovery route has undergone major paradigm shifts in the last decade. The older version, the time tested empirical path is mainly driven by chemistry while the modern process, referred to as the mechanistic path, is largely driven by biological chemistry. This review discusses the major elements in each of these paths with specific reference to the discovery of novel antituberculosis drugs.

The empirical path in comparison with the mechanistic path

The empirical path starts with the identification of an 'active principle' consisting of either a pure compound or a mixture of structurally related compounds, with potent antimicrobial activity on bacterial cells *in vitro*. The active principle is usually identified either by chance or random screening. The compound is then tested in appropriate animal models of infection and those showing antimicrobial activity are selected and progressed for testing in humans. Historically, the most successful antimicrobial 'active principles' are natural products, with potent *in vitro* cidal activity against the microbe of interest. Many of the natural products being chemically complex, did not offer scope for modifications by the synthetic chemist. Hence a pharmacology-based approach was adopted to optimize and select compounds with increased potency. The successes with this approach triggered the era of large-scale investigations into microbial secondary metabolites and their biological importance resulting in the identification of a number of natural products with antimicrobial properties, a few of which could be successfully developed into potent antimicrobial drugs. Elucidation of the structures of some of these natural product drugs like penicillin, enabled chemists to make modifications on the scaffolds leading to the generation of novel chemical entities with different and desirable properties[1]. The key limitation in the empirical path was the lack of knowledge of metabolic pathways or the specific target within the microbe that was being inhibited by the chemical. Thus chemical modifications on the compounds were carried out empirically and compounds were chosen based only on their potency on whole bacterial cells. This resulted in the high rates of failure of compounds because of toxicity problems.

The modern drug discovery path or the 'mechanistic' path addresses this shortcoming at the very first step. The

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era of molecular biology and genome structures has enabled the identification of targets, which are specific to the microbe, absent or structurally different in the human host. Such targets serve as the pivotal points for drug discovery. This knowledge-based strategy facilitates a better choice of the screening systems used in the identification of inhibitory molecules. Quantitative biochemical assays to monitor the function of the target and its use to test libraries of synthetic compounds for the identification of potent inhibitory molecules are essential features of this route for drug discovery. Availability of the atomic structure of the target and the ability to design chemical molecules based on co-crystals of the target with the inhibitor have significantly improved the chances of identifying specific and selective antibacterial drugs.

Apart from the influence of molecular biology on the 'mechanistic' path, rapid strides in a number of technologies applicable to chemistry have also been incorporated in this approach to increase the chances of finding potent inhibitor molecules and to reduce the time taken for finding new chemical entities. Robotic liquid handling and signal reading instruments have enabled the testing of a large number of chemical compounds rapidly in very small reaction volumes[2]. Similarly, novel combinatorial and parallel synthetic chemistry combined with robotics allows the synthesis of a large number of compounds with diverse structures that support the large and fast screening process.

The present day therapeutic regimen recommended for the treatment of tuberculosis was established following the empirical route. Important features of the disease process and the pathogenicity of *M. tuberculosis* were revealed during the search for novel antituberculosis drugs following the empirical route. The following two sections highlight the evolution of the antituberculosis therapy and discuss them in the context of the modern drug discovery path.

Evolution of the antituberculosis therapy

The evolution of present day therapy for the treatment of tuberculosis and its success is an outcome of elegant clinical trials. Mitchison and co-workers[3] *et al.* have reviewed data obtained from various British MRC trials during the period 1946 to 1986. The major milestones leading to the establishment of the regimen were achieved in a step-wise fashion.

