

## New Drugs for Tuberculosis: Current Status and Future Prospects

Richard J. O'Brien, MD<sup>a,\*</sup>, Mel Spigelman, MD<sup>b</sup>

<sup>a</sup>Foundation for Innovative New Diagnostics, Case Postale 93, 1216 Cointrin/Geneva, Switzerland

<sup>b</sup>Global Alliance for TB Drug Development, 80 Broad Street, 31st Floor, New York, NY 10004, USA

Following nearly 3 decades of neglect, there is now renewed interest in the development of new drugs for the treatment and prevention of tuberculosis [1]. Three reasons are usually given for needing new tuberculosis drugs: (1) to improve current treatment of active tuberculosis by shortening the total duration of treatment or by providing for more widely spaced intermittent therapy; (2) to improve the treatment of multidrug-resistant tuberculosis (MDR-TB), and (3) to provide more effective treatment of latent tuberculosis infection (LTBI) in low-incidence countries where this intervention is a component of the control strategy. Of these, the first is most compelling.

Despite the great decrease in tuberculosis incidence throughout the latter half of the twentieth century in industrialized countries, the disease remains a significant global health problem, particularly among adults in developing countries [2]. In countries affected by the AIDS epidemic, notably those in sub-Saharan Africa, rates of tuberculosis have increased dramatically, overwhelming control programs [2]. The World Health Organization (WHO) has recently promoted the directly observed treatment, short course (DOTS) strategy as an effective intervention that will lead to reduced tuberculosis transmission and decreasing numbers of tuberculosis cases [3]. This strategy has been shown to be among the most cost-effective global health interventions available today [4]. An important component of that strategy is the provision

of high-quality drugs in standardized regimens of short-course, rifampin-based treatment given under direct supervision.

The current treatment regimens, however, suffer from a number of drawbacks. With the combination of available drugs, the duration of treatment required for curing patients cannot be reduced below 6 months without a significant increase in relapses. When given under suboptimal conditions, these regimens are associated with high rates of patient nonadherence, with the consequence of increased mortality and creation of chronic, infectious, drug-resistant cases [5]. It is recommended that treatment be directly observed by a health care provider, especially during the first 2 months and whenever rifampin is used. The infrastructure required is cumbersome, labor intensive, and expensive. Thus, shorter treatment regimens or those that could be administered once or twice a week would significantly improve treatment outcome.

Development of drug resistance is far more likely when supervised treatment is not given, when recommended regimens are not used, and when drugs with poor bioavailability are used. All these factors are frequently present in countries where DOTS has not been established. WHO has documented an increasing problem of MDR-TB that threatens to undermine recent progress in global efforts to control the disease [6]. The second-line drugs that are used for treatment of MDR-TB are much more expensive, more toxic, or less effective than first-line drugs. Although the development of more effective therapy for MDR-TB would not alone solve the problem, providing better treatment would be an important personal health benefit for those afflicted by MDR-

\* Corresponding author.

E-mail address: rick.obrien@finddiagnostics.org (R.J. O'Brien).

TB and would improve the effectiveness of the WHO-supported MDR-TB treatment programs known as DOTS-Plus [7].

The resurgence of tuberculosis in the United States beginning in the late 1980s, coupled with the outbreaks of MDR-TB largely associated with HIV infection, led to increased federal support for both domestic and global tuberculosis control [8]. That support has resulted in continued declines in tuberculosis in the United States beginning in 1993 and a renewed call for the elimination of tuberculosis as a public health problem [9]. An important component of the tuberculosis elimination strategy in the United States is the treatment of individuals who have LTBI and are at increased risk of developing active TB [10]. The most widely used LTBI treatment regimen, 9 months of isoniazid, is associated with significant nonadherence, however. Thus, a more easily administered LTBI treatment regimen is a priority in a number of low-incidence countries.

#### **Tuberculosis drug development—a changing environment**

Increased resources directed toward tuberculosis drug development are now being marshaled from both the public and private sectors. Governmental organizations, such as the United States National Institutes of Health (NIH), are investing in basic research aimed at the identification of new drug targets and a better understanding of the phenomena of mycobacterial latency. Foundations, such as the Bill and Melinda Gates Foundation, are supporting research and development to enhance the understanding of the basic biology of tuberculosis and to develop new tuberculosis drugs. A number of small biotech companies have programs focused on the identification of new chemical entities with antimycobacterial activities that could become lead compounds in the drug-development process. Several large pharmaceutical companies, such as GlaxoSmithKline (Brentford, United Kingdom), AstraZeneca (London, United Kingdom), and Novartis (Basel, Switzerland), have launched programs directed at the discovery and development of new tuberculosis drugs. Other companies, notably Aventis and Bayer, have made compounds available for clinical studies.

At the same time, the clinical trials infrastructure, which had been greatly eroded beginning in the early 1980s, is being reestablished with the formation of groups such as the United States Tuberculosis Trials Consortium (TBTC) [11] sponsored by the Centers for Disease Control and Prevention (CDC) and the

Clinical Trials Program of the International Union Against Tuberculosis and Lung Disease. With support from the European Community, the European and Developing Countries Clinical Trials Partnership aims to provide €600 million over 5 years to perform clinical trials and to establish capacity for the conduct of high-quality clinical trials, including those for tuberculosis, throughout Africa [12]. Underpinning all this effort is the Global Alliance for TB Drug Development (TB Alliance), a recently established organization that is forging public-private partnerships with the objective of building a portfolio of new tuberculosis drugs and bringing a major new tuberculosis drug to market in the next decade [13].

This article reviews two classes of compounds that have advanced into phase II and III clinical trials, long-acting rifamycins and fluoroquinolones, and a number of other drugs that have entered or it is hoped will enter clinical development in the near future.

#### **Rifapentine: the search for widely spaced intermittent treatment**

Rifampin is the cornerstone of modern short-course tuberculosis treatment, but rifampin-based regimens must be administered for at least 6 months for optimal effectiveness. Although this treatment is also highly effective when given three times per week throughout the course of treatment [14], more widely spaced regimens are less effective and may be associated with acquired drug resistance in HIV-infected patients, even when properly taken.

A number of rifamycin derivatives with much longer serum half-lives than that of rifampin (2–4 hours) have been evaluated in regimens given intermittently. The first of these compounds to undergo clinical investigation was rifabutin [15]. The initial clinical trials of the drug focused on the prevention of *Mycobacterium avium* complex (MAC) infection in HIV-infected patients [16]. Although the drug was approved for MAC prophylaxis in the United States and for the treatment of tuberculosis in several other countries, it now is used primarily as a substitute for rifampin in patients who cannot use that drug because of drug-drug interactions [17]. A TBTC trial of a rifabutin-containing regimen given twice weekly in HIV-infected patients found high rates of acquired rifamycin resistance among patients who had more advanced immunosuppression, leading to CDC recommendations against the use of widely spaced treatment of tuberculosis with rifamycin regimens in such patients [18].

