# Challenges Associated with Current and Future TB Treatment

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**Abstract:** Current tuberculosis (TB) treatment is based on a combination of drugs that were developed mostly in the central decades of the last century. Cure rates are high for drug sensitive strains of *Mycobacterium tuberculosis* (*M. tb*) when the recommended complex and lengthy treatment protocols are adhered to. However the difficulty in correctly prescribing and adhering to these protocols, the emergence of *M. tb* strains resistant to multiple drugs, and drug-drug interactions that interfere with optimal treatment of HIV and TB co-infected patients have generated a pressing need for improved TB therapies. Together with the ominous global burden of TB, these shortcomings of current treatment have contributed to a renewed interest in the development of improved drugs and protocols for the treatment of tuberculosis. This article highlights hurdles related to the optimized use of existing drugs and challenges related to the development of novel, improved products, focusing in particular on aspects inherent in TB drug clinical development. Concluding comments propose processes for more efficient development of new TB therapies.

**Keywords:** Tuberculosis (TB) drug development, pipeline, fluoroquinolones, nitroimidazoles, rifamycins, clinical trials.

## THE BURDEN

Despite being a curable disease, tuberculosis (TB) kills approximately 1.7 million people every year [1]; its global incidence is increasing by approximately 1% per year, with an estimated 8.8 million new cases of active disease annually [2]. Globally, approximately one third of the human population is infected with the causative agent of TB, *Mycobacterium tuberculosis* (*M. tb*). Tuberculosis is the leading killer of HIV infected people, causing an estimated quarter million deaths per year in that population [1,3]. As TB is primarily a disease of the poor and the TB burden disproportionately affects the lower income countries [1], the pharmaceutical industry has shown relatively little interest in developing new products for the treatment of TB [1]. The drugs currently used to treat TB have been developed in the central decades of the last century, at a time when both the process of developing new drugs and the regulatory environment differed significantly from today's. The success rates achievable with these drugs are significant, but require complex and long treatment protocols. Lack of compliance and other factors represent significant hurdles to the optimized use of existing drugs. These hurdles, described below, have contributed in recent years to a resurgence of interest in developing improved therapies to treat TB. As a result, there are now a larger number of TB drugs under development than at any previous point in history (these drugs and projects are collectively referred to as the TB drug "pipeline"). This article reviews the major challenges presented by the development of this pipeline.

## BACKGROUND

### Current Therapy for TB

Current TB treatment is based on principles of combination chemotherapy. Thus, multiple drugs are used both to increase efficacy and to prevent the emergence of resistant organisms. Based on mechanism of action, TB drugs can be classified as inhibitors of: bacterial protein synthesis (aminoglycosides), electron transport across the bacterial membrane (a proposed mechanism of action for pyrazinamide), nucleic acid synthesis (rifampin, quinolones) and cell wall synthesis (isoniazid, ethambutol, ethionamide and cycloserine). See Fig. (**1**). Perhaps the greatest hurdle to optimal TB therapy with the current drugs is the long treatment time necessary to achieve cure. The requirement for this long duration of treatment is generally attributed to physiologic heterogeneity of TB bacteria - that is, the hypothesis that there are subpopulations of organisms that span the spectrum from actively growing bacteria to metabolically quiescent ones. It appears that one or more of these bacterial subpopulations, although they are genetically drug-sensitive, can display phenotypic drug-resistance in response to altered environmental signals and thereby survive long periods of drug treatment in an animal or human host. These bacteria have been called "persisters" [4,5]. These persistent bacteria may or may not be in the same physiologic state as the mycobacteria in the majority of individuals with *M. tb* infection – i.e., those with latent TB infection (LTBI) who are clinically asymptomatic and noninfectious.

Drugs having different mechanisms of action are most likely needed to kill different bacterial sub-populations. The most effective of the current TB drugs at killing actively replicating tubercle bacilli is isoniazid, while rifampin, an inhibitor of RNA synthesis, is active against both replicating and non-replicating or slowly replicating bacteria [6]. Pyrazinamide, which is believed to act by inhibiting energy metabolism across the cell membrane, is a pro-drug requiring acidic conditions for activation. Clinical benefit from use of pyrazinamide is only seen during the first 2 months of therapy and the drug is believed to be effective against relatively slowly replicating bacilli [7]. The combination of rifampin and pyrazinamide played a major role in shortening the duration of treatment of active disease from the original 18 to 24 months to the current 6 to 9 months [8].

The treatment protocols for drug sensitive TB vary slightly in different parts of the world, but they are based on a combination of three, or more typically, four drugs, i.e. isoniazid, rifampin, pyraziminamide and ethambutol. These drugs offer the best combination of efficacy and tolerability amongst the available TB drugs and are therefore recommended for use as "first line" therapy. Less efficacious and tolerable drugs are used in cases of resistance to the first line drugs - and are referred to as "second line" products [9]. See Table (**1**). They include streptomycin, capreomycin, kanamycin, amikacin, ethionamide, para-aminosalicylic acid, cycloserine, ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gatifloxacin and clofazimine. These products have, in general, a lower therapeutic index, and almost all of them are significantly more expensive than the first line drugs.

### Limitations and Hurdles to Optimal Use of Current Therapy

As noted above, the first significant hurdle to the successful treatment of TB with current drugs is the length and complexity of the treatment protocols, which negatively impact patient adherence and play a significant role in the emergence of drug resistant TB. When delivered under a strictly regulated program, the cure rates

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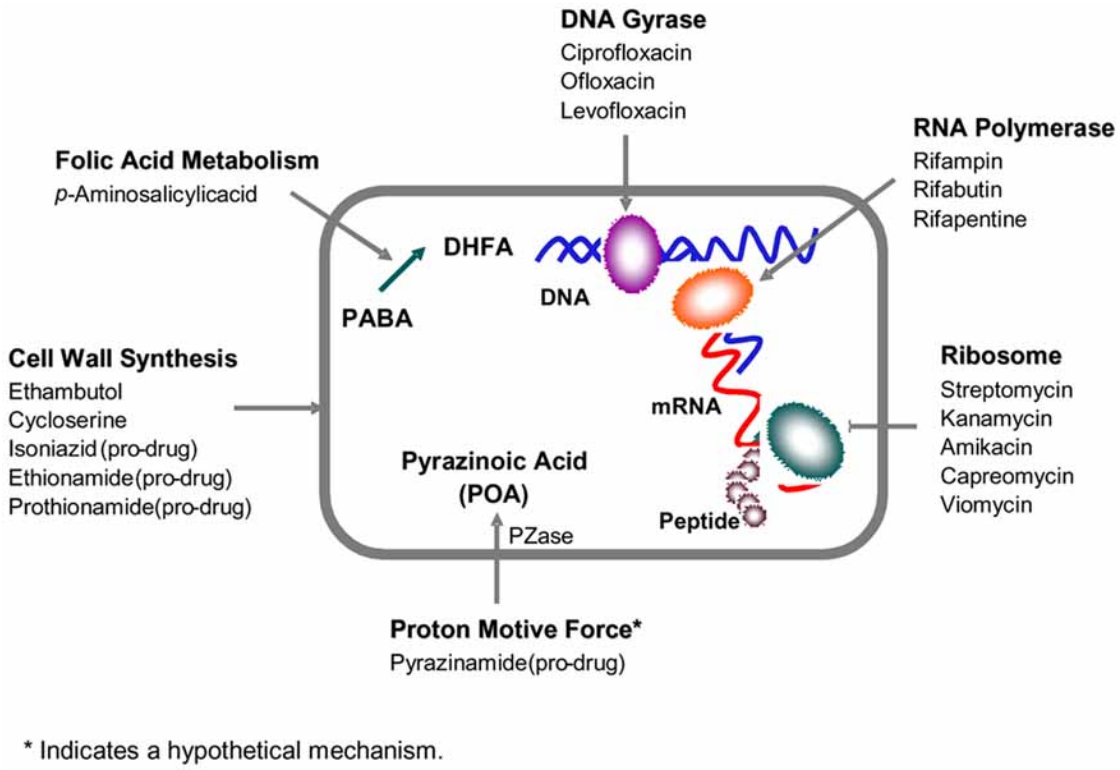


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**Fig. (1).** Schematic illustration of the sites of action for the available anti-tuberculosis agents.

with the standard regimen are quite high, exceeding 90% [10,11]. The World Health Organization has promoted a program known as Directly Observed Treatment – Short course (DOTS), which includes direct observation by trained personnel of the consumption of the TB medications. This has proven to be one of the most cost-effective global health interventions available today [12] but its level of implementation varies as it is quite demanding for patients and for health care staff. Specific reasons for inadequate treatment include incomplete implementation of regimens in terms of duration of treatment, number of drugs and/or their dosages and quality. The consequences of inadequate treatment can be severe for both the individual and for public health, most significantly, the selection of strains that are resistant to one or more of the drugs used. Over the decades, resistance has appeared to each one of the existing drugs; strains that are resistant to at least isoniazid and rifampin are referred to as "multi-drug resistant" (MDR-TB). Recently strains have appeared that are resistant to a very large number of products; those that are resistant to isoniazid, rifampin, fluoroquinolones, and at least one second line injectable drug (capreomycin, kanamycin, or amikacin) are defined as "Extremely Drug Resistant" (XDR-TB) [13]. During 2000--2004, of 17,690 *M. tb* isolates examined in a global survey carried out by the WHO, 20% were MDR and 2% were XDR. Other population-based estimates report a relative incidence of approximately 10% MDR-TB among all new TB cases [14]. In addition, population-based data on drug susceptibility of *M. tb* isolates obtained from the United States (for 1993--2004), Latvia (for 2000--2002), and South Korea (for 2004), showed that 4%, 19%, and 15% of MDR TB cases, respectively, were XDR. The treatment regimens for MDR TB are much less well defined and tested than those for drug-sensitive TB. Treatment of MDR TB must rely on second line drugs which are less effective and more toxic than first line therapy, as well as up to 110-fold more expensive overall [15-17]. The mortality rate in a recent outbreak of XDR approached 100% [a]. XDR TB has thus come to worldwide attention as a major therapeutic challenge and potential threat to public health.