Need for combination therapy

The first drug introduced for the treatment of tuberculosis was Streptomycin (Sm) in 1946. Though the initial clinical results with streptomycin indicated a reduction in the number of tubercle bacilli in the sputum of the infected patient, the cure rates at the end of the treatment period were very poor because of the appearance of streptomycin-resistant mutants[4]. Para amino salicylic acid (PAS) when administered along with Sm led to definite improvement in cure rates. This was the first significant milestone in the evolution of anti-tuberculosis therapy, indicating the need of combination therapy for successful treatment[5]. Identification of Isoniazid (Inh) as a potent anti-tuberculosis agent in 1952 was a turning point in the treatment of tuberculosis. The remarkable *in vitro* potency of Inh encouraged its use as a monotherapeutic agent in humans. However, the high incidence of therapy failure due to the appearance of Inh-resistant strains was quickly recognized. Significant cure rates could be obtained on administering Inh in combination with PAS and Sm, thus strengthening and establishing the need for combination therapy[6]. This marked the beginning of triple drug therapy that not only showed tremendous improvement in preventing the emergence of drug resistance, but also cure of patients who had primary resistance, i.e. resistant to one of the drugs even at the onset of treatment. The major shortcoming that continued to hinder tuberculosis treatment was the prolonged duration of therapy and the high relapse rates: relapse occurred in about 60% of the patients treated for six months, 20% of those treated for one year and 5% treated for 2–3 years[7].

Introduction of domiciliary treatment

The potent bactericidal activity of Inh reduced the bacillary load in the sputum of infected patients rapidly, thus reducing the infectivity of these patients. Studies on the number of contacts who contracted the disease when patients were under the domiciliary course of treatment or at the hospital were found to be similar. The domiciliary treatment provided the first step towards the ability to treat larger groups of patients with specific antituberculosis drugs[8].

Relapse rates and the duration of therapy

Even after the introduction of Inh in combination with Sm and PAS, relapse rates remained high when patients were treated for a period less than a year. Relapse was defined as the reappearance of clinical symptoms with positive sputum cultures during a two-year follow-up period. In recent years, a true relapse case is being categorized by matching restriction fragment length polymorphism (RFLP) finger printing of pre-treatment cultures with remerging cultures[9]. Contrary to the previously observed treatment failure due to the appearance of resistant mutants, *M. tuberculosis* cultures isolated from relapsed patients were 'drug sensitive'[10]. Rifampicin (Rif) originally called Ansamycin A, a potent antimycobacterial compound was first recognized in 1965, and was quickly introduced into the combination regimen by early 1970s. Rif pro-

vided the major breakthrough in reducing the relapse rates to approximately 10% in a combination regimen administered for six months[11]. No clinical trials of Rif as monotherapy were performed even during its development phase, emphasizing the acceptance of combination therapy as a prerequisite for successful tuberculosis treatment. Ethambutol (Emb) replaced PAS because of its potency and reduced side effects[12]. Pyrazinamide (Pza) discovered at Squib Pharmaceuticals in their antituberculosis programme in 1970 and introduced into combination regimens[13] was found to have modest antituberculosis activity in the animal model but had a pronounced effect in reducing relapse rates. Thus, the introduction of Rif and Pza into the combination regimen along with Inh increased cure rates to > 90% when administered for a duration of six months. Attempts to reduce the duration of therapy to less than six months by varying the combination or the dosage of the individual components were not successful[14].

The recommended regimen today: Short course chemotherapy

The combination and duration is broadly classified into three categories. Category 1 consists of an initial phase of two months with thrice weekly doses of Inh, Rif, Pza and Emb. This is followed by a continuation phase of four months with thrice weekly doses of Inh and Rif, for newly smear-positive and seriously ill smear negative patients or seriously ill patients with extrapulmonary manifestation. Category 2 is recommended for previously treated smear-positive patients who come for re-treatment either due to relapse, failure or default. The regimen consists of an initial phase of two months with thrice weekly doses of Inh, Rif, Pza, Emb and Sm followed by a continuation phase of one month with thrice weekly doses of Inh, Rif, Emb with or without Pza. Category 3 is recommended for the treatment of new smear-negative patients and those with extra pulmonary manifestations, but not seriously ill. The combination consists of an initial phase of two months with thrice weekly doses of Inh, Rif and Pza followed by a continuation phase of four months with thrice weekly doses of Inh and Rif[15].

Salient features of the disease revealed by the drug regimen

Combination therapy. The introduction of combination therapy for the treatment of tuberculosis initially was to overcome the problem of the appearance of drug-resistant mutants during the course of treatment. Drug resistance in MTB has been shown to be the result of mutations in the targeted gene and not due to inactivating enzymes[16] or efflux pumps. The requirement of a combination therapy of more than two drugs is difficult to rationalize based on the mutability of MTB or the 'mutation frequency' to any particular drug. On the other hand, definitive studies by Mitchison and co-workers[17–19] have shown that the combination therapy is required not only to reduce the rate of appearance of drug-resistant mutants but the individual drugs in the combination contribute differentially to the eradication of the microbe. This is substantiated by the fact that varying efficiencies of cure are seen by changing the individual components of the combination[20,21].