Another long-acting rifamycin derivative, rifalazil, has an even longer half-life and potent activity in animal models suggesting that it might be used in ultrashort treatment regimens [19]. One attractive feature of the compound is its rather low potential for enzyme induction and drug interactions [20]. Initial phase I tolerability studies, however, found relatively high rates of side effects manifesting as a flulike syndrome when the drug was administered as a single 50-mg dose [21]. The hypothesized mechanism causing the dose limiting side effect is release of cytokines with evidence for increased interleukin-6 levels in the serum. Following an early bactericidal activity (EBA) study that did not demonstrate drug activity of once-weekly rifalazil (at 10- and 25-mg doses) plus isoniazid given for 2 weeks [22], further clinical development stopped. It is believed that closely related compounds can be identified that are better tolerated and lack the propensity for enzyme induction. Currently, there is significant interest in the use of rifalazil for the treatment of chlamydia infections [23].

The greatest interest and investment in long-acting rifamycins has been in rifapentine, a cyclopentyl-substituted rifampin with a half-life of 14 to 18 hours in normal adults. Following a 600-mg dose, serum levels in excess of the minimum inhibitory concentration (MIC) persist beyond 72 hours, suggesting that the drug might be useful in intermittent regimens (Fig. 1). A series of experimental studies in mice found that a once-weekly continuation phase of rifapentine and isoniazid for 4 months following a standard 2-month induction phase with daily isoniazid, rifampin, and pyrazinamide was as effective as stan-

dard therapy given daily for 6 months [24]. These studies provided the scientific underpinning for the large phase III trial that was begun by CDC in 1995 and subsequently became known as TBTC Study 22.

Study 22 was an unmasked clinical trial that randomly assigned adults who had newly diagnosed, drug-susceptible pulmonary tuberculosis to a 4-month (16-week) continuation-phase regimen of either once-weekly rifapentine-isoniazid or twice-weekly rifampin-isoniazid following successful completion of a standard 2-month induction phase [25]. The primary study end points were treatment failure and relapse and safety and tolerability of rifapentine. The rifamycins were dosed at 600 mg and isoniazid at 900 mg. Although the trial focused on HIV-negative patients, HIV-positive patients were also enrolled initially to gain experience with this important subset of patients. Enrollment of HIV-positive patients was stopped early in the trial, however, following the finding of a high rate of relapse with acquired rifampin monoresistance among HIV-positive patients assigned to the rifapentine arm [26].

A total of 1003 HIV-negative patients were enrolled into the completed study. The crude rate of failure and relapse was significantly higher in the rifapentine arm (9.2% versus 5.6%,  $P=0.04$ ). In a multivariate analysis, the factors statistically associated with an adverse outcome were the presence of cavitory disease on chest radiograph, sputum culture positivity at study entry (ie, at the end of the intensive phase of therapy), white race, and weight less than 90% of ideal body weight at time of the diagnosis of tuberculosis. The treatment regimen was not associ-

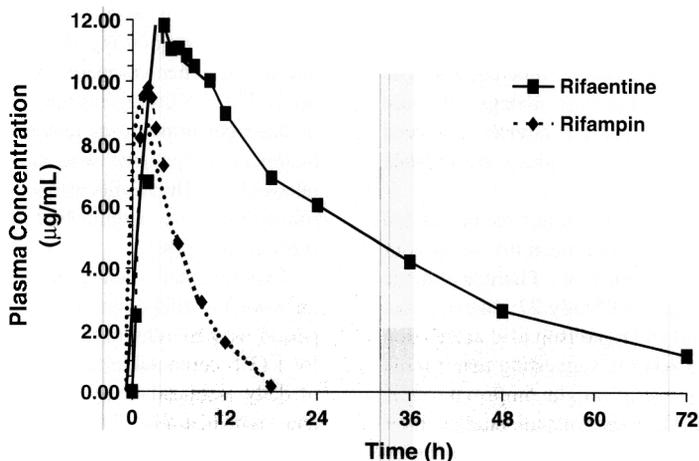


Fig. Rifampin and rifapentine time-concentration curves following 600-mg dose in normal adults.

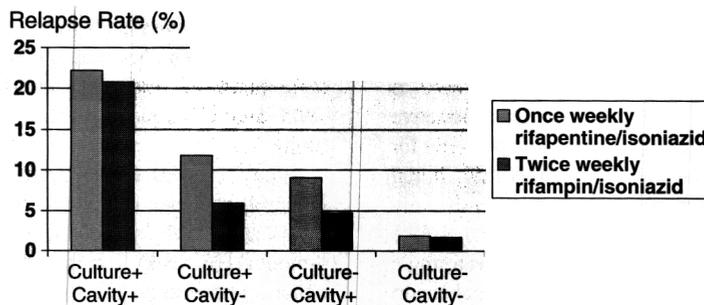


Fig. 2. Tuberculosis Trials Consortium study 22. Relapse rate by arm, cavitory chest radiograph, and 2-month culture. (Adapted from Tuberculosis Trials Consortium. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. *Lancet* 2002;360:528–34; with permission.)

ated with an adverse outcome. Cavitory disease and culture positivity after 2 months were also predictors of an adverse outcome among patients in the rifampin arm (Fig. 2). Among patients who had noncavitory 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 both groups discontinuing treatment because of a drug-related adverse event. These results were similar to those from a study in Hong-Kong that used Chinese-manufactured rifapentine of inferior bioavailability [27] and with those from a company-sponsored trial that enrolled patients largely from Africa [28].

The TBTC study results led to new recommendations for the use of the rifapentine-isoniazid continuation-phase regimen for HIV-negative adults who have drug-susceptible, noncavitory tuberculosis and negative acid-fast bacillus (AFB) smears at 2 months [29]. This category includes approximately 40% of patients in the United States who have newly diagnosed pulmonary tuberculosis. The regimen provides substantial cost savings for these patients, because encounters for directly observed treatment during the continuation phase are reduced by 50% [30].

Rifapentine-based treatment is not recommended for patients who have more advanced tuberculosis or patients who have HIV infection. Pharmacokinetic studies undertaken as part of Study 22 indicated that low levels of isoniazid and rapid isoniazid acetylation were associated with relapse, suggesting that a more effective companion drug might improve once-weekly treatment [31]. Experimental studies have also suggested that, in addition to a better companion drug, higher doses of rifapentine might also result in more effective treatment [24].

Following the completion of Study 22, the TBTC undertook a large phase II trial of higher rifapentine doses. In Study 25, 150 HIV-negative patients who had drug-susceptible pulmonary tuberculosis and completed initial-phase treatment were randomly assigned to 600, 900, and 1200 mg rifapentine given once weekly with isoniazid for 16 weeks. The rifapentine dose was masked with the use of dummy tablets of rifapentine. The primary study end points were adverse events and drug discontinuation. All regimens were well tolerated, and only one patient assigned to the 1200-mg dose stopped treatment because of a possible drug-related adverse event [32]. Because the results of Study 22 were known when this study began, the protocol was modified to provide extended treatment for an additional 3 months (or 12 weeks) for patients who had cavitory disease and had positive sputum cultures at entry (ie, at 2 months). Twenty such patients were enrolled, received extended treatment, and were followed prospectively for relapse. Only one patient who was assigned to the 600-mg dose relapsed. The relapse rate of 5%, when compared with historical data from Study 22 (22%), suggests that extended treatment and higher rifapentine doses may provide more effective treatment for patients who are at increased risk of relapse [33]. The results also suggest that the 900-mg rifapentine dose would be appropriate to use in subsequent trials.