A second significant hurdle in the treatment of TB is the high prevalence of co-infection with *M tb*. and the human immuno-deficiency virus (HIV). It is estimated that half the people living with HIV/AIDS develop active TB [18], approximately 12 million individuals are co-infected, and roughly 15% of AIDS patients globally die of TB every year [19]. These two infections are synergistic. The risk of progression from latent TB to active disease is estimated to be on average fifty fold higher in HIV + individuals compared to HIV -, with the risk of progression in an individual increasing in proportion to the degree of cellular immune suppression [20-22]. Interactions of rifampin, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) with cytochrome P450 3A4, create a significant therapeutic obstacle in the treatment of patients co-infected with TB and HIV. These drug-drug interactions render co-treatment with first-line TB drugs and antiretrovirals problematic in many high burden settings. Additionally, anti-retrovirals and isoniazid can both cause peripheral neuropathy, and their toxicity is enhanced when used together. Therefore, concomitant therapy with rifampin and PIs or NNRTIs is not recommended [23-25], particularly in resource-constrained settings where close drug level monitoring and resultant dose adjustments are not practical.

[a] Gandhi, N.R.; Mol, A.; Pawinski, R. *XVI International AIDS Conference, Abstract THLB0210*, Toronto, August 13-18, 2006.

**Table 1. Year of Discovery, Main Characteristics and Most Frequently Reported Adverse Reactions of First and Second Line TB Drugs**

| First Line Drugs | | | | | |
|---|---|---|---|---|---|
| **Drug** | **Year discovered** | **MOA** | **Route** | **Daily Dose** | **Major Adverse Reactions** |
| **Isoniazid** | 1952 | Cell wall syntheis inhibitor | P Os | 5 mg/kg (max 300 mg) | Hepatitis, peripheral neuropathy, lupus-like syndrome, drug interactions |
| **Rifampin** | 1966 | RNA synthesis Inhibitor | P Os | 10 mg/kg (max 600 mg) | Drug interactions, orange color of body fluids, GI, hepatitis, fever, acute renal failure, hemolytic anemia |
| **Pyrazinamide** | 1952 | Disruption of electron transport across the membrane ♦ | P Os | 15-30 mg/kg (max 2 g) | Hyperuricemia, gouty arthritis, rarely nephritis |
| **Ethambutol** | 1961 | Cell wall synthesis inhibitor | P Os | 15-25 mg/kg | Optic neuritis, exfoliative rash |
| **Second Line Drugs** | | | | | |
| **Drug** | **Year discovered** | **MOA** | **Route** | **Daily Dose** | **Major Adverse Reactions** |
| **Streptomycin** | 1944 | Protein synthesis inhibitor | IV/IM | 15 mg/kg | Cochlear and vestibular toxicity, nephrotoxicity |
| **Capreomycin** | 1956 | Protein synthesis inhibitor | P Os | 15-30 mg/kg | Cochlear and vestibular toxicity, nephrotoxicity |
| **Kanamycin** | 1957 | Protein synthesis inhibitor | IV/IM | 15-30 mg/kg | Cochlear and vestibular toxicity, nephrotoxicity |
| **Amikacin** | 1974 | Protein synthesis inhibitor | IV/IM | 15-30 mg/kg | Cochlear and vestibular toxicity, nephrotoxicity |
| **Ethionamide** | 1956 | Inhibition of mycolic acid synthesis (cell wall) | IV/IM | 15-20 mg/kg | GI toxicity/hepatitis/dizziness |
| **PAS** | 1946 | Inhibition of folic acid | P Os/IV | 15-20 mg/kg | GI toxicity, fever, rash |
| **Cycloserine** | 1952 | Inhibition of peptidoglycan synthesis | P Os | 15-20 mg/kg | Dizziness, depression, CNS |
| **Ciprofloxacin** | 1986 | Inhibition of DNA gyrase | P Os/IV | 750-1550 mg/d | GI toxicity, CNS, tendon rupture |
| **Ofloxacin** | 1995* | Inhibition of DNA gyrase | P Os/IV | 600-800 mg/day | GI toxicity, CNS, tendon rupture |
| **Levofloxacin** | 1996* | Inhibition of DNA gyrase | P Os/IV | 500 mg/d | GI toxicity, CNS, tendon rupture |
| **Moxifloxacin** | 1999* | Inhibition of DNA gyrase | P Os/IV | 400 mg/d | GI toxicity, CNS, tendon rupture |
| **Gatifloxacin** | 1999* | Inhibition of DNA gyrase | P Os/IV | 400 mg/d | GI toxicity, CNS, dysglycemia |
| **Clofazimine** | 1954 | Binding to mycobacterial DNA and mRNA ♦ | P Os/IV | 100-300 mg/d | GI toxicity, cutaneous, ocular discoloration/ pigmentation QT prolongation, dizziness |

* Year of first US patent

♦ proposed

## Towards a Better Treatment

The lengthy duration and complex nature of current TB therapy and the consequent emergence of MDR-TB and XDR-TB, and the incompatibility of key anti-tuberculous drugs with antiretroviral therapy (ART), all support the need to develop novel, better drugs and regimens for the treatment of TB. Improving TB treatment focuses on achieving several goals:

- shortening the duration of treatment for active TB to improve compliance, lessen the burden on public health infrastructure, and reduce the occurrence of MDR-TB.

- developing safe, tolerable drugs with novel mechanisms of action that will therefore be effective against resistant disease (MDR-TB and XDR-TB).

- developing TB drugs that lack liver cytochrome P450 enzyme induction and inhibition, to avoid drug-drug interactions, especially with ART, and facilitate treatment of patients co-infected with *M. tb* and HIV.

- developing safe and effective drugs to shorten the treatment of LTBI thus making it possible to address the problem of the biologic reservoir of *M. tb*.

Several organizations, including governmental institutions, philanthropic organizations, academia, and public/private partnerships are working towards achieving these goals. Additionally, in recent years, increased awareness of the public health threat has led some pharmaceutical and biotechnology companies to include TB within their efforts, despite the lack of strong market incentives.

### *The Pipeline of New TB Drugs*

The portfolio of compounds currently in research and development is commonly referred to as a '*pipeline*'. As a result of the combined efforts of the institutions described in previous sections and driven by the severity of the global public health needs, there is now a growing pipeline of compounds for the treatment of TB. Of the projects that one might include in the discovery and pre-clinical phases of the pipeline, limited public information is available for many, and it is often difficult to establish where exactly a given product is in the research and development cycle based on non-proprietary information. The next section will review broad categories and a few examples of projects in the discovery phase for which more robust published data are available. A more detailed review of chemical classes and compounds in the discovery and pre-clinical stages of the TB drug development pipeline is beyond the scope of this article and can be obtained elsewhere [26].

### *Discovery Phase*

To reach a robust, steady state that will meet the need for novel, optimized combination treatments, the number of new products in the pipeline of TB drug development must continue to grow in the coming years. Recent initiatives such as the 'Facilitation of Tuberculosis Drug Discovery' initiative, also known as the "TB drug accelerator", which was recently announced by the Bill and Melinda Gates Foundation [27] and is specifically aimed at accelerating drug discovery for tuberculosis, should help achieve this needed growth, but a marked increase in effort and resources is still needed.

Existing discovery projects in the pipeline can be categorized as either representing: a) a new chemical class addressing a known target, b) a new chemical class addressing a novel target, or c) an existing chemical class addressing a known target but being optimized for the treatment of TB. The next section of this article will review one representative from each of these categories as a means to providing insight into the main issues related to the discovery phase of new TB drugs.

#### A New Chemical Class Addressing a Known Target: Pleuromutilins

Pleuromutilins are a novel class of antimicrobial compounds that inhibit bacterial protein synthesis, and are active against a variety of pathogenic bacteria [28]. Pleuromutilins interact with the 50S bacterial ribosomal subunit [29]. This mechanism of action is not yet represented among TB drugs and therefore drugs utilizing this mechanism should be equally active against current drug-resistant and drug-sensitive TB strains. When pleuromutilin resistance does emerge, it occurs slowly, and in a stepwise fashion [30]. Combination drug therapy would make development of such resistance unlikely.

Two semi-synthetic pleuromutilins (tiamulin and valnemulin) have been introduced for treating mycoplasma infections in farm animals [31] but to date, no pleuromutilin derivative has been successfully developed for use in human medicine. Pleuromutilins are metabolized by liver enzymes and eliminated from the body at a very high rate — a major obstacle to developing the class for human oral treatment [32]. GlaxoSmithKline (GSK) has been working to develop an oral pleuromutilin for the treatment of respiratory tract infections. Together with the TB Alliance they are also working to identify a semi-synthetic pleuromutilin optimized against both replicating and non-replicating *M. tb* with respect to potency, pharmacokinetic, metabolic and safety characteristics. When used as part of an appropriate combination therapy, such an agent – if identified –could have the potential to help shorten current TB therapy and treat drug-resistant TB and TB-HIV co-infections [33]. Cross- resistance has, however, been reported between pleuromutilins and oxazolidinones with other microorganisms, [34] so this aspect will require careful evaluation in TB.