Relapse: The treatment and relapse data compiled from various clinical trials are illustrated in Figure 1. At the end of two months therapy, bacilli cannot be cultured from the sputum, the patient's clinical symptoms have disappeared but the symptoms and the microbe reappear if treatment is discontinued at this stage. Six months of therapy is required to overcome the 'relapse' factor indicating the continued presence of a residual bacterial population termed 'persisters' during the treatment period. Sustained efforts over the last five decades using a continuously evolving array of techniques to establish the presence of live bacilli in lung tissue has had very limited success. Recent reports on the detection of specific mycobacterial mRNA from lesions of TB patients lends support to the persister hypothesis[22].

The mechanism-based drug discovery path

The process leading to the introduction of novel anti-infective drugs from the laboratory bench to the clinics can be broadly classified into two halves. The first dealing mostly with the microbe, termed Discovery or Pre-clinical research, involves activities in the laboratory up to the testing of the compound in relevant animal models and the second dealing mostly with the human host, is

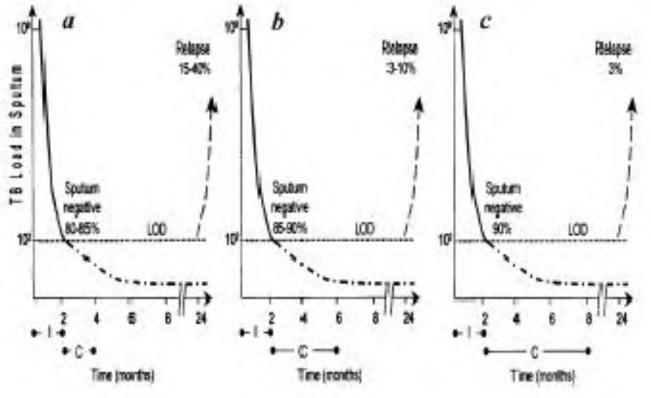

Figure 1. Schematic representation of percentage sputum conversion and relapse among tuberculosis patients following chemotherapy. ***a***, outcome from patients who received SHRZ during the 2-month initial phase (I) followed by H, HZ, HR or HRZ during the 2-month continuation phase (C); ***b***, Outcome from patients who received SHRZ during I phase followed by H, HZ, or HR during the 4-month C phase; ***c***, Outcome from patients who received SHRZ during I phase followed by H during the 6-month C phase. Data summarized from East and Central African/BMRC trials[55–57].

termed Development. Development activities are predominantly studies of the chemical compound on normal human host and infected patients. Activities during the discovery phase with specific reference to the discovery of anti-tuberculosis drugs are discussed below.

The discovery phase: The discovery phase involves two major goals: (i) Identification and validation of a target macromolecule in the microbe suitable for drug discovery, and (ii) Identification of a compound, which (a) inhibits the biological activity of the target macromolecule in the microbe resulting in cidal or static effect on the *in vitro* growth of the microbe, termed as lead identification process; (b) inhibits the biological activity of the target molecule in the microbe selectively in an animal model, resulting in the clearance of the microbe from the host tissues–lead optimization.

Identification and validation of targets suitable for antituberculosis drug discovery

The present era of genome sequencing has maximally benefited drug discovery in this effort. The criteria on which a target is evaluated are:

Specificity: Targets that are unique to mycobacteria, which are not represented in the human genome, are preferred for antimicrobial drug design. Using comparative genomics, targets are evaluated for (i) Presence or absence of a human/yeast homologue, (ii) Structural similarity of the primary sequence between the MTB target and the mammalian homologue, (iii) The role of the target enzyme in the metabolic pathways of the microbe as compared to that in the human host: for example, two different pathways in the human host, the *de novo* and the salvage pathways can generate nicotinamide adenine dinucleotide (NAD). MTB cells can generate NAD only by the *de novo* route. Inhibition of the *de novo* pathway by targeting the enzyme QAPRTase will be lethal to MTB while cells of the mammalian counterpart will remain unaffected[23].