Experimental studies have also suggested that once-weekly rifapentine and isoniazid for as short a period as 3 months may provide effective treatment for LTBI, comparable to that conferred by 6 months of daily isoniazid or by 2 months of daily rifampin and pyrazinamide [34]. Based on these findings, the TBTC has embarked on an ambitious study of rifapentine/isoniazid for LTBI treatment, intending to enroll and randomly assign 8000 patients to either

9 months of daily self-administered isoniazid or 12 doses of once-weekly rifapentine/isoniazid. Because of the large sample size required and the capacity of the TBTC sites to enroll eligible patients, study completion is not expected before 2008.

### Moxifloxacin: the next treatment-shortening drug?

During the past decade, fluoroquinolone antibiotics have become the most important second-line drugs for treating patients who have MDR-TB. Until recently, however, these drugs have not been considered for the treatment of drug-susceptible disease, in part because the few randomized, controlled trials of fluoroquinolones for drug-susceptible tuberculosis that have been conducted have not demonstrated a benefit. This perspective began to change with the publication of a clinical trial conducted by the Tuberculosis Research Centre in Chennai, India. This study, which did not have a standard control group, randomly assigned patients who had newly diagnosed pulmonary tuberculosis to one of four ofloxacin-containing regimens [35]. Rates of 2-month sputum culture conversion, a marker of the sterilizing activity of tuberculosis drug regimens [36], ranged from 92% to 98%, which compares favorably to an expected rate of approximately 80% with standard four-drug treatment [25]. Rates of relapse during the 2 years following completion of treatment were 2% and 4% in patients randomly assigned to 3 months of daily isoniazid, rifampin, pyrazinamide, and ofloxacin,

followed by twice-weekly isoniazid and rifampin for 1 and 2 months, respectively. These results suggest that fluoroquinolones might permit substantial shortening of tuberculosis treatment from the current minimum of 6 months.

Recent experimental data also suggest that fluoroquinolones may be potent sterilizing drugs that could allow shortened regimens for the treatment of active tuberculosis, including MDR-TB, and be effective against LTBI. Thus, newer fluoroquinolones have the potential to achieve all three objectives of a new tuberculosis drug. Several fluoroquinolones with markedly enhanced in vitro activity against *M. tuberculosis* are now available. Of these, the most potent are moxifloxacin and gatifloxacin. The MICs of these two agents are fourfold lower than that of levofloxacin, the fluoroquinolone that is currently preferred for the treatment of drug-resistant tuberculosis [37,38]. Moxifloxacin also has excellent activity against *M. tuberculosis* in animal models [39,40]. A recent evaluation of fluoroquinolones in a model of mycobacterial persistence found that moxifloxacin had the greatest sterilizing activity [41]. The pharmacokinetic profile of moxifloxacin, with a relatively long half-life and high area under the time concentration curve, also suggests that this agent may be an ideal antimycobacterial drug [42].

A series of studies of moxifloxacin in mouse models of acute tuberculosis have also contributed to the interest in this drug. The initial study, in which infected mice were treated for 1 month with several fluoroquinolones, found that moxifloxacin has the greatest bactericidal activity, comparable to that of isoniazid (Fig. 3) [39]. A second study suggested that

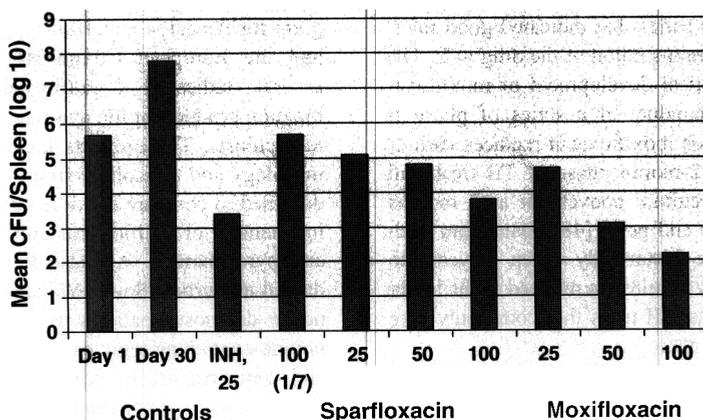
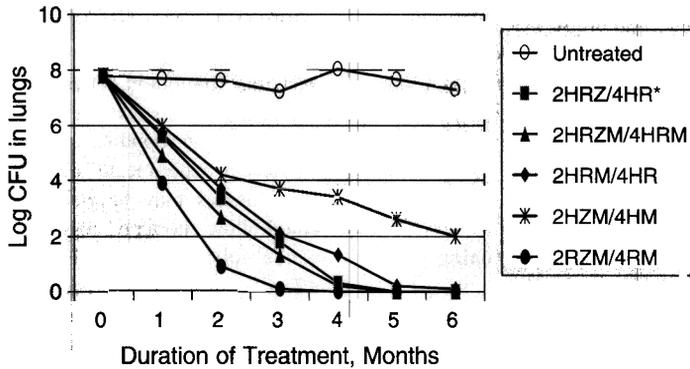


Fig. 3. Thirty-day experimental study of isoniazid (INH), sparfloxacin, and moxifloxacin in a mouse model of acute tuberculosis. Drug doses in mg/kg. (Adapted from Ji B, Lounis N, Maslo C, et al. In vitro and in vivo activities of moxifloxacin and cinafloxacin against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1998;42:2066–9; with permission.)



\*H=isoniazid, R=rifampin, Z=pyrazinamide, M=moxifloxacin

Fig. 4. Experimental study of moxifloxacin-containing regimens in murine tuberculosis. (Adapted from Nuernberger EL, Yoshimatsu T, Tyagi S, et al. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *Am J Respir Crit Care Med* 2004;169:334–5; with permission.)

moxifloxacin also has potent sterilizing activity and might substantially improve the efficacy of once-weekly rifapentine treatment, replacing isoniazid that has been shown in clinical studies to be a poor companion drug [43]. The most recent study found that the combination of rifampin, pyrazinamide, and moxifloxacin had substantially greater sterilizing activity than the standard regimen, again suggesting the possibility that the drug would permit significant shortening of treatment (Fig. 4) [44].

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, the most potent bactericidal drug in EBA studies [45,46]. The only other published experience with moxifloxacin treatment of tuberculosis is a small case series that indicated good tolerability to chronic administration of the drug [47]. The next step in the clinical development of moxifloxacin for TB is the conduct of a series of phase II clinical trials in which moxifloxacin replaces various drugs in the initial 2-month phase of TB treatment and where sputum culture conversion at 2 months is the primary study end point [48]. Data from such studies, which have historically taken 2 years to complete, are usually required to proceed to the larger and more costly phase III trials that commonly take much longer to complete.