#### A New Chemical Class Addressing a Novel Target: Malate Synthase Inhibitors

As previously stated, the primary reason why current TB therapy requires a long treatment duration is that some of the bacteria in the infection can enter what is defined as a "persistent" state and become phenotypically, although not genetically, resistant to the treating drugs [5]. The development of drugs that target proteins essential for persistence should enable more rapid clearance of the infection. It has been very difficult to identify these drug targets and validate them as being essential to the persistent bacteria. In *M. tb*, persistence appears to involve a switch of metabolism to the glyoxylate shunt, and therefore a shift in carbon source to acetyl CoA generated by β-oxidation of fatty acids [35]. The best characterized mycobacterial enzyme in this metabolic pathway is isocitrate lyase, encoded in fact by two genes in the *M. tb* genome – *icl1* and *icl2*. Inhibition of both appears to be required for complete inhibition of *M. tb* growth in the persistent state [36]. Icl1 and icl2 have been the object of intensive high-throughput screening (HTS), however, due to the need to inhibit both enzymes and the fact that the isocitrate lyase active site is very small and hydrophilic, this effort has not been successful to date and the target is considered not easily "druggable". As a result, effort has now focused on the next enzyme in the glyoxylate shunt, malate synthase, which appears to be a more promising target based on its larger, hydrophobic active site. It is currently the focus of joint efforts by researchers in academia (Texas A&M University and Rockefeller University), the pharmaceutical industry (GSK), and the TB Alliance to complete target validation and identify specific inhibitors. To provide direct evidence of this target's relevance, the first step will be the demonstration that inhibition of malate synthase *in vivo* kills persistent *M tb*. Compounds that show good potency will undergo further characterization and will be examined in whole cell and animal models of acute and persistent infections. If successful, a lead malate synthase inhibitor could be entered into preclinical development within the next two to three years and would represent a completely novel class for the treatment of TB. Such an agent could potentially shorten TB treatment through potent killing of persistent bacteria, and provide a novel treatment in the armamentarium against drug-resistant as well as drug-sensitive disease.

#### An Existing Chemical Class Optimized for the Treatment of TB: Oxazolidinones

Oxazolidinones were first described in 1978 for their utility in treating plant diseases [37]. Antibacterial properties were discovered six years later. Structural variations led to greatly improved antibacterial properties relative to their progenitor compounds [38,39]. This is one of two new classes of antibiotics discovered and successfully developed for human use in the clinic over the past 40 years (the other one being cyclic lipopeptides). Linezolid was first described in 1996 [40] and has since served as a lead compound. Linezolid acts by interacting with the 50S ribosomal subunit. It was approved by the Food and Drug Administration in 2000. Linezolid was shown to inhibit growth of MDR-TB strains *in vitro* [41]; because of the lack of effective therapeutic agents to treat MDR-TB, linezolid has been used occasionally in patients with MDR-TB. Anecdotal reports appear to confirm linezolid's biologic activity as evidenced by sputum culture conversion [42,43]. However the prolonged use of linezolid is associated with peripheral and optic neuropathy and with bone marrow suppression, although these have been reported relatively rarely [44].

Activities are currently ongoing to test other members of this class against *M. tb*. The most active compound published to date appears to be PNU-100480, the activity of which appears to be similar to that of isoniazid and/or rifampin in an acute mouse model [45].

### *Clinical Development*

The probability of any given product moving successfully through the various phases of the drug development process is limited; of the thousands of compounds screened only a handful make it to the pre-clinical phase, and a fraction of those enter human experimentation. Therefore, as expected, the number of products in clinical development is considerably smaller than those in earlier stages of the pipeline. We are currently aware of seven new products in clinical trials for TB. The next section will summarize the available information related to these products.

### *Phase I*

#### Diamine SQ-109

SQ109 is a diamine, a small molecule with a novel structure and potentially novel mode of action, being developed by Sequella, Inc. Although it was originally discovered during a collaborative effort with investigators at the U. S. National Institutes of Health (NIH) to identify promising analogs of the first-line drug ethambutol (EMB), its structural dissimilarity to EMB and the potential differences in its intracellular target(s) suggest that it may have a novel mechanism of action. While its exact target has not been identified, SQ109's general mechanism of action appears to be that of a cell wall inhibitor. It has shown potency against *M. tb in vitro* (including drug-resistant strains) and *in vivo*, with a high degree of specificity for mycobacteria [46]. SQ109 has also been reported to demonstrate synergistic activity with rifampin and isoniazid both *in vitro* and *in vivo* [47]. While blood concentrations in a murine model are very low, SQ109 distributes into lungs and spleen in concentrations exceeding the MIC [48]. Oral administration of SQ109 (10-25 mg/kg in mice) once per day is reported to maintain drug levels above the MIC without accumulation of the drug in the target tissues. Cytochrome P450 reaction phenotyping suggests exclusive involvement of CYP2D6 and CYP2C19 in the metabolism of this compound [49]. An IND for SQ109 was granted by the US Food and Drug Administration (FDA) in September, 2006 [50]. A single dose, double blind, placebo-controlled, dose escalation study (5 doses) of this compound in healthy, adult volunteers was started in December 2006. By the completion of the first four doses (fasting) no serious adverse events had been reported. At the time of this writing, the single dose Phase I study is still ongoing [51].

#### Pyrrole LL3858

Lupin Ltd., headquartered in Pune, India, has identified a lead compound called sudoterb or LU-3858. Sudoterb belongs to a class known as pyrroles, which is derived from plant alkaloids. Sudoterb has been reported to have potent anti- *M. tb* activity *in vitro* and *in vivo*, against both drug-sensitive and drug-resistant strains of *M. tb*, suggesting that it works via a novel mechanism of action. Lupin Ltd. reported that, *in vitro*, sudoterb has bactericidal activity similar to isoniazid and is synergistic with rifampin, and that the combination of sudoterb with isoniazid, rifampin, and pyrazinamide has led to complete sterilization of sensitive and resistant *M. tb* strains in infected mice within two months. In combination with rifampin and pyrazinamide, sudoterb also sterilized lungs and spleens of infected animals in a shorter timeframe than conventional therapy[b]. These results suggest sudoterb could potentially reduce the time of TB treatment. Sudoterb has started Phase I trials [52] but results have not been published to date.

#### Nitroimidazole PA-824

PA-824 is a novel nitroimidazo-oxazine being developed by the TB Alliance. It has the potential for both first-line treatment of active tuberculosis and for therapy of MDR/XDR TB as it has a novel mechanism of action. While being explored in the 1970s by Ciba-Geigy as possible radio-sensitizing agents for use in cancer therapy, a series of nitroimidazoles were found to have anti-tuberculous activity. However, because the lead compound (CGI-17341) was demonstrated to be mutagenic in the Ames assay [53], further work in TB was not pursued. In the 1990s, PathoGenesis decided that this class of compounds warranted further exploration for the potential treatment of tuberculosis, and discovered PA-824 and related nitroimidazoles. PA-824 was found to be the most active of these compounds against *M. tb* in a murine infection model [54].

When Chiron purchased PathoGenesis in 2000, they stopped the development of PA-824. In 2002, the TB Alliance and Chiron signed an exclusive license agreement granting the TB Alliance worldwide rights to develop PA-824 and nitroimidazole derivatives for TB. *In vitro* studies indicated that PA-824 should be efficacious against both drug-sensitive and drug-resistant tuberculosis [55]. Additional *in vitro* studies demonstrated that PA-824 is active at a minimum inhibitory concentration (MIC) similar to that of isoniazid [56,57]. The compound is highly selective, and its activity among the mycobacterial species tested is limited to BCG and *M tb*., with no significant activity against a broad range of gram-positive and gram-negative bacteria (with the exception of *H. pylori* and some anaerobes) [58]. Additional *in vitro* studies with anaerobic culture models indicate that PA-824 has activity against non-replicating bacilli [54]. Finally, PA-824 has shown activity against strains with known resistance to standard TB treatment [56]. PA-824 significantly inhibits both protein and lipid synthesis but does not affect nucleic acid synthesis. It induces an accumulation of hydroxymycolic acid and a concomitant reduction in ketomycolic acids, indicating inhibition of the enzyme responsible for the oxidation of hydroxymycolate to ketomycolate [54]. Similar to isoniazid, PA-824 is a pro-drug that undergoes activation via an F420-dependent mechanism (although by a different F420-dependent mechanism than is involved in isoniazid-activation). Mutations in the gene encoding the F420 enzyme are responsible for some instances of PA-824 resistance identified *in vitro* [58]. PA-824 appears to undergo nitro-reduction producing highly reactive intermediates which interact with multiple intracellular targets. Therefore, its anti-mycobacterial mechanism of action is likely complex. *In vitro* studies indicate that PA-824 neither inhibits nor is metabolized by major P450 enzyme isoforms suggesting that it should have minimal drug-drug interactions, for example, with antiretroviral agents. PA-824 – differently from Ciba-Geigy's CGI-17341 - has not shown mutagenic or genotoxic potential in the Ames test.