Selectivity: Targets present only in MTB and absent in other bacteria are ideal as this increases the chances of identifying compounds that inhibit MTB but are inactive on soil bacteria and bacteria of the gut flora. Such selectivity avoids resistance priming in gut bacteria exposed to the drug during the extended treatment required for antituberculosis therapy. However, in practice strict adherence to such criteria results in restricting the spectrum of targets of mycobacteria to either those involved in cell wall biosynthesis or those implicated in virulence. Thus the compromise is to identify targets with limited homology among other microbes and rely upon 'permeability' of the chemical to enhance selectivity.

Environmental niches and their influence on target selection

MTB survives and multiplies in different environmental niches, extracellular, intracelluar within the vacuole of the macrophage, and in closed or open granulomas, the milieu being different in each case. These different environments alter the physiological and the metabolic processes within the microbe. The metabolic processes active within the microbe depend upon not only the nature of the substrates available as nutrients but also on the relative abundance of these[24]. Identification of pathways and targets essential for survival in the different environmental milieu would facilitate the selection of compounds with a broad spectrum of antituberculosis activity. Figure 2 depicts the hypothesized environmental changes that MTB cells can encounter during the course of the treatment. A striking feature of the graph is the influence of the environmental niche on the rate of killing of MTB cells with the present combination of drugs.

Growth phase and its influence on target choice

Almost all known antibacterial drugs efficiently kill the replicating microbe. Minimum inhibitory concentration (MIC) determinations are done on logarithmically growing bacteria in media optimized to support maximum growth. However, most of these antibacterial drugs have poor antibacterial activity on non-replicating bacteria[25] or

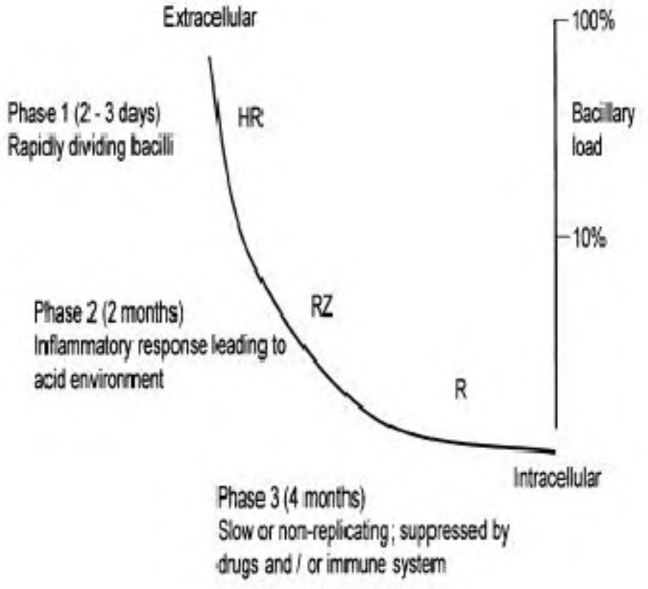

Figure 2. Schematic representation of the hypothesis postulated by Mitchison[58,59], with modifications. The drugs H, R and Z are shown at phases where they are most relevant in terms of their proposed role in curing tuberculosis. The intracellular state in the later phases of the treatment is emphasized in this model. The three phases and the corresponding drugs shown are not indicative of the initial and continuation phases of the short course chemotherapeutic regimen.

when tested on bacteria in stationary phase[26]. Selection of compounds with potent activity only against the rapidly growing phase will not translate to potent antituberculosis efficacy, as there are several lines of evidence to indicate that subpopulations of MTB cells in the host are in the non-growing/non-replicating phase: (i) In animal models used to study tuberculosis infection, bacterial numbers in the different organs remain constant once the immune system is activated[27]; (ii) The number of MTB cells found in closed granulomas is few and do not increase over time[22]. These arguments make it imperative that a preferred target for drug discovery against MTB should be essential for the survival of the organism in the non-growing phase also.