To develop clinical data that would justify larger phase III efficacy trials of moxifloxacin, the TBTC has embarked on a phase II trial of the drug, Study 27. This study randomly assigns newly diagnosed, AFB-positive, HIV-positive and -negative patients who have suspected pulmonary tuberculosis to

one of four 2-month intensive-phase regimens: two standard-treatment regimens given either daily or three times weekly or similar regimens in which moxifloxacin replaces ethambutol, with assignment masked by placebo moxifloxacin and ethambutol. The primary study end points are 2 month sputum culture conversion and withdrawal because of adverse events. Investigators from Johns Hopkins University are working with colleagues from Rio de Janeiro on a similar study that is supported by the United States Food and Drug Administration Office of Orphan Products Development (R. Chaison, personal communication, 2004).

A product development team supported by the United Nations Childrens Fund/United Nations Development Program/World Bank/WHO Special Program for Research and Training in Tropical Diseases and the European Commission is embarking on several studies of a gatifloxacin fixed-dose combination product for the treatment of drug-susceptible tuberculosis. These efforts include preclinical pharmacology and toxicology studies and a phase I study designed to compare the drug–drug/pharmacokinetic interactions of gatifloxacin and isoniazid, rifampin, and pyrazinamide. A phase II study is being conducted in Durban, South African, randomly assigning newly diagnosed patients to one of three fluoroquinolone-containing regimens (ofloxacin, moxifloxacin, and gatifloxacin) in combination with isoniazid, rifampin, and pyrazinamide during the first 2 months of treatment. A variety of bacteriologic markers are being evaluated as potential surrogate markers of treatment response. A large phase III trial of gatifloxacin included in a 4-month regimen that intends

to enroll over 2000 patients at centers in five countries in sub-Saharan Africa was expected to begin in late 2004 (C. Lienhardt, personal communication, 2004).

### The emerging tuberculosis drug pipeline

In addition to the rifamycin derivatives and fluoroquinolones, a variety of other compounds or classes of compounds are under investigation as potential antimycobacterial drugs. These include a diarylquinoline (R207910), a nitroimidazopyran (PA-824), a nitro-dihydroimidazo-oxazole (OPC 67,683), a pyrrole (LL3858), macrolides, oxazolidinones, and a diamine (SQ109).

#### *Diarylquinolines (R207910)*

The diarylquinolines, under investigation by Johnson & Johnson (New Brunswick, New Jersey), have been shown to have potent *in vitro* activity against *M. tuberculosis* and seem promising in an animal model [49]. The lead compound, R207910, is currently in clinical testing in phase I studies. R207910 is equally active against drug-sensitive *M. tuberculosis* (MIC 0.03 µg/mL) and strains resistant to a variety of commonly used drugs such as isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide, and fluoroquinolones. Similar potency was also found against other mycobacteria, such as *M. smegmatis*, *M. bovis*, *M. avium*, and *M. fortuitum*, but the compound is not active against several other bacterial species, such as *Nocardia asteroides*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Hemophilis influenzae*. Two resistant *M. smegmatis* isolates were not cross-resistant to a wide range of antibiotics, including the fluoroquinolones. Thus, the mechanism of action of R207910 seems to be unique among the commonly used antimicrobials.

In addition to the *in vitro* activity of R207910, the compound has also shown excellent *in vivo* activity in mouse models of established and nonestablished disease. When R207910 was administered by gavage 5 days/week from day 1 to day 28 after intravenous inoculation of Swiss mice with 7-log colony forming units (CFU) of strain H37Rv *M. tuberculosis* (nonestablished infection model), the compound was able to prevent mortality at the lowest dosage used (1.5 mg/kg), prevent gross lesions at 6.5 mg/kg, and reduce CFU counts in lungs and

spleens at 12.5 mg/kg to the same extent as isoniazid (25 mg/kg). When therapy was started on day 14 after inoculation and continued until day 70 (established infection model), 12.5 mg/kg of R207910 was at least as active in decreasing CFU count in lung as was isoniazid (25 mg/kg) or rifampin (10 mg/kg). At a dose of 25 mg/kg, R207910 was even more active than at 12.5 mg/kg, reducing lung CFU count from 6 to 0.4 log. The combination of R207910 with any two of the three commonly used drugs (isoniazid, rifampin, and pyrazinamide) was more effective than the standard regimen of isoniazid, rifampin, and pyrazinamide. In fact, the combination of R207910, isoniazid, and pyrazinamide and the combination of R207910, rifampin, and pyrazinamide both resulted in negative spleen and lung cultures after 8 weeks of therapy.

Pending results of the phase I studies, the ability of R207910 to shorten the therapy of active TB will be tested.

#### *Nitroimidazopyrans (PA-824)*

The TB Alliance is developing PA-824, a novel nitroimidazopyran with a molecular weight of 359, for first-line therapy of active tuberculosis and for the treatment of MDR-TB. The history of the nitroimidazoles goes back to the 1970s, when Ciba-Geigy (Basel, Switzerland) explored a novel series of nitroimidazole compounds as radiosensitizing agents for use in cancer therapy. Subsequent studies described these compounds' antimicrobial activity, including activity against *M. tuberculosis*. Ciba-Geigy halted development when their lead compound (CGI-17341) was found to be mutagenic in the Ames assay. In the 1990s, PathoGenesis (Seattle, Washington) decided this class of compounds warranted further exploration for potential tuberculosis therapy and synthesized more than 700 novel compounds. They determined that the nitroimidazopyran PA-824 was the most active of these compounds against *M. tuberculosis* in a murine infection model [50].

Following Chiron's (Seattle, Washington) purchase of PathoGenesis in 2000, development of PA-824 was halted because of the company's decision to focus on other therapeutic areas. In 2002, the TB Alliance and Chiron signed an exclusive license agreement granting the TB Alliance worldwide rights to PA-824 and nitroimidazole derivatives. Since then, the TB Alliance has continued the development of PA-824.

A series of *in vitro* pharmacology studies indicate that PA-824 may be efficacious against both drug-

sensitive and drug-resistant tuberculosis. In vitro studies demonstrate that the MIC of PA-824 against a variety of drug-sensitive tuberculosis isolates ( $\leq 0.015$ – $0.25$   $\mu\text{g/mL}$ ) is similar to that of isoniazid ( $0.03$ – $0.06$   $\mu\text{g/mL}$ ). PA-824 is highly selective, with potent activity only against bacille Calmette-Guerin (BCG) and *M. tuberculosis* among the mycobacterial species tested, and without significant activity against a broad range of gram-positive and gram-negative bacteria (with the exception of *H. pylori* and some anaerobes). In vitro studies using anaerobic culture models indicate that PA-824 has activity against nonreplicating bacilli, whereas isoniazid does not have activity in these models. Finally, PA-824 has been shown to have activity against strains of tuberculosis with known resistance to standard anti-tuberculosis therapies, indicating a novel mechanism of action.

To evaluate in vivo activity, PathoGenesis tested PA-824 in a mouse model of tuberculosis, employing an *M. tuberculosis* reporter strain expressing firefly luciferase. PA-824 was administered orally at 25, 50, and 100 mg/kg/day in mice for 10 days, with isoniazid used in the control arm. Administration of PA-824 at all doses significantly reduced *M. tuberculosis* levels in both spleen and lung compared with controls and demonstrated a linear dose response. In longer-term studies, PA-824 at 50 mg/kg/day demonstrated reductions in bacillary burden similar to isoniazid at 25 mg/kg/day in murine lungs, and all mice treated with PA-824 survived infection, whereas all untreated control animals died by day 35. Daily oral administration of PA-824 at 37 mg/kg/day for 35 days in a guinea pig aerosol infection model also caused statistically significant reductions of *M. tuberculosis* in lungs and spleens compared with controls, reductions comparable to those caused by isoniazid.