In pre-clinical studies, Nuermberger *et al*. confirmed that the substitution of PA-824 for isoniazid in standard treatment in the mouse leads to significantly lower lung CFU counts after 2 months of treatment and to more rapid culture-negative conversion than standard therapy. There was no significant difference in the proportion of mice relapsing after completing 6 months of the PA-824-based regimen compared to control in this experiment where none of the control mice relapsed [59]. Therefore, further pre-clinical work and then clinical testing are planned to identify an optimized PA-824-containing TB treatment regimen. Such complexities are inherent to the development of novel, improved regimens for TB treatment as discussed later in this review.

Preclinical evaluation has also included two acute (14-day) GLP toxicology studies, one in the rat and one in the monkey; both demonstrated a no observed adverse effects level (NOAEL) of 50 mg/kg. Three-month toxicity studies are planned in the rat and monkey, to be followed by longer term toxicity studies to support ultimate administration of this drug to patients for extended periods of time. PA-824 entered Phase I clinical development in 2005. To date four studies have been completed and one is ongoing (See Table (**2**).

[b] American Chemical Society Meeting, Anheim CA, **2004**, Abstract 63.

The first Phase I study (CL-001) was a single dose study carried out on 53 male healthy volunteers at doses of 50, 250, 500, 750, 1000, 1250, and 1500 mg. The study showed that PA-824 was well tolerated, with no dose-limiting adverse events or abnormal laboratory results. No effects were seen on ECG, vital signs, or on physical examination. The time to maximum concentration ($T_{max}$) was 4-5 hours, and the half-life ($t_{½}$) was approximately 18 hours. The second study (CL-002) was a 7 day multiple-dose study carried out on 24 healthy volunteers of both genders at doses of 200, 600, and 1000mg; a further cohort was planned to be treated at 1400 mg/day, but was never enrolled because the cohort treated with 1000 mg/d showed moderate creatinine elevation after 5 days of dosing. The creatinine elevation was reversed during a 7-day washout period, and no consistent effect was observed on blood urea nitrogen (BUN). The study confirmed a $T_{max}$ of about 4-5 hours and a $t_{½}$ of approximately 17 hours. The third study (CL-004) was an Absorption, Distribution, Metabolism and Excretion study (ADME) carried out with radio-labeled PA-824 [$^{14}$C] given as oral solution equivalent to 840 mg of PA-824. The study showed that 91% of the dose was recovered in urine and feces (about 65% in the urine and 26% in the feces), and pharmacokinetic parameters appeared consistent with the earlier studies. The analysis of metabolites from this study is still in progress. A fourth study (CL-005), evaluating potential renal effects of PA-824 in healthy volunteers has just been completed. It was designed to establish the underlying mechanism of the serum creatinine elevations seen in CL-002 and was carried out on 48 healthy volunteers of both genders with multiple doses (8 days) of 800 and 1000 mg. Complete data analysis is underway but preliminary analyses indicate that the creatinine elevations are a benign phenomenon clinically and support the decision to move into proof of concept studies. An extended early bactericidal activity (EBA) trial is being planned for adult patients with newly diagnosed pulmonary tuberculosis. A fifth Phase I study (CL-003) is underway to evaluate the PK properties of PA-824 in the fed vs. the fasted state; drug-drug interaction studies are also being planned with a special focus on potential interaction with anti-retroviral drugs (ARVs).

### *Phase II*

#### Nitroimidazole OPC-67683

A second nitroimidazole in development, OPC-67683, is a nitro-dihydroimidazo-oxazole derivative under development by Otsuka Pharmaceutical Co. Ltd. To date it has been evaluated in a number of Phase I studies in healthy volunteers and in an early bactericidal activity study in TB patients. The compound has potent *in vitro* anti-microbial activity against *M. tb*, and has shown no cross-resistance with any of the currently used first-line tuberculosis drugs, consistent with its novel mechanism of action (See discussion under PA-824 above). Like PA-824, this compound therefore could prove effective in the treatment of MDR/XDR TB. Pre-clinical studies in a chronic mouse model of tuberculosis showed superior efficacy of OPC-67683 to the currently used drugs. The dose that provided effective plasma concentration was 0.625 mg/kg, confirming the remarkable *in vivo* potency of OPC-67683. In other pre-clinical *in vitro* and *in vivo* studies, OPC-67683 did not show antagonistic activity with other first-line drugs but rather either synergistic effect or no appreciable interactions.

Otsuka has completed a small single dose level Phase II EBA study with OPC-67683 and has started a larger, multiple dose level EBA trial. This larger trial compares the safety, efficacy and pharmacokinetics of 100mg, 200mg, 300mg and 400mg once daily OPC-67683, administered orally for 14 consecutive days vs. standard therapy (Rifafour e-275), in patients with uncomplicated, smear-positive pulmonary TB. The study, which started in November 2006 aims to enroll 54 patients total, and is expected to be completed by April 2007 [60,61].

#### Diarylquinoline TMC207

TMC207 is a diarylquinoline discovered and under development by Tibotec Pharmaceuticals Ltd., a subsidiary of Johnson & Johnson. It has a novel mechanism of action, inhibiting the mycobacterial ATP synthase proton pump — one of the mycobacterial sources of energy [62]. This mechanism of action appears to be unique among the commonly used antimicrobials, and

**Table 2. Synopsis of Clinical Trials with PA-824 Carried out to Date**

| Study | CL-001 | CL-002 | CL-003 | CL-004 | CL-005 |
|---|---|---|---|---|---|
| **Design** | Single-dose | 7 day Multi-dose | 2-dose Food Interaction | ADME | Renal Effects Study |
| **Doses (mg)** | 50, 250, 500, 750, 1000, 1250, 1500 | 200, 600, 1000, (1400) | 1000 | [$^{14}$C] PA-824 OS* | 800, 1000 |
| **Population (N)** | Males only (53) | Males and Females (24) | Males and Females (16) | Males only (6) | Males and Females (45) |
| **Site** | MDS, Lincoln, NE | MDS, Neptune, NJ | MDS, Lincoln, NE | Covance, Madison, NJ | DCR, Minneapolis, MI |
| **Main Results** | Well tolerated, no dose-limiting AEs or abnormal laboratory results. No effects on ECG, vital signs or PE<br>$T_{max}$ 4-5 hrs.<br>$t_{1/2}$ ~ 18 hrs. | 1000 mg/d, 5 days moderate creatinine elevation; reversed during 7-day washout period. No consistent effect on BUN. 1400 mg cohort not enrolled.<br>$T_{max}$ 4-5 hrs.<br>$t_{1/2}$ ~ 18 hrs. | Ongoing | 91% of dose recovered in urine and feces (~65% urine, ~26% feces).<br>No significant radioactivity captured as [$^{14}$C]-CO2.<br>Metabolite analysis in process.<br>$T_{max}$ 4.5 hrs.<br>$t_{1/2}$ ~ 17 hrs | Ongoing |

* Oral Suspension

explains the absence of reports of cross-resistance in pre-clinical studies to current TB drugs. In pre-clinical studies, TMC207 has shown potent anti-TB activity [62]. In the mouse model, the combination of TMC207 with any two of the three first-line drugs (isoniazid, rifampin and pyrazinamide) was more effective than the standard regimen of isoniazid, rifampin and pyrazinamide. In fact, the combination of TMC207, isoniazid and pyrazinamide as well as the combination of TMC207, rifampin and pyrazinamide resulted in negative spleen and lung cultures after 8 weeks of therapy [63].

TMC207 has completed several Phase I studies to evaluate safety, tolerability and pharmacokinetic parameters. These include a single ascending dose study (25-700mg), a multiple ascending dose study (25-400mg, 14 days), drug-drug interaction studies with rifampin, isoniazid and pyrazinamide, and an interaction study with ketoconazole. Results showed a positive food effect (2-fold increase in exposure), metabolism by CYP450 3A4, and a resultant interaction with rifampin (standard doses of rifampin, an inducer of CYP450 3A4, lower TMC207 levels by 50%). Overall, phase I studies in healthy human volunteers suggest that the drug is safe and, with a half-life greater than 24 hours, may allow for dosing at frequencies less than once per day.

Currently in Phase II development, an EBA trial with 75 TB patients has been completed [61]. The study compared three doses of TMC207 (25, 100 and 400 mg/day) vs. isoniazid or rifampin, given for 7 days. The patients treated with 400 mg/day showed a decrease in colony forming units/ml/day over seven days, which reached statistical significance beginning on the fourth day of treatment, but which was less than the decrease observed with either isoniazid or rifampin over the same time period. The lower dosages of TMC207 did not achieve significant decreases in cfu/ml/day. The linear PK seen in healthy volunteers was confirmed in patients. No serious adverse events related to the drug have been reported among the 189 subjects treated to date with TMC207. A Phase II dose-finding study in MDR patients, which includes safety and efficacy endpoints, is planned to start in the second quarter of 2007 [61].

### *Phase III*

Two products, both fluoroquinolones, are in the TB drug pipeline in Phase III development. The fluoroquinolones, originally introduced in the 1980s, have a broad spectrum of activity, and offer a favorable pharmacokinetic profile for the treatment of TB [64,65].