The drug discovery pathway and its influence on the choice of targets

The mechanism-based approach relies on identifying compounds that inhibit a specific function of the target. This makes it essential to develop an *in vitro* read-out to monitor the *in vivo* function. Targets that do not have an *in vitro* read-out are not preferred because a biochemical assay of the target function is a prerequisite to carry out high throughput screening, a method of choice for identifying inhibitors. Furthermore, chemistry-based optimization of compounds to improve potency is monitored using the biochemical assay and this inhibition in turn is correlated to the *in vitro* whole cell-killing potency of the compound.

Validation of targets

Knockout of genes of interest to determine essentiality under specific growth conditions can be accomplished in MTB also, though the process of validation takes longer than it would take in rapidly growing bacteria. A more reliable method to validate targets and grade the biological effects of inhibiting them is by determining the effects of reducing the levels of the active target protein on the survival fitness of the bacterial cell[28]. This allows the identification and selection of targets, inhibitors of which will have pronounced effects on the viability of the bacterial cell. These targets are referred to as 'vulnerable' targets[29,30]. Techniques to carry out these manipulations in Gram-negative and Gram-positive bacteria are well established. However, current knowledge on the regulatable systems that would allow such manipulations in mycobacteria is still rudimentary which limits the application of this concept to characterise mycobacterial targets. A number of biological parameters such as the mycobacterial cell wall, intracellular compartmentalization, slow growth, non-replicating phase, etc. are major barriers to the efficacy of antituberculosis drugs. The selection of highly 'vulnerable targets' increases the chances of translating *in vitro* inhibition of target function to potent antimycobacterial activity.

Lead identification

The major goal during this phase is the identification of 'chemicals' which inhibit the function of a validated target and correlation of the inhibition to the cidal activity on MTB cells. The establishment of such a correlation proves that the pathway through which the microbe is killed is a consequence of inhibition of the specific target of interest. Establishment of a direct relationship between the inhibition of the target enzyme and the mode of killing of the microbe provides confidence to inferring structure–activity relationships of the compound. Knowledge of these parameters also reduces the risk of toxicity of the molecules when tested on mammalian systems.

The general methods followed to find inhibitory compounds are:

- Throughput screening: Biochemical assays reflecting the function of the target protein are developed, miniaturized and made compatible with robotic dispensing of reagents and reading of end points. Large numbers of compounds, synthesized on a chemical diversity basis or purified from natural resources, are tested for their ability to inhibit the assay.
- Structure-based design of inhibitors: This approach can be employed as a support to the former where identified inhibitors are 'docked' into the crystal structure or co-crystallized and the deduced atomic structure is used to increase the affinity of the compound to the target. The alternate approach is the *de novo* design of chemical molecules based on the structure of the active site followed by optimization based on docking of these into the active site.

Chemical compounds confirmed to inhibit the target, referred to as 'hits' are analysed for their drug-like properties[31] and those amenable to chemical diversification are selected for further investigations. A series of related analogues of the original 'hit' are synthesized and tested in the biochemical assay. This iterative process, continues till a satisfactory inhibitory potency is obtained. These inhibitors are then tested for their antimicrobial potency on a panel of microbes. The mode of killing of the microbe by the selected compound needs to be established before further optimization of the compound begins. Compounds with an established mode of action are synthesized in larger scale and tested in suitable animal models to determine the pharmacokinetic parameters of the compound. Chemical structures with the desired potency in the *in vitro* biochemical assay and the antimicrobial assay, having pharmacokinetic properties compatible with the desired dosing regimen are desig-

nated as lead compounds. One of the features of this phase of drug discovery is that following the screening of a large number of compounds, a smaller number of structurally related classes or scaffolds meeting requisite criteria are identified.

An unanswered question is the specific activity of MTB enzymes. Are they similar to those studied in other bacteria or will they reflect the growth kinetics of the tubercle bacilli? The specific activity influences quantities of reagents required for enzyme assays during high throughput screening. An additional challenge is the 'mode of action' studies with MTB. Monitoring macromolecular synthesis using radioactive precursors is an established methodology for organisms like *E. coli* and *S. aureus*. However, there are several limitations of applying the same to MTB cells because of their stringent nutritional requirements.