The activity of PA-824 against MDR-TB isolates and against both replicating (aerobic) and nonreplicating (anaerobic) *M. tuberculosis* bacilli indicates this compound has a novel mechanism of action. PA-824 seems to inhibit significantly both protein and lipid synthesis but does not affect nucleic acid synthesis. PA-824 produces an accumulation of hydroxymycolic acid with a concomitant reduction in ketomycolic acids, suggesting inhibition of an enzyme responsible for the oxidation of hydroxymycolate to ketomycolate.

Unlike the Ciba-Geigy lead compound, CGI-17341, PA-824 has not demonstrated mutagenicity in the Ames test (with or without S9 activation), and initial toxicity studies indicated the doses needed for therapeutic activity in murine and guinea pig infec-

tion models are below the acute and chronic toxic thresholds observed for PA-824 in mice.

More recent studies by Grosset et al [51] have indicated that, in a murine model, the minimum effective dose (defined as the minimum dose which prevents the development of gross lung lesions and splenomegaly) of PA-824 is 12.5 mg/kg/day, that the absence of lung lesions on gross inspection correlates well with bacteriostatic activity measured by CFU count, that the minimum bactericidal dose (defined as the minimum dose which reduces the long colony forming unit counts by 99%) is 100 mg/kg/day, and that the activity of PA-824 at 100 mg/kg is comparable to the activity of isoniazid at 25 mg/kg.

The potential genotoxicity of PA-824 was examined further with chromosomal aberration, mouse micronucleus, and mouse lymphoma tests. The results indicate that PA-824 is not genotoxic. Furthermore, in vitro studies indicate that PA-824 neither inhibits nor is metabolized by major P450 enzyme isoforms.

Pharmacokinetic studies have been performed in the rat, dog, and monkey, because the systemic exposure in dogs is low for both males and females secondary to poor absorption and rapid metabolism. Results of the single-dose studies indicate that the half-life of PA-824 is approximately 2 to 5 hours in male rats and monkeys and trends toward a longer half-life in female rats (8–9 hours). The half-life in dogs is shorter (1–2 hours). In monkeys, single doses of PA-824 are rapidly absorbed with a time to maximal concentration ( $T_{\text{max}}$ ) of 3.33 hours or less, whereas  $T_{\text{max}}$  in the rat ranges up to 8 hours. There was no significant effect of sex on rate of absorption in any species. There was not a significant food-effect on PA-824 pharmacokinetics in the rat.

The pharmacokinetics of PA-824 was determined in plasma, heart, liver, kidney, spleen, and lung following a single 100-mg/kg oral dose of PA-824 in rats. The time to reach maximal concentrations of PA-824 in these tissues was 4 hours as compared with 6 hours in plasma. Exposure (area under the curve) in tissues was approximately three- to eight-fold higher than that in plasma. These data suggest that, in the rat model, penetration of PA-824 into lung, spleen, and other tissues is extensive. In repeated dose studies, there was no evidence of accumulation in the rat or monkey.

Two 14-day good-laboratory practice toxicology studies, one in the rat and one in the monkey, have been completed. The results of these studies indicate that toxicity is observed when exposures at or above approximately 500  $\mu\text{g}/\text{hour/mL}$  are achieved. Phase I

studies of PA-824 are planned for the first quarter of 2005.

#### *Dihydroimidazo-oxazoles (OPC-67683)*

OPC-67683 is a newly synthesized nitro-dihydroimidazo-oxazole derivative under development by Otsuka Pharmaceutical Company (Tokyo, Japan) for the treatment of tuberculosis and is currently in phase I study in normal volunteers (Otsuka Pharmaceutical Company, personal communication, 2004). The compound has potent *in vitro* antimicrobial activity against *M. tuberculosis*, with MICs against H37Rv and 67 clinically isolated strains ranging from 0.006 to 0.024  $\mu\text{g}/\text{mL}$ . Furthermore, OPC-67683 shows no cross-resistance with any of the currently used first-line tuberculosis drugs, most likely indicating a novel mechanism of action. Therefore the compound may be of benefit both in shortening duration of therapy in the treatment of active disease and in the treatment of MDR-TB.

*In vivo* studies using a chronic mouse model of tuberculosis have demonstrated the efficacy of OPC-67683 to be superior to that of the currently used tuberculosis drugs. In the mouse model, the dose that provided the effective plasma concentration of 0.100  $\mu\text{g}/\text{mL}$  was 0.625 mg/kg, confirming the remarkable *in vivo* potency of OPC-67683.

In other nonclinical *in vitro* and *in vivo* studies, OPC-67683 does not have any antagonistic activity with other first-line tuberculosis drugs when used in combination. Combinations with other first-line therapeutic drugs reveal synergistic, additive, or no appreciable interactions.

#### *Pyrrole (LL3858)*

Pyrrole derivatives were first described by Deidda et al [52] as having fairly potent antimycobacterial activities against several strains of *M. tuberculosis*. The MICs were between 0.7 and 1.5  $\mu\text{g}/\text{mL}$  for the most potent derivative, 1,5-diaryl-2-methyl-3-(4-methylpiperazin-1-yl) methyl-pyrrole (BM212). The activity of BM212 against various drug-resistant strains of *M. tuberculosis* was similar to its activity against sensitive strains, probably indicating a novel mechanism of action. Although some nontuberculous mycobacterial strains seemed to be sensitive, the MICs were higher than for *M. tuberculosis*.

A novel pyrrole compound, LL3858, is currently in development for tuberculosis by Lupin Limited (Mumbai, India). This compound has submicromolar MICs and seems to be very active in a mouse model of tuberculosis. In combination with currently used

antituberculous drugs, LL3858 sterilizes lungs and spleens of infected animals in a shorter timeframe than conventional therapy.

#### *Macrolides*

The Institute for Tuberculosis Research, College of Pharmacy at the University of Illinois at Chicago, in conjunction with the TB Alliance, is currently studying the potential for macrolide antibiotics in the treatment of tuberculosis. Among approved antimicrobial agents that do not include tuberculosis as an indication, the macrolides are one of the more promising to yield a clinically useful tuberculosis drug. This potential is based on their oral bioavailability and distribution to the lungs, low toxicity, infrequent adverse reactions, extensive intracellular concentration and activity, anti-inflammatory activity, and, perhaps most importantly, demonstrated clinical utility and bactericidal activity in infections caused by several pathogenic and opportunistic mycobacteria, including *M. avium*, *M. leprae*, *M. chelonae*, and *M. fortuitum*.

Erythromycin, the first-generation prototypical macrolide, is a natural product derived from *Streptomyces erythreus*. The compound interferes with protein synthesis and possesses most of the favorable properties mentioned previously but suffers from a short serum half-life and acid lability, which results in gastric motility-based discomfort. In addition, activity is restricted to gram-positive bacteria.