These antibiotics have been used for several years for other indications. In spite of the fact that there are few published clinical data to support their use in TB, fluoroquinolones have become part of the recommended second-line regimen for the treatment of MDR-TB [66]. Most demonstrate good oral bioavailability and achieve peak serum concentrations above the MIC. They are distributed widely in the body, including intracellularly. Fluoroquinolones act by inhibiting mycobacterial DNA gyrase (see elsewhere in this issue). Given the common target for all fluoroquinolones, it is not surprising that cross-resistance has been reported among different members of this class [67,68]. However, since the assessment of resistance to fluoroquinolones is not carried out routinely, information about the extent of the problem is not readily available. Sporadic data indicate that the prevalence of resistance to fluoroquinolones is low in North America (1.8% reported for ciprofloxacin [69], but considerably higher in at least some parts of South East Asia (between 18.2 and 53.4% reported)[c]. The fluoroquinolones are cleared by the kidney and/or by the liver, with varying serum half-lives [70,71]. Fluoroquinolones are generally well tolerated but little experience exists on their long-term use.

[c] Tupasi, T.E. MDR in the Philippines, 4th World Congress on TB, Washington DC, 2002.

Data published by the Tuberculosis Research Centre in Chennai, India on a clinical trial with ofloxacin-containing regimens, showed rates of sputum culture conversion to negativity at two months ranging between 92% and 98%, compared to an expected rate of approximately 80% with a standard four-drug treatment. This trial unfortunately did not include a standard control group, but rather randomized patients with newly diagnosed pulmonary tuberculosis to one of four ofloxacin-containing regimens [72].

In patients randomized to three months of daily isoniazid, rifampin, pyrazinamide, and ofloxacin, followed by twice weekly isoniazid and rifampin for one or two months, the relapse rates observed in the two years following completion of treatment were 4% and 2%, respectively. These results suggest that fluoroquinolones have the potential to shorten the duration of tuberculosis treatment.

Moreover, recent data have shown that among the fluoroquinolones, gatifloxacin and moxifloxacin have more potent activity against *M. tb* than the older members of this class, including ofloxacin [73]. A recent evaluation of fluoroquinolones in an *in vitro* model of *M. tb* persistent infection also found that moxifloxacin had the greatest sterilizing activity of the fluoroquinolones tested [73].

Gatifloxacin and moxifloxacin are now being developed specifically for the treatment of TB, and have reached Phase III. These programs could lead to the first new class of drugs approved for the treatment of tuberculosis in over 30 years.

### **Gatifloxacin**

Gatifloxacin was approved in 1999 by the FDA for the treatment of patients with pneumonia, bronchitis, uncomplicated gonorrhea, and various infections including those of the urinary tract, kidneys, and skin. Gatifloxacin has shown bactericidal activity against *M. tb* both *in vivo* and *in vitro* [73,78,74]. When tested in mice in combination with pyrazinamide and ethionamide at high doses (450 mg/kg), gatifloxacin cleared the lungs of infected animals after 2 months [75].

The gatifloxacin clinical development program is being conducted by the OFLOTUB consortium[♦]. It includes a Phase II study conducted in Durban, South Africa, randomizing newly diagnosed patients to one of three fluoroquinolone-containing regimens (ofloxacin, moxifloxacin, or gatifoxacin) in combination with isoniazid, rifampin, and pyrazinamide during the first two months of treatment ("Oflotub Phase II surrogate marker study"). A variety of bacteriological endpoints were evaluated as potential biomarkers of treatment response, with a particular focus on serial sputum colony counts. The study found that when substituted for ethambutol in standard therapy, both moxifloxacin and gatifloxacin killed *M. tb* significantly faster than the control or ofloxacin-based regimens, supporting a potential for these fluoroquinolones to be able to reduce treatment duration by one, or possibly two months. The OFLOTUB consortium has continued the evaluation of the gatifloxacin-substituted regimen vs. standard 6 months treatment in a Phase III design [76]. The Phase III portion of this study is a multicenter, open-label, randomized, controlled trial of a 4-month gatifloxacin-containing regimen vs. a standard 6-month regimen for the treatment of adult, pulmonary TB [77]. This study is testing the non-inferiority of a regimen of 2 months of gatifloxacin, isoniazid, rifampin and pyrazinamide followed by two months of gatifloxacin, isoniazid and rifampin vs. 2 months of ethambutol, isoniazid, rifampin and pyrazinamide followed by 4 months of rifampin and

[♦] OFLOTUB is a consortium of ten partners from Europe and Africa that was initiated in 2002 to undertake Phase II and Phase III trials to test the safety and efficacy of a gatifloxacin-containing 4-month treatment regimen for the treatment of TB. It was established under the auspices of the European Commission and is coordinated by the Institut de Recherche pour le Dévelopement (IRD) in Paris, France.

isoniazid, according to standard treatment guidelines. The study at the time of writing has enrolled about half of the targeted sample size of 2070 individuals. The primary endpoint will be the percent of relapses at 24 months; secondary endpoints will include time to relapse, defined from the date of treatment cure to date of relapse, percent culture conversion at 8 weeks and percent patients cured in each arm by the end of treatment, and time to a composite endpoint of treatment failure. The study has been impacted by the recent identification through post-marketing surveillance activities, of the effects of gatifloxacin on glucose control. In February 2006, the FDA issued a specific warning [78] of increased incidence of serious hypoglycemia and hyperglycemia in patients, especially the elderly and/or diabetic, receiving gatifloxacin. Subsequently the marketing of gatifloxacin in the US and Canada was stopped. The study is continuing, but has been modified to reflect the increased needs for stringent monitoring of glucose levels. To date, to the authors' knowledge, no dysglycemic events have been identified in this trial.

**Moxifloxacin**

Moxifloxacin was first approved in 1999 by the FDA [79]. It is produced and marketed by Bayer Healthcare for the treatment of acute bacterial exacerbation of chronic bronchitis, acute bacterial sinusitis, community-acquired pneumonia and uncomplicated skin and skin structure infections.

In 2005, Bayer and the TB Alliance entered a partnership to undertake a global clinical development program to register moxifloxacin for a TB indication. Jacques Grosset and colleagues at Johns Hopkins University conducted a series of studies in the mouse model, supported by the TB Alliance and the US NIH, which contributed significantly to the interest in this drug. The initial study, in which infected mice were treated for one month with sparfloxacin, clinafloxacin, moxifloxacin or isoniazid [80], found that moxifloxacin had the greatest bactericidal activity, comparable to that of isoniazid, (the most potent bactericidal drug in EBA studies) [81]. A second study suggested that moxifloxacin also had potent sterilizing activity and might substantially improve the efficacy of once-weekly rifapentine treatment [82]. In a more recent study, using a mouse model reflective of chemotherapy for human tuberculosis, Nuermberger *et al.* demonstrated that the combination of moxifloxacin, rifampin, and pyrazinamide reduced the time needed to eradicate *M. tb* from the lungs of infected mice by up to 2 months when compared with the standard regimen of isoniazid, rifampin, and pyrazinamide. These findings suggest that this regimen has the potential to substantially shorten the duration of therapy needed to cure human tuberculosis [83]. The results of two small EBA studies have demonstrated that moxifloxacin has bactericidal activity superior to that of rifampin and perhaps comparable to that of isoniazid [84,85].

These pre-clinical data and human data from Chennai and the EBA studies led to the planning and implementation of a number of Phase II and Phase III clinical trials of two different moxifloxain-containing regimens. A synopsis of these trials is provided in Table (**3**). The first was a Phase II trial (Study 27) carried out by the US Centers for Disease Control and Prevention Tuberculosis Trial Consortium (CDC/ TBTC) [86] which randomized HIV-positive and negative patients with newly diagnosed, acid-fast bacilli (AFB)-positive, pulmonary tuberculosis to one of four 2-month intensive phase regimens: two standard regimens given either daily or three times weekly, and two analogous regimens in which moxifloxacin replaced ethambutol. Moxifloxacin and ethambutol placebos were used to ensure that the study was carried out under double blind conditions. The primary study endpoints were two-month sputum culture conversion rate and withdrawal due to adverse events. Although the study showed no significant difference between standard and moxifloxacin-based regimens in percent patients whose sputum converted to negative by 8 weeks, the arm treated with moxifloxacin showed higher rates of sputum conversion after four and six weeks of therapy in the moxifloxacin arm compared to the control arm, results consistent with the mouse model findings, supporting further evaluation of a regimen substituting moxifloxacin for ethambutol in standard therapy. The study also demonstrated a marked difference in the rates of two-month culture-conversion between African participants and North American participants (60% vs. 85% respectively; this difference was not affected by HIV status). This difference has not yet been fully explained but may relate at least in part to differences in degree of lung cavitation at diagnosis in these patient populations. A similar Phase II study of a moxifloxacin substituted for ethambutol-based regimen is currently being conducted by the Johns Hopkins University in Brazil. The results of this study are also expected within 2007.

CDC/TBTC is currently conducting Study 28, a multicenter, randomized, controlled, double-blind study designed to test the hypothesis that the replacement of isoniazid with moxifloxacin in standard therapy during the intensive phase of treatment for newly-diagnosed, drug-sensitive, adult, pulmonary TB would result in a significantly greater proportion of patients whose sputum is culture negative at 2 months, compared with the standard regimen. The primary endpoints of the study, similar to those in Study 27, are the proportion of patients having a negative sputum culture at 2 months of therapy and the proportion of patients who discontinue assigned study therapy for any reason during the first 2 months. The study completed enrollment of the target sample size (420 patients) in March 2007. Results are expected before the end of 2007.