Lead optimization

Lead compounds are subjected to synthetic chemistry guided by medicinal chemistry to improve the *in vivo* potency of the compound. The compounds are tested in the relevant animal disease model to establish proof of principle that inhibiting the specific target results in the inability of the microbe to cause disease *in vivo*. A series of analogues are designed to achieve the required *in vivo* efficacy, which is a combination of not only the antimicrobial potency of the compound but also the pharmacokinetic and pharmacodynamic properties of the chemical entity. The 'lead identification' phase focused on establishing a relationship between the inhibition potency in the biochemical assay and the *in vitro* cidal effect on the microbe. In the 'lead optimization' stage a relationship between the *in vitro* cidal effects on the microbe with the ability to eradicate the microbe from infected animal models is established.

Simultaneously the therapeutic index, a ratio of the toxic dose to the effective dose is closely monitored to achieve a safety profile acceptable to regulatory agencies. A compound meeting these criteria is nominated as a candidate drug and the compound begins its long journey in the development phase starting with detailed toxicity studies on a rodent and a non-rodent species. One of the major bottlenecks in tuberculosis drug discovery is encountered during the lead optimization phase. A key activity of the lead optimization phase is the iterative process of modifications of compounds based on data obtained from animal studies. The cumbersome and lengthy nature of the animal model for tuberculosis stifles this iterative process.

Hurdles in the antituberculosis drug discovery path

Three major hurdles, one a black hole, the second a bottleneck and the third an enigma, hinder rational identification and progression of compounds in the drug discovery route described above.

Sterilization: The black hole of TB drug discovery: Antibacterial potency is evaluated on logarithmically growing cells, and quantitatively represented in terms of their kill kinetics and MIC values. These parameters determine the efficacy of the compound to 'cure' the host of the infection. The cure is the result of the eradication of the pathogen from all tissues, a property referred to as 'sterilization'. However, an analysis of known antituberculosis drugs, suggests that neither the *in vitro* MIC potency nor the kill kinetics correlates with the ability to 'cure', as many compounds with potent MIC fail to eradicate the microbe from all host tissues. This discrepancy cannot be explained by the pharmacokinetic/pharmacodynamic properties of the compound.

The *in vivo* efficacy of antituberculosis drugs can be seen as two distinct properties: (i) Reducing bacterial numbers to achieve culture-negativity of sputum – bactericidal efficacy, and (ii) Eradication of the microbe from all tissues – sterilizing efficacy.

While achieving the 'culture negative sputum state' is a prerequisite for eradication, all antituberculosis compounds with this property may not be successful in sterilizing the host. Table 1 lists the number of drugs with potent MIC values against MTB known to be either used in clinics or attempted in clinical trials and compares them for their ability to sterilize. Only two of the frontline drugs used in the current regimen are 'sterilizing'. This anomaly can be explained by hypothesizing that most antituberculosis compounds have a cidal activity on many but not all the different fractions of the microbial population since heterogeneous physiological states for the microbial population exist in the host, Thus, even though compounds can be selected based on their rapid *in vitro* kill kinetics, their ability to sterilize has to be experimentally determined in animal models. In patients too, sterilization measured by the relapse factor, takes 18 months of post-treatment monitoring. Thus, there is an urgent need for rapid and reliable surrogate biological markers to predict the 'sterilization' property of antituberculosis drugs. The lack of definitive understanding of what constitutes the sterilizing potential of an antituberculosis compound is a prominent 'black hole' for antituberculosis drug discovery.

The bottleneck: Progressing compounds

Determining efficacy of potential lead compounds. Testing the bactericidal efficacy of an experimental compound in mice or the guinea pig aerosol infection model takes about 12 weeks, of which four weeks are for establishing the infection, 4–6 weeks of treatment followed by sacrifice and finally microbial monitoring which takes

an additional three weeks[32,33]. These models however, only test the efficacy of the compound against acute infection during which, bacilli are in either log or early stationary phase thus easily detected in the tissues of control animals. Testing the efficacy of a compound in any of the presently available animal models representing the dormant or non-replicating MTB, involves a large number of animals and a period of at least 24 to 36 months[34].