Therefore, second-generation macrolides with superior acid stability and serum half-life were developed. Clarithromycin, roxithromycin, and azithromycin represent the most successful second-generation macrolides. It quickly became apparent that the second-generation macrolides were, along with rifabutin, the most active clinical agents against the MAC. With the exception of azithromycin (an azalide that possesses a spectrum of activity different from that of other macrolides), these compounds also were found to possess potent activity against *M. leprae* in macrophages and mice and were shown to be effective in clinical trials. Clarithromycin is currently recommended by the WHO for treatment of leprosy in cases of rifampin resistance or intolerance. Other studies demonstrated low MICs or clinical utility of second-generation macrolides against *M. kansasii*, *M. marinum*, *M. xenopi*, and other opportunistic mycobacterial pathogens. The impressive activity of second-generation macrolides unfortunately did not include *M. tuberculosis*.

The third-generation macrolides, represented largely by the ketolides, were developed with the

intention of overcoming the ribosome-modification and efflux-resistance mechanisms found in gram-positive cocci. Telithromycin was the first such agent to be brought to market. A comparative study of the antimycobacterial activity of clarithromycin versus telithromycin (as well as the fluorinated analogue of telithromycin) revealed the superior activity of clarithromycin for both the moderately clarithromycin-susceptible mycobacteria *M. bovis* BCG, *M. avium*, *M. ulcerans*, and *M. paratuberculosis*, and the clarithromycin-resistant mycobacteria *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. simiae* [53]. Thus, although the general resistance mechanisms to macrolides of gram-positive cocci and mycobacteria seem to be similar, there are significant differences in their structure–activity relationships.

Studies conducted several years ago confirmed that clarithromycin was the most active antimycobacterial macrolide among 15 first- and second-generation macrolides (S. Franzblau, personal communication, 2004). The most potent of the commercially available macrolides, cethromycin, still has a MIC that is higher than the maximum plasma concentration ( $C_{max}$ ) that is obtainable in man. Further testing of modifications of the substituents on the macrolide structure have produced much more potent antimycobacterial compounds with low toxicity. These compounds form the basis for the ongoing work in optimizing the macrolide structure for activity against *M. tuberculosis*.

### Oxazolidinones

Oxazolidinones represent a relatively new class of antimicrobial agents, initially discovered by scientists at DuPont (Wilmington, Delaware) in the 1970s [54,55]. They act by inhibiting protein synthesis by binding to the 70S ribosomal initiation complex [56,57]. The spectrum of activity of the oxazolidinones includes anaerobic and gram-positive aerobic bacteria, such as methicillin-resistant *S. aureus* and *S. epidermidis*, the enterococci, and also mycobacteria [58,59].

Linezolid is the first commercially available oxazolidinone antibiotic. Although not approved for use in mycobacterial disease, there are convincing *in vitro* data that the drug is active against *M. tuberculosis*. A few oxazolidinones have been evaluated for their activity in murine *in vivo* systems. The most active compound seems to be PNU-100480, the activity of which seems to be similar to that of isoniazid or rifampicin [59].

Because of the lack of effective therapeutic options for patients who have MDR disease, linezolid has been used sporadically in patients who have MDR-TB. Although all reports are anecdotal, linezolid does seem to have biologic activity as evidenced by sputum culture conversion [60]. Somewhat distressing, however, is the reported occurrence of peripheral and optic neuropathy associated with prolonged use of linezolid [61].

Overall, the class of oxazolidinones seems to hold promise for the treatment of tuberculosis. Unfortunately, there has not yet been a truly concerted effort to optimize activity of the oxazolidinones for *M. tuberculosis*. In the meantime, the evidence for potential neuropathies associated with long-term use of linezolid will require careful use of this drug as it becomes used more commonly in the treatment of MDR-TB.

### SQ109

*N*-adamantan-2-yl-*N'*-(3,7-dimethylocta-2,6-dienyl)-ethane-1,2-diamine (SQ109) was originally developed as a second-generation antibiotic from a first-line tuberculosis drug, ethambutol, to improve efficacy of the drug against *M. tuberculosis* and lower its toxicity. Although SQ109 is a diamine, its structural dissimilarity to ethambutol and differences in its intracellular target(s) suggest that it is a new antimycobacterial agent, not an ethambutol analogue (Fig. 5).

In collaboration with Dr. Clifton Barry at the NIH, Sequella, Inc. (Rockville, Maryland) synthesized a diverse combinatorial library of compounds with the

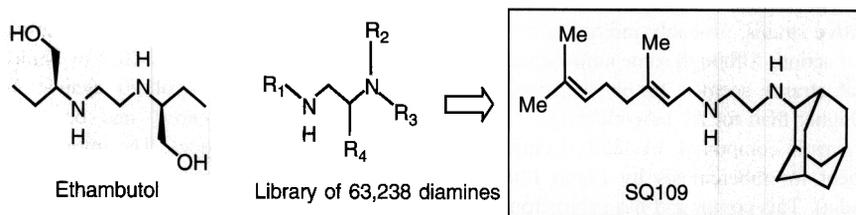


Fig. 5. Chemical structures of ethambutol, compounds in the original combinatorial library, and SQ109.

1,2-diamine pharmacophore of ethambutol and tested them for activity against *M. tuberculosis* using an MIC- and target-based (cell wall) reporter high-throughput screening assay [62]. These efforts found 2796 mostly lipophilic compounds to be active against *M. tuberculosis* in vitro, and 26 demonstrated in vitro activity equal to or greater than (up to 14-fold) ethambutol. Sixty-nine of the most potent hit compounds were later studied in a sequential set of in vitro and in vivo tests: MIC followed by cytotoxicity screen, followed by activity in infected macrophages, followed by permeability evaluation, followed by in vivo efficacy testing, followed by pharmacokinetic studies. SQ109 was identified as the most potent compound in the series and was then subjected to intensive pharmacokinetic/ pharmacodynamic testing.

SQ109 is a lipophilic, nonsymmetric derivative of 1,2-ethylenediamine with unsaturated geranyl and bulky adamantane fragments present. SQ109 has been synthesized as a stable dihydrochloride salt on a multikilogram scale with high chemical purity (99.7%). The formulation to be used in clinical development, hard gelatin capsules, has been developed.

SQ109 has an MIC against *M. tuberculosis* in the range of 0.1 to 0.63  $\mu\text{g}/\text{mL}$  (broth microdilution, Alamar blue, BACTEC [Becton Dickinson, Franklin Lakes, New Jersey]). The compound is bactericidal with 99% inhibition of *M. tuberculosis* growth in macrophages at its MIC. When tested in vivo (in mice), SQ109 is able to reduce infection in lungs and spleen by 2 to 2.5 log. It is active against MDR strains of *M. tuberculosis* in vitro. SQ109 has a low mutational frequency in *M. tuberculosis* in vitro ( $2.18 \times 10^{-9}$ ) and demonstrates enhanced antimycobacterial activity in vitro and in vivo when used in combination with rifampicin and isoniazid (rapid mouse model and chronic infection model).