Finally, a large Phase III trial of moxifloxacin-based therapy is planned by the University College London in cooperation with the British Medical Research Council and with the support of the European and Developing Countries Clinical Trials Partnership (EDCTP) and the TB Alliance at a number of sites in Africa. This

**Table 3. Clinical Trials for the Development of Moxifloxacin in the Treatment of TB**

| Trial (Sponsor) | Study Design (Phase) | Countries | N | Status |
|---|---|---|---|---|
| TBTC # 27 (CDC) | Moxifloxacin replaces Ethambutol (Ph II) | USA, Canada, Uganda, South Africa | 336 | Completed 6/05 |
| TBTC #28 (CDC) | Moxifloxacin replaces Isoniazid (Ph II) | USA, Canada, Uganda, South Africa, Brazil, Spain | 420 | Completed enrollment 03/07 |
| JHU | Moxifloxacin replaces Ethambutol (Ph II) | Brazil | 170 | Completed enrollment 03/07 |
| REMox TB (UCL/MRC) | Moxifloxacin replaces Ethambutol (Ph III)<br>Moxifloxacin replaces Isoniazid (Ph III) | Kenya, South Africa, Tanzania, Zambia | 1500 | Trial initiation planned for July 2007 |

double-blind, randomized, controlled, multi-center trial, called "Rapid Evaluation of Moxifloxacin in TB" (REMox TB) will test two different hypotheses: 1) two months of moxifloxacin, isoniazid, rifampin and pyrazinamide followed by two months of moxifloxacin, isoniazid and rifampin will be non-inferior to six months of standard therapy, and 2) two months of moxifloxacin, rifampin, pyrazinamide and ethambutol, followed by two months of moxifloxacin and rifampin will be non-inferior to six-months of standard therapy for newly-diagnosed, drug-sensitive, adult, pulmonary TB patients. This study will address the question of whether, by substituting moxifloxacin for either isoniazid or ethambutol and continuing moxifloxacin for a total of four months, the treatment of TB can be safely and efficaciously shortened by 2 months. The study aims to enroll 1,500 patients, mostly or entirely in Africa, and is expected to start in mid 2007.

**The Search for an Intermittent Treatment**

The efforts of the current Phase III fluoroquinolone trials described in the previous section are focused on the shortening of TB treatment, from the current six months to four. If successful, this change could have a very significant impact on the treatment of TB, presumably improving adherence, decreasing development of drug resistance and lessening the extensive public health resources required by DOTS in TB high-burden countries. Another ongoing parallel search seeks to simplify treatment by identifying *intermittent* regimens using currently available drugs that would have comparable efficacy and safety to present therapy, but without promoting the occurrence of resistance. Currently, the focus of this treatment-simplification research is based on use of long-acting rifamycins.

Rifampin, a rifamycin, is one of the key components in the armamentarium of modern "short-course" tuberculosis treatment. Like all other regimens for the treatment of active TB, rifampin-based regimens must be administered for at least six months. This treatment however has been shown to be as effective when administered three times weekly after the first two weeks of therapy as when given daily [87], while more widely spaced regimens are less effective, and may be associated with acquired drug resistance in HIV-infected patients, even when properly supervised.

Rifampin has a half-life of 2-4 hours; a number of other rifamycin derivatives with much longer serum half-lives have been evaluated in intermittent regimens. The first clinical trial on these compounds addressed the use of rifabutin [88]. The initial trials with this product focused on prevention of infection with *Mycobacterium avium* complex (MAC) in HIV+ patients [89]. Although rifabutin is approved for the prophylaxis of MAC in the US and for the treatment of tuberculosis in several other countries, its primary use currently is as a substitute for rifampin in patients who are being simultaneously treated with anti-retroviral therapy, since it interferes less with the CY P-450 system and therefore with antiviral drug levels than rifampin [90]. A trial carried out by the TBTC (Study 23) of a rifabutin-containing regimen administered twice weekly in HIV+ TB patients found unacceptably high rates of acquired rifamycin resistance among patients with more advanced immunosuppression[d] . This finding led the CDC to recommend against the use of widely spaced treatment of tuberculosis with rifamycin-based regimens in such patients [91].

Rifalazil, another long-acting rifamycin derivative, has an even longer half-life and showed potent activity in animal models suggesting its possible use in simplified treatment regimens [92]. One interesting feature of this compound is its rather low potential for enzyme induction and drug-drug interactions [93]. However, an initial Phase I study demonstrated relatively high rates of adverse events manifesting as a "flu-like" syndrome [94]. This could represent a mechanism-based phenomenon due to the release of cytokines; there is evidence of increased interleukin-6 (IL6) serum levels in individuals treated with rifalazil. An EBA study did not demonstrate significant drug activity of once-weekly rifalazil administered with isoniazid for two weeks, [95] and consequently rifalazil development for TB treatment was stopped. However, it is believed that closely related compounds that are better tolerated and also lack propensity for enzyme induction can be identified [96].

Rifapentine, a cyclopentyl-substituted rifampin with has a half-life of 14-18 hours in normal adults, is registered for the treatment of TB. After the administration of a 600 mg dose, serum levels in excess of the MIC persist beyond 72 hours, suggesting that the drug might in fact be effective with intermittent regimens [96,97]. A series of experimental studies in mice conducted by Jacques Grosset and colleagues found that a once-weekly continuation phase of rifapentine and isoniazid for 4 months following a standard two-month induction phase with daily isoniazid, rifampin, and pyrazinamide was as effective as standard therapy given daily for 6 months [98]. These studies provided the scientific rationale for a large phase III trial that was begun by TBTC in 1995 (Study 22) [99].

This was an open-label trial that randomized patients with newly diagnosed, drug-susceptible pulmonary tuberculosis to a 4-month continuation phase regimen of either once weekly rifapentine-isoniazid or twice-weekly rifampin-isoniazid following successful completion of a standard two-month induction phase. The rifamycins were dosed at 600 mg and isoniazid at 900 mg. The primary end-points were the combined rate of treatment failure and relapse, and safety and tolerability of rifapentine. Although the trial focused on HIV-negative patients, initially HIV-positive patients were also enrolled in order to gain experience with this important subset of patients. However, early in the trial, following the finding of a high rate of relapse with acquired rifampin-monoresistance among HIV-positive patients assigned to the rifapentine arm, enrollment of HIV-positive patients was stopped [100]. A total of 1003 HIV-negative patients were enrolled into the completed study. Among these the crude rate of failure and relapse was significantly higher in those enrolled in the rifapentine arm (9.2% vs 5.6%, $p = 0.04$). In a multivariate analysis, the factors statistically associated with an adverse outcome were the presence of cavitary disease, being sputum culture positive at study entry, white race, and being more than 10% under ideal body weight at time of diagnosis; treatment regimen on the other hand was not associated with an adverse outcome [101]. Cavitary disease and two-month culture positivity were also predictors of an adverse outcome among patients in the rifampin arm. Among patients with non-cavitary tuberculosis and negative 2-month sputum cultures, the relapse rate was low in both arms. Rifapentine was well tolerated, and rates of adverse events were similar in both treatment groups, with 3% of patients in each group discontinuing treatment because of a drug-related adverse event. These results were similar to those from a study carried out in Hong-Kong that utilized Chinese-manufactured rifapentine of inferior bioavailability, [102] as well as those from a company-sponsored trial that enrolled patients largely from Africa [103].

These results led to new recommendations for the use of the rifapentine-isoniazid continuation phase regimen for HIV-negative adults with drug-susceptible, non-cavitary tuberculosis and negative AFB smears at two months [104]. As a result of these recommendations, necessary encounters with patients for the direct observation of treatment are reduced by 50%, which has a considerable positive impact on costs [105]. However, rifapentine is not recommended for the treatment of patients with more advanced tuberculosis or for HIV + TB patients. Pharmacokinetic evaluations conducted within Study 22 indicated that low levels of isoniazid and rapid acetylation of isoniazid were associated with relapse. These results suggest that with the use of a more pharma-

[d] Burman, W.; Benator, D.; Vernon, A.. Abstracts of the 10th Conference on Retroviruses and Opportunistic Infections [abstract 136], Boston MA, 2003, 106.

cokinetically appropriate companion drug the efficacy of once weekly rifapentine-based treatment might improve [101]. Experimental studies suggest that higher doses of rifapentine might also result in more effective treatment [98].

As a result of the above data, TBTC more recently undertook a large Phase II trial of higher doses of rifapentine. In TBTC Study 25, 150 HIV-negative patients with drug-susceptible pulmonary tuberculosis who completed standard initial phase treatment were randomly assigned to 600, 900, and 1200 mg rifapentine administered once weekly together with isoniazid for 16 weeks in a double blind double dummy study. The primary study endpoints were adverse events and drug discontinuation. Only one patient enrolled in the 1200 mg arm stopped treatment because of a possible drug-related adverse event [106].

Because the results of Study 22 had been disclosed by the time this study began, the protocol was modified to extend treatment for an additional three months for patients with cavitary disease and positive sputum cultures at entry (i.e., at two months). The twenty patients who met these criteria received extended treatment and were followed for relapse, which occurred only in one patient, who was enrolled in the 600 mg arm. The relapse rate of 5% compared to the higher rate seen in Study 22 (22%) suggests that extended treatment and higher rifapentine doses may in fact provide a better outcome in patients who are at increased risk of relapse [107].