A progressive feature in antibacterial drug discovery is the ability to predict the efficacy of a class of compounds based on the pharmacokinetic-pharmacodynamic (PK/PD) relationships that include measurement of the *in vitro* kill kinetics and free plasma levels of the compound. The critical parameter is the recognition of the PK/PD driver for the particular class of compounds, i.e. whether it is time (%t > MIC), concentration (C_{max}/MIC) or exposure (AUC/MIC) dependent[35]. The efficacy of compounds within the same class can then be inferred based on the *in vitro* kill kinetics and free plasma levels of the compounds. Both these properties can be determined rapidly and the best compounds tested in the relevant animal infection model. These features circumvent the need for carrying out the evaluation of a large number of compounds in animal models for determining efficacy even though models for testing broad-spectrum anti-infectives are only a week in duration.

PK/PD parameters as predictors of in vivo antituberculosis efficacy

In comparison, limited information exists on the PK/PD relationships of drugs against MTB. Attempts to identify the PK/PD drivers based on pharmacokinetic and efficacy data available in the literature have been futile, since the dosing studies in experimental animals have been designed based on the dose regimens recommended for human patients. Typically PK/PD drivers are identified by dose fractionation studies that remove the interdependence between the 'time', 'concentration' and 'exposure' components. It is not currently known why the first-line anti-TB drugs are active when given as infrequently as twice weekly despite having PK parameters that would predict the need for multiple daily doses[36]. Most investigators use broth MIC and to a limited extent intracellular MIC as well as post-antibiotic effect[37] as *in vitro* parameters to correlate with bactericidal efficacy or the ability to achieve a culture-negative state of the sputum[38]. Recent PK/PD studies with Rif indicate that the overall exposure (AUC/MIC) is the main determinant for bactericidal efficacy in experimental airborne tuberculosis[39]. Based on this information, efficacy of new derivatives of the rifamycin structural class can be predicted based on the AUC/MIC achieved with the maximum tolerated dose. Similar studies with other frontline antituberculosis drugs correlating PK/PD parameters to bactericidal efficacy resulting in the culture-negative state in the animal model are in progress[40].

In contrast, sterilization, the key endpoint in TB chemotherapy does not have predictive *in vitro* parameters. For example, Pza has weak *in vitro* MIC on MTB at neutral pH, penetrates rapidly into macrophages[41], shows weak activity on intracellular bacteria[42] yet shows excellent sterilization *in vivo*[43]. In contrast, Inh is potent i*n vitro* with a MIC < 0.05 mg/l, but shows poor sterilization *in vivo*[43]. At this time very little is understood *vis-à-vis* the PK/PD drivers for sterilization and thus the choice of compounds to test for this property is merely empirical, based on their ability to successfully achieve total bacterial clearance at completion of therapy in experimental animals.

An enigma – the latent microbe

The terms latent, dormant and persister have often been used interchangeably, however, the first and second terms are synonymous to a physiological state of the microbe in individuals who have been infected but do not show clinical symptoms and thus did not receive treatment with

Table 1. Comparison of MIC with EBA and sterilizing potency of common tuberculosis drugs in the context of serum levels at standard therapeutic doses

Drug	MIC (μg/ml)*	C_{max} (μg/ml)*	C/MIC	$T_{1/2}$ (h)*	T > MIC (h)	EBA#	Sterilizing Potency¶
Amikacin	1	30	30	2.3	12	–	–
Ciprofloxacin	0.5	4	8	3.5	15	–	–
Ethambutol	1.5	5	3	3.3	6	+	–
Ethionamide	1.2	2	2	2.1	4	NA	–
Isoniazid	0.02	7	350	2	18	++++	–
Ofloxacin	0.5	10	20	7	35	–	–
PAS	1.5	7.5	5	1	2.5	NA	–
Pyrazinamide	6.2	65	10	18	36	–	+++
Rifampicin	0.2	10	50	3.5	28	+++	++++
Streptomycin	0.5	3	6	2.3	8	+	–
Thiacetazone	0.4	3.2	8	12.9	> 50	NA	–

NA, Not available.
*Data from ref. 50; #Data depicted from refs 51 to 54; ¶Data inferred from ref. 43; T, time; C, concentration.

antituberculosis drugs. The third term refers to a specific sub-population of MTB in the host selected during the course of the treatment of active tuberculosis. The underlying feature of the latent or the persister population is the inability to detect these microbes using conventional culture techniques, the failure of the immune system to eradicate them and the ability of this population to cause active tuberculosis in the host. The major limitation in identifying individuals with 'latent or dormant' tuberculosis is the lack of a proven diagnostic technique. The major technical barrier for discovering drugs specific for the treatment of latent tuberculosis is the lack of authenticated *in vitro*, *ex vivo* or disease models in animals.