The mechanism of action of SQ109 seems to be that of a cell wall inhibitor because, like the cell wall-targeting antibiotics (ethambutol, isoniazid, ethionamide, and thiacetazone), it induces a promoter, Rv0341, that was employed in the original luciferase high-throughput screening assay. Because the Rv0341 luciferase reporter responds with light production to inhibition of a wide variety of enzyme targets involved in cell wall construction, the specific target of SQ109 is not known. To address the issue, a proteomic study was initiated to identify proteins in H37Rv *M. tuberculosis* that are affected by the drug in comparison with ethambutol and isoniazid. The results of this study suggest that most of the 44 distinct proteins whose expression is increased (ESAT-6 and others) or decreased (MPT64 and others) by SQ109 were similar to those affected by ethambutol. Only two gene products whose functions are unknown were regulated differently by ethambutol and SQ109. Similarly, two different genes were affected, but in opposite directions, by exposure of *M. tuberculosis* to SQ109 or ethambutol [63].

The pharmacokinetic/pharmacodynamic profiles of SQ109 were evaluated in three species (mice, rats, and dogs). Single-dose pharmacokinetic studies in mice indicate that SQ109 has 4% oral bioavailability as measured by drug concentration in plasma. The high potency of SQ109 in vivo at low doses (1 mg/kg) combined with tissue distribution data argue, however, that, despite low bioavailability, SQ109 antimicrobial effects can be attributed to effective concentrations achieved at the sites of bacterial infection. Although blood concentrations remain low, SQ109 distributes into lungs and spleen (target sites of the bacterial infection), greatly exceeding the MIC (Fig. 6). Oral administration of SQ109, 30 to 75  $\text{mg}/\text{m}^2$  (10–25 mg/kg in mice)

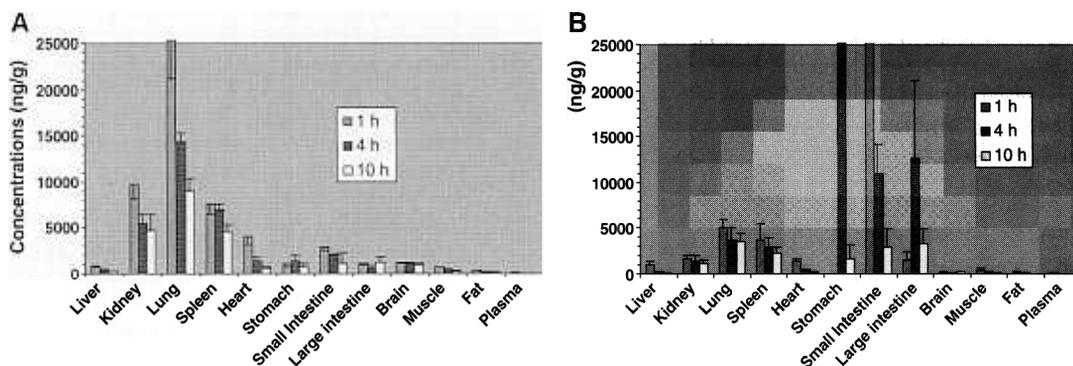


Fig. 6. Tissue levels of SQ109 after intravenous administration of 3 mg/kg (A) and oral administration of 25 mg/kg (B) to mice.

one time per day maintains drug levels above the MIC without accumulation of the drug in the target tissues.

The liver may have a first-pass effect on SQ109 metabolism, resulting in low content of the drug in plasma after oral dosing. P450 reaction phenotyping suggests exclusive involvement of CYP2D6 and CYP2C19 in SQ109 metabolism; analysis of metabolites formed upon incubation of SQ109 with human, mouse, dog, and rat microsomes suggest similar metabolism of the drug in all tested species. SQ109 is undergoing formal preclinical 90-day pharmacology and toxicology studies in preparation for human clinical trials.

In summary, SQ109 is a novel 1,2-diamine-based drug candidate with in vitro and in vivo activity against *M. tuberculosis*. It has pharmacokinetic/pharmacodynamic properties that are characterized by a rapid and broad distribution into various tissues (ie, lungs) that is advantageous for tuberculosis infection.

## Summary

During the recent decade, significant progress has been made in reinvigorating the almost nonexistent pipeline of novel agents for the treatment of tuberculosis and in reestablishing the infrastructure for the conduct of clinical trials of new tuberculosis drugs and treatment regimens. Recent studies of long-acting rifamycin derivatives and potent fluoroquinolone antibiotics are leading to improved regimens for the treatment of active and latent tuberculosis. A number of other compounds in late preclinical and early clinical development show great promise. The rapid increase in knowledge of mycobacterial pathogenesis is leading to the identification of new drug targets, including those believed to play a role in latent infection or in the phenomenon of persistence. A major challenge will be to sustain and increase funding for continued developmental and clinical work if the promise of tuberculosis elimination, or at least significant lessening of the global tuberculosis epidemic, is to be achieved.

## References

- [1] O'Brien RJ, Nunn PP. The need for new drugs against tuberculosis. Obstacles, opportunities, and next steps. *Am J Respir Crit Care Med* 2001;163:1055–8.
- [2] Corbett EL, Watt CJ, Walker N, et al. The growing burden of tuberculosis: global trends and interactions

- with the HIV epidemic. *Arch Intern Med* 2003;163:1009–21.
- [3] Raviglione MC, Pio A. Evolution of WHO policies for tuberculosis control, 1948–2001. *Lancet* 2002;359:775–80.
- [4] Murray CJ, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. *Bull Int Union Tuberc Lung Dis* 1990;65:6–24.
- [5] Datta M, Radhamani MP, Selvaraj R, et al. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapy under the district tuberculosis programme. *Tuberc Lung Dis* 1993;74:180–6.
- [6] Dye C, Espinal MA, Watt CJ, et al. Worldwide incidence of multidrug-resistant tuberculosis. *J Infect Dis* 2002;185:1197–202.
- [7] Stop TB. Working Group on DOTS-Plus for MDR-TB. A prioritised research agenda for DOTS-Plus for multidrug-resistant tuberculosis (MDR-TB). *Int J Tuberc Lung Dis* 2003;7:410–4.
- [8] Schneider E, Castro KG. Tuberculosis trends in the United States, 1992–2001. *Tuberculosis (Edinb)* 2003;83:21–9.
- [9] Centers for Disease Control and Prevention. Tuberculosis elimination revisited: obstacles, opportunities, and a renewed commitment. Advisory Council for the Elimination of Tuberculosis (ACET). *MMWR Recomm Rep* 1999;48(RR-9):1–13.
- [10] American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). Targeted tuberculosis testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;161:S221–47.
- [11] Tuberculosis Trials Consortium. The Tuberculosis Trials Consortium: a model for clinical trials collaborations. *Public Health Rep* 2001;116(Suppl 1):41–9.
- [12] Medaglini D, Hoeveler A. The European research effort for HIV/AIDS, malaria and tuberculosis. *Vaccine* 2003;21(Suppl 2):S116–20.
- [13] Global Alliance for TB Drug Development. Available at: <http://www.tballiance.org>.
- [14] Hong Kong Chest Service/British Medical Research Council. Controlled trial of 2, 4, and 6 months of pyrazinamide in 6-month, three-times-weekly regimens for smear-positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampin, and pyrazinamide. Results at 30 months. *Am Rev Respir Dis* 1991;143:700–6.
- [15] O'Brien RJ, Lyle MA, Snider Jr DE. Rifabutin (ansamycin LM 427): a new rifamycin-S derivative for the treatment of mycobacterial diseases. *Rev Infect Dis* 1987;9:519–30.
- [16] Nightingale SD, Cameron DW, Gordin FM, et al. Two controlled trials of rifabutin prophylaxis against *Mycobacterium avium* complex infection in AIDS. *N Engl J Med* 1993;329:828–33.
- [17] Centers for Disease Control and Prevention. Updated guidelines for the use of rifabutin or rifampin for the treatment and prevention of tuberculosis among HIV-infected patients taking protease inhibitors or non-nucleoside reverse transcriptase inhibitors. Centers for