Results of other studies suggest that once-weekly administration of rifapentine and isoniazid for three months may provide effective treatment for latent tuberculosis infection, comparable to that conferred by six months of daily isoniazid or by three months of daily rifampin and pyrazinamide [108]. On the basis of these observations, the TBTC in 2002 initiated a study of rifapentine/ isoniazid for latent TB infection (LTBI) treatment (Study 26). This is a trial of short-course treatment of LTBI among contacts of active cases, using a 3-month, once-weekly regimen of isoniazid 900 mg and rifapentine 900 mg, compared to standard 9-month daily therapy with isoniazid 300 mg., aiming to enroll 8000 patients. Given the large sample size and capacity issues of the TBTC sites, which are largely North American, study completion is not expected before 2008.

### Challenges in Clinical Development of Novel TB Therapies

The process of advancing compounds from discovery through clinical development is complex and lengthy and will not be described in any detail in this article, but its major stages are summarized in Table (**4**).

Developers of improved therapies specific for TB face, in addition to the significant issues that all drug developers encounter, a number of challenges directly related to aspects of TB which will be described in the next section.

Phase I trials for TB drugs are generally similar to those for other drugs; each individual compound must be tested for safety, tolerability and to define its pharmacokinetic/pharmacodynamic profile. The first challenge directly related to the field of TB drug clinical development is the way in which 'proof of concept' (POC) is achieved for TB drugs. In TB, POC - which must be obtained before moving into the Phase II studies that will be described in the next paragraphs - typically involves evaluation of the early bactericidal activity (EBA) of an individual new drug [109,110]. Evaluation of EBA is accomplished through the treatment of TB patients with a short course of monotherapy with the experimental drug, aimed at establishing its efficacy in killing *M. tb* in pulmonary cavities, although what is actually measured is numbers of live bacilli in the sputum during the first days of treatment. Given the regulatory need to test the new product as monotherapy, but the clinical imperative to treat TB with a combination regimen, these studies present significant ethical and logistic challenges. They can be carried out only for brief treatment periods and by experienced sites capable of performing the required sophisticated quantitative mycobacterio-logical tests. The endpoint used in EBA studies is the

**Table 4. Synopsis of the Stages of Drug Development**

| | Discovery | Pre-Clinical | Clinical |
|---|---|---|---|
| **Areas Involved** | - Biology<br>- Medicinal Chemistry | - Process Chemistry<br>- Safety<br>- Drug Metabolism<br>- Pharmaceutical R &D | - Clinical Pharmacology<br>- Clinical Research<br>- Regulatory<br>- Health Economics |
| Issues Addressed | - Identification of potential targets<br>- Identification of compounds active on those targets<br>- Optimization and development of lead candidates<br>- Evaluation of in-vitro binding activity<br>- Elucidation of Structure-Activity Relationships (SAR) | Animal models:<br>- PK/PD<br>- ADME<br>- Safety<br>- Toxicology<br>- Bioavailability<br>- Pharmaceutical R&D and formulation<br>- Scaling up of production | Study on humans:<br>- PK/PD<br>- ADME<br>- Drug interactions<br>- Food interaction<br>- Bioavailability<br>- Formulation<br>- Dose<br>- Safety<br>- Tolerability<br>- Efficacy<br>- Effectiveness |
| Objective | - Identification of lead candidates for animal studies | - Identification of lead candidates for studies in humans | - Registration |

PK/PD: Pharmacokinetics/Pharmacodynamics

ADME: Adsorption, Distribution, Metabolism, Excretion

log change in colony forming units (CFU) per ml of sputum over the first few days of treatment as determined by quantitative sputum culture. This is a continuous outcome measure, which allows detection of differences with much smaller sample sizes than those necessary for dichotomous variables (e.g., culture-positive vs. culture-negative). As a cones-quence EBA studies have typically been carried out on as few as 4 to 10 patients per study arm [111]. These studies can also examine the effects of the human pharmacokinetic behavior of a new drug in patients. A major drawback of standard EBA studies is that – by the nature of their short duration - they do not measure the sterilizing activity of a drug against persistent bacilli [112]. In some studies, investigators have attempted to overcome this limitation by conducting an "extended EBA" trial in which study treatment is continued for up to 14 days. Longer durations of study with monotherapy are considered unethical because of the possibility drug resistance developing and the desire not to withhold curative treatment for an excessive period of time. Importantly, one can envision how a similar "EBA" approach might be taken towards evaluating the efficacy in patients of a novel drug *combination*. In such a study, treatment duration could more readily be extended to even longer than two weeks, as long as the combination contains more than one bactericidal agent, since development of drug resistance should be prevented.

The need for TB treatment to consist of a *combination regimen* rather than a single drug creates a second challenge inherent in TB drug development. This need renders it impossible to demonstrate fully that any single new compound is safe, tolerable and efficacious in patients. As noted, only combination regimens can be tested in man for an extended period of time. The identification of the best drug combination(s) in which to use a novel compound is a critical and complex decision that needs to be based first on *in vitro* and animal data and then confirmed in man [113].

The need for extensive data on drug-drug interactions and on the safety of the proposed drug combination is significantly more extensive than is required by clinical development of most single, non-TB products. For example, drug-drug interaction data are needed not only on interactions between the TB drugs, but also between TB drugs and ARVs. Given the frequent co-infection of individuals by *M. tb* and HIV, minimizing drug-drug interactions is an important element of target product profiles for new TB drugs and an aspect that needs to be addressed early in the discovery phase of TB drug R&D.

After the identification of optimized drug combinations in preclinical models, and once a sufficient body of safety, tolerability and pharmacokinetic and pharmacodynamic data has been accumulated on each individual drug as well as on the combination, the combination's further evaluation must then be pursued in relatively small Phase II efficacy trials. Typically these studies require a sample size varying between 50 and 150 patients per arm, depending on the study assumptions, hypotheses, and selected endpoints. These trials are usually designed to test the hypothesis that the new therapy will confer an efficacy advantage in the target population relative to standard therapy, with a frequent endpoint being the rate of sputum culture conversion after the first two months of therapy as a marker for treatment-shortening potential. Endpoints may also include average time to negative sputum culture, and rate of fall in viable colony forming units in the sputum or serial sputum colony counts (SSCC) over two months [114]. These endpoints represent only an indicator of efficacy, but they may provide – within a reasonably short amount of time – the guidance needed to decide whether to advance the novel combination into later stage trials or terminate the program.

A third challenge of particular relevance to TB drug development is the long timeline required for TB clinical trials. Once a new regimen successfully completes Phase II, it needs to go through the much lengthier process of Phase III evaluation. In order to provide the final and unquestionable demonstration of the regimen's safety and efficacy, these trials require a different set of endpoints. The 'gold standard' efficacy endpoint in TB Phase III trials is the combined rate of treatment failure and relapse of TB disease within one to two years post treatment completion, as this gives the best measure of the curative capability of a given treatment. So, one must add a six month treatment duration and 1-2 year follow-up post-treatment to the usual time needed for study preparation (development of the protocol, identification and enrollment of the sites, training, regulatory issues like sourcing the appropriate study drugs and materials, obtaining all necessary approvals from the regulatory authorities and from the relevant ethical review committees), trial implementation (patient enrollment monitoring and data collection) and data clean up and analysis.

It can be easily understood why the development of new TB drugs requires a long time. It has been estimated that clinical evaluation of a drug combination containing a single new compound requires a minimum of six years. Significantly reducing these development times will require surrogate endpoints that can be measured earlier than one to two years after the end of treatment. The identification of one or more validated surrogate markers of drug efficacy would allow the shortening of pivotal TB trials, and possibly a quantum leap in the development of new treatments for TB, similar to what happened in the field of HIV research with the identification and validation of CD4 cell counts and viral load as surrogate markers for the AIDS 'gold standard' endpoint, i.e. survival. For an endpoint to be considered an adequate 'surrogate' marker, that is a reliable predictor of the main outcome of interest, it needs to be validated first through full-scale clinical trials comparing the candidate marker to the "gold standard" endpoint. The process of candidate marker identification is ongoing in TB drug development, and a number of possible markers are under consideration [115-119].

A fourth challenge in TB drug clinical development is posed by the very high efficacy of current first-line TB treatment when administered under clinical trial conditions. The superiority design traditionally used in pivotal Phase III trials for the development of many non TB drugs implies the comparison of two treatments using a given set of statistical tests that allow one to conclude whether in fact the experimental treatment is superior (as defined by the primary endpoint(s)). The sample size for a clinical trial is determined by the size of the comparator treatment effect, i.e. of the expected difference between the two treatments, and by its variability. Given that the efficacy of the existing standard, first-line TB treatment is very high under trial conditions (≥95%) [120], and that a possible incremental improvement could only be of a few percentage points, a very large sample size would be needed to prove a statistically significant superiority in the efficacy rates with a given regimen, and – even if it were to be done - the clinical relevance of the difference might not justify the number of patients treated (and exposed to the risks of a new regimen) and the amount of resources that would be required for such a study design. Most importantly, the goal of new TB treatment is not necessarily to improve efficacy beyond an already impressive 95% but rather to shorten and/or simplify treatment while maintaining this high efficacy. For these reasons, Phase III trials for new TB drugs use a different design, known as a 'non inferiority' design [121]. In this approach the investigators, based on clinical information, identify a range within which the primary study endpoint for the treatment arms needs to fall in order to be able to conclude that the experimental arm is not statistically inferior to the comparator arm with respect to efficacy. The superiority of the novel treatment would derive from its ability clinically to shorten or simplify treatment relative to the control regimen (for example, by demonstrating that a four month treatment with an experimental regimen has an efficacy that is statistically 'non inferior' to that of a six month treatment with standard therapy). A frequently contro-

versial issue in this type of design is the identification of the range of values within which the experimental treatment may be considered non-inferior to the control therapy, also referred to as the "delta". The delta must be adequately justified from a clinical point of view.