An alternate strategy to identify compounds with potential activity on the latent microbe is to assume identical metabolic states for both the latent and the persister population, though the triggers or signals inducing this state may have been different. The fundamental characteristic of both these physiological states being the 'non-replicating microbe', compounds cidal on the non replicating microbe can be expected to be active on both the latent and persister forms of MTB.

Addressing latency and sterilization in the drug discovery path

The assumptions, which form the basis for the selection of compounds with the potential to 'sterilize', are: the microbe numbers are small; the microbe is in a nonreplicating state; the microbe is 'drug tolerant' as a consequence of its physiological state or environmental niche; the low numbers of the microbe is also an influence of the immune system of the individual.

Three of the four assumptions underscore the fact that keeping the numbers low in the host is a specific 'virulence' mechanism developed by the microbe to survive without damaging the host. The need to maintain low numbers could be regarded as a facet of the microbe's pathogenesis. As with most virulence mechanisms the microbe will evolve multiple paths to achieve this stage. A prediction of this hypothesis would be that the non-replicating cryptic stage could be achieved through a triggering of several independent signals and pathways. Incorporating this hypothesis into the drug discovery path involves testing of experimental compounds on MTB cells in the non-replicating state maintained under different physiological conditions, such as oxygen depletion[44–46], nutrient depletion[25], stationary phase[47], exposure to drugs[26], or immunity[48].

These models present environmental conditions, which trigger the microbe into a non-replicating phenotype. Recent studies have shown that gene expression in granulomas from tuberculosis patients is comparable to that seen in a variety of *in vitro* models[22]. Thus the metabolic pathways operating to maintain the non-replicating phenotype under any or all of these *in vitro* conditions can be assumed to be the same or similar to those operating in the non-replicating microbe *in vivo*. The challenge is in the identification of compounds active on the microbe in these models.

Conclusion: The new antituberculosis drug

The primary mandate for a new antituberculosis drug is to reduce the duration of therapy. Today tuberculosis treatment administered via DOTS can be extended to only 20 to 25% of all patients world wide, one of the constraints being the operating budget. It is estimated that a reduction in the duration to four months or less would enable DOTS to reach a significantly larger number of tuberculosis patients with the same budget and also have an impact on the control of the disease. This defines the minimum requirement in the improvement of therapy.

What properties of a new drug can influence the duration? An ideal drug would be a compound with cidal activity on replicating and non-replicating bacteria, in an extracellular and an intracellular niche. This is a far-fetched goal at the present juncture of our understanding of the pathogenesis of the microbe. However, as is apparent in the foregoing discussions, cidal activity on non-replicating bacteria can be expected to eradicate the persister population, the main source of the relapsed infection. Whether this population is intracellular or extracellular is not clear, though evidence points to an intracellular niche[49]. This reinforces the requirement of intracellular cidal activity on non-replicating MTB as a critical property for a new drug capable of reducing the duration of therapy.

Will the new drug be a part of the combination regimen? The ability of MTB to survive in different physiological states in the human host can be compared to a mixed infection with multiple pathogens. Attempts to eradicate a mixture of pathogens with a single drug are impractical. A new drug with marked activity on specific sub-populations of MTB will have to be combined into the regimen replacing one or two members of the current regimen. The cidal properties of the new compound will dictate the most effective combination.

Finally a critical parameter to be addressed is the compatibility of the pharmacokinetic/pharmacodynamic properties of the compound with the dosing regimen. The PK/PD parameters of the current drugs are incompatible with the recommended dosing regimen. Studies on the PK/PD properties of the frontline antituberculosis drugs could redefine a more efficient dosing regimen, which will also contribute to a reduction in the duration of therapy.

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