A fifth challenge inherent in TB drug development is ensuring available site capacity for conducting clinical trials that meet regulatory standards. In the last two decades, regulatory requireements have significantly increased; guidelines have been issued that regulate clinical trials in many countries. A process is in place (*The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use - ICH*) for the harmonization of regulatory requirements for registering new products in Europe, Japan and the United States. The ICH process has embraced a strict set of guidelines known as Good Laboratory Practice (GLP) and Good Clinical Practices (GCP) which govern clinical trial procedures [122]. These guidelines, consistent with the Declaration of Helsinki [123] require that all trials use clearly documented protocols specifying research objectives, patient recruitment and treatment allocation, study procedures and endpoints, independent monitoring of study implementation, adverse events documentation and reporting, and data management and analysis. They also spell out clearly the roles and responsibilities of all the parties involved in the trial. Previous guidelines are outdated, and compliance with these new complex regulations requires extensive training and significant resources. Activities aimed at registration of new TB treatments have been sparse for the past several decades, and as a consequence there are only a limited number of sites currently capable of implementing late stage TB trials that are compliant with the existing regulations. During the past decade, as already mentioned, thanks to the combined activities of several organizations, there has been a resurgence of activities in TB trials, which in turn has led to some initial build-up of capacity and resources at a number of sites and the specific training of their staff in the existing guidelines. The US CDC has established the TB Trials Consortium, CDC TBTC [124], with trial sites in the Americas, Europe and Africa conducting studies of new drugs and regimens [125]. The U.S. NIH supports the Tuberculosis Research Unit, which has conducted Phase I and II clinical trials largely outside the United States. The International Union Against Tuberculosis and Lung Disease has also been active in this area by developing sites in Africa, Asia and Latin America. The EDCTP [126] has recently pledged €600 million over five years to establish capacity for and conduct of high-quality clinical trials, including those for tuberculosis, throughout Africa. In addition, some public sector institutions in countries such as Brazil, India, and South Africa have accumulated significant expertise in the conduct of tuberculosis drug trials. Finally, a global survey of capacity to conduct GCP/GLP-compliant TB drug registration trials has been implemented recently by the TB Alliance [127]. To date, the survey has involved the review of facilities (clinical and laboratory) and staff of 51 sites in 25 countries, and has assessed the sites for their capacity and readiness to participate in late stage registration TB drug trials (and EBA studies in some cases) in accordance with the ICH guidelines [128]. Results of this survey are currently being compiled and analyzed.

A sixth challenge inherent in TB drug development is regulatory in nature, and related to the limited activity in the field of TB drug development over the last several decades. This lack of activity has impacted the regulatory agencies, where specific regulatory guidances for TB drug development have not been issued. Development of TB-specific guidances by major Regulatory Agencies would be important in clarifying and streamlining the development of novel, improved TB treatment regimens. Discussions of many of the key issues have recently (2005, 2006) been initiated in a series of Open Fora, [129] bringing together regulators from industrialized and high burden countries, sponsors, public health officials, interested academic researchers, patient representatives and other stakeholders [130].

Finally, a seventh challenge to the development of TB drugs is the lack of incentives for pharmaceutical companies to develop new compounds for the treatment of TB. Not only does TB research and development face significant challenges, including the i) limited understanding of *M. tb* biology, ii ) difficulty of identifying active new compounds, and iii) complexity of clinical development of promising new TB compounds, but tuberculosis is not currently considered to be a major threat to the industrialized world, i.e. to the major markets. The estimated total cost for a standard treatment course currently is as little as $19 [131], which is not sufficient to generate major interest from the pharmaceutical industry.

## THE FUTURE OF TB DRUG DEVELOPMENT

The regimens currently recommended for treatment of drug-sensitive TB are considerably simpler than the initial TB treatment regimens, having been shortened from two years to six months; however they are still far from optimal. A major focus of current efforts in TB drug development therefore is the identification and registration of shorter, simpler treatments. The development of a two to three month regimen with once weekly dosing of three to four drugs would result in decreasing the duration of treatment from the currently recommended twenty-eight weeks to eight to twelve weeks, and from approximately one hundred and thirty doses of a combination regimen to ten. Such a change should have a significant positive impact on control of the disease by improving patient adherence, and on inhibiting development of drug resistance by improving treatment completion rates. However, reaching this objective of a two month regimen will likely require a substantially new therapeutic armamentarium. As previously stated, one of the key challenges in the field of TB drug development is that the therapeutic unit is a combination regimen, not a single drug. It has been estimated that the clinical testing of a TB combination regimen containing a single new compound requires a minimum of six years. Assuming that the adoption of a new combination would be immediate, and that one could move from one new combination to the next one with no idle time, a simple mathematical operation allows one to conclude that in order to go from an existing and accepted combination of four drugs to a totally new four-drug combination by substituting the four components serially would require a minimum of twenty-four years. This is clearly not an acceptable timeframe given the severity of the TB epidemic globally, and therefore alternative development approaches must be found. A new paradigm is needed for the rational selection and development of new drug combinations. Pre-clinical combination testing is an essential step in the search for a combination that will allow shortening treatment duration to two months, have no interactions with ARVs and be effective against MDR-TB. Preclinical combination testing should proceed in parallel with testing individual new drugs in standard Phase I and early proof of concept studies, followed by testing optimized combinations as the developmental unit in human studies to evaluate their PK interactions, safety, tolerability, early bactericidal and sterilizing activity. An extensive effort of this kind should lead to the identification of a very small number of drug combinations deserving further pre-clinical and clinical development. This approach will require a new cooperation amongst sponsors and a true paradigm shift, both among researchers and regulators. Finally, this approach should ideally be accompanied by the availability of surrogate markers to streamline development timelines, and by the build-up of clinical trial site capacity for the conduct of modern, registration-standard clinical trials.

If new drugs developed for active TB are based on novel mechanisms of action relative to current TB therapy, and are screened early to avoid compounds with undesirable drug-drug interactions, successful products should prove equally effective for

drug-sensitive and MDR/XDR TB, and for treatment of HIV-negative and HIV-positive patients. Achieving the ultimate objective, i.e. a TB treatment regimen consisting of a ten day or two week course of antibiotics, comparable to present treatments for acute respiratory infections, will likely require a much deeper understanding of the biological mechanisms of *M. tb* persistence that underlie the need for current prolonged antibiotic therapy.

The combined effort of stakeholders, including funding agencies - both governmental and private - basic researchers in the fields of biology and chemistry, clinicians and regulators will be instrumental in addressing the challenges described above, and in consolidating and expanding the pipeline of novel drugs for TB. This in turn would have a meaningful impact towards reaching important goals, which now appear reachable, such as low cost, dramatically shortened, safe and effective treatment for drug-sensitive and MDR/XDR TB, in both HIV-positive and HIV-negative patients.

## ABBREVIATIONS

| | | |
|---|---|---|
| ADME | = | Absorption, Distribution, Metabolism and Excretion |
| AIDS | = | Acquired Immune Deficiency Syndrome |
| ART | = | Antiretroviral Therapy |
| ARVs | = | Anti-retroviral agents |
| BUN | = | Blood Urea Nitrogen |
| CDC | = | U.S. Centers for Disease Control and Prevention |
| DNA | = | Deoxyribonucleic acid |
| DoH | = | Declaration of Helsinki |
| DOTS | = | Directly Observed Treatment – Short Course |
| EBA | = | Early Bactericidal Activity |
| EDCTP | = | European and Developing Countries Clinical Trials Partnership |
| EMB | = | Ethambutol |
| FDA | = | U.S. Food and Drug Administration |
| GCP | = | Good Clinical Practices |
| GLP | = | Good Laboratory Practices |
| GSK | = | Glaxo-Smith-Kline |
| HIV | = | Human Immunodeficiency Virus |
| HTS | = | High-throughput Screening |
| ICH | = | The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| IND | = | Investigational New Drug |
| IRD | = | Institut de recherche pour le développement |
| IUATLD | = | International Union Against Tuberculosis and Lung Disease |
| LTBI | = | Latent Tuberculosis Infection |
| *M. tb* | = | *Mycobacterium tuberculosis* |
| MAC | = | Mycobacterium avium Complex |
| MDR-TB | = | Multi-drug Resistant tuberculosis |
| NIH | = | U.S. National Institutes of Health |
| NNRTIs | = | Non-nucleoside Reverse Transcriptase Inhibitors |
| NOAEL | = | No Observed Adverse Effects Level |
| PK | = | Pharmacokinetics |
| POC | = | Proof of Concept |
| REMox TB | = | Rapid Evaluation of Moxifloxacin in TB |
| RNA | = | Ribonucleic acid |
| SSCC | = | Serial Sputum Colony Counts |
| $t_{½}$ | = | Half-life |
| TB | = | Tuberculosis |
| TB Alliance | = | Global Alliance for TB Drug Development |
| TBTC | = | Tuberculosis Trial Consortium |
| $T_{max}$ | = | Time to maximum concentration |
| WHO | = | World Health Organization |
| XDR-TB | = | Extreme Drug Resistant Tuberculosis |

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