- Disease Control and Prevention. MMWR Morb Mortal Wkly Rep 2000;49:185–9.
- [18] Centers for Disease Control and Prevention. Notice to readers: acquired rifamycin resistance in persons with advanced HIV disease being treated for active tuberculosis with intermittent rifamycin-based regimens. MMWR Morb Mort Wkly Rep 2002;51:214–5.
- [19] Klemens SP, Cynamon MH. Activity of KRM-1648 in combination with isoniazid against *Mycobacterium tuberculosis* in a murine model. Antimicrob Agents Chemother 1996;40:298–301.
- [20] Mae T, Hosoe K, Yamamoto T, et al. Effect of a new rifamycin derivative, rifalazil, on liver microsomal enzyme induction in rat and dog. Xenobiotica 1998;28:759–66.
- [21] Rose L, Vasiljev-K M, Adams P, et al. Safety and pharmacokinetics of PA-1648, a new rifamycin in normal volunteers. Am J Respir Crit Care Med 1999;159(Suppl):A495.
- [22] Dietze R, Teixeira L, Rocha LM, et al. Safety and bactericidal activity of rifalazil in patients with pulmonary tuberculosis. Antimicrob Agents Chemother 2001;45:1972–6.
- [23] Rothstein DM, Hartman AD, Cynamon MH, et al. Development potential of rifalazil. Expert Opin Investig Drugs 2003;12:255–71.
- [24] Daniel N, Lounis N, Ji B, et al. Antituberculosis activity of once-weekly rifapentine-containing regimens in mice. Long-term effectiveness with 6- and 8-month treatment regimens. Am J Respir Crit Care Med 2000;161:1572–7.
- [25] Tuberculosis Trials Consortium. Rifapentine and isoniazid once a week versus rifampin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomized clinical trial. Lancet 2002;360:528–34.
- [26] Vernon A, Burman W, Benator D, et al. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. Lancet 1999;353:1843–7.
- [27] Tam CM, Chan SL, Lam CW, et al. Rifapentine and isoniazid in the continuation phase of treating pulmonary tuberculosis. Initial report. Am J Respir Crit Care Med 1998;157:1726–33.
- [28] US Food and Drug Administration. Review of new drug application from Aventis for Prifitin. Available at: <http://www.fda.gov/cder/foi/nda/98/21024.htm>. Accessed March 10, 2005.
- [29] American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America. Treatment of tuberculosis. Am J Respir Crit Care Med 2003;167:603–62.
- [30] Taylor Z, Qualls N, Vernon A, et al. A prevention effectiveness study of rifapentine in the continuation phase of therapy for active pulmonary tuberculosis. Am J Respir Crit Care Med 2000;161(Suppl):A524.
- [31] Weiner M, Burman W, Vernon A, et al. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am J Respir Crit Care Med 2003;167:1341–7.
- [32] Bock NN, Sterling TR, Hamilton CD, et al. A prospective, randomized, double-blind study of the tolerability of rifapentine 600, 900, and 1,200 mg plus isoniazid in the continuation phase of tuberculosis treatment. Am J Respir Crit Care Med 2002;165:1526–30.
- [33] Bock NN, Sterling TR, Khan A, et al. Extension of continuation phase therapy to reduce relapse rates among HIV-negative TB patients at high risk for relapse. Am J Respir Crit Care Med 2003;167(Suppl):A433.
- [34] Chapuis L, Ji B, Truffot-Pernot C, et al. Preventive therapy of tuberculosis with rifapentine in immunocompetent and nude mice. Am J Respir Crit Care Med 1994;150:1355–62.
- [35] Tuberculosis Research Centre. Shortening short course chemotherapy: a randomized clinical trial for treatment of smear positive pulmonary tuberculosis with regimens using ofloxacin in the intensive phase. Ind J Tub 2002;49:27–38.
- [36] Mitchison DA. Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months. Am Rev Respir Dis 1993;147:1062–3.
- [37] Alvarez-Freites EJ, Carter JL, Cynamon MH. In vitro and in vivo activities of gatifloxacin against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2002;46:1022–5.
- [38] Rodriguez JC, Ruiz M, Climent A, et al. In vitro activity of four fluoroquinolones against *Mycobacterium tuberculosis*. Int J Antimicrob Agents 2001;17:229–31.
- [39] Ji B, Lounis N, Maslo C, et al. In vitro and in vivo activities of moxifloxacin and cinafloxacin against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1998;42:2066–9.
- [40] Miyazaki E, Miyazaki M, Chen JM, et al. Moxifloxacin (BAY12–8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. Antimicrob Agents Chemother 1999;43:85–9.
- [41] Hu Y, Coates AR, Mitchison DA. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2003;47:653–7.
- [42] Wright DH, Brown GH, Peterson ML, et al. Application of fluoroquinolone pharmacodynamics. J Antimicrob Chemother 2000;46:669–83.
- [43] Lounis N, Bentoucha A, Truffot-Pernot C, et al. Effectiveness of once-weekly rifapentine and moxifloxacin regimens against *Mycobacterium tuberculosis* in mice. Antimicrob Agents Chemother 2001;45:3482–6.
- [44] Nuermberger EL, Yoshimatsu T, Tyagi S, et al. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. Am J Respir Crit Care Med 2004;169:334–5.
- [45] Gosling RD, Uiso LO, Sam NE, et al. The bactericidal activity of moxifloxacin in patients with pulmonary

- tuberculosis. *Am J Respir Crit Care Med* 2003;168:1342–5.
- [46] Pletz MW, De Roux A, Roth A, et al. Early bactericidal activity of moxifloxacin in treatment of pulmonary tuberculosis: a prospective, randomized study. *Antimicrob Agents Chemother* 2004;48:780–2.
- [47] Valerio G, Bracciale P, Manisco V, et al. Long-term tolerance and effectiveness of moxifloxacin therapy for tuberculosis: preliminary results. *J Chemother* 2003;15:66–70.
- [48] Global Alliance for TB Drug Development. Scientific blueprint for tuberculosis drug development. *Tuberculosis (Edinb)* 2001;81(Suppl 1):1–52.
- [49] Andries K, Verhasselt P, Guillemont J, et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005;307:223–7.
- [50] Stover CK, Warrener P, VanDevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* 2000;405:962–6.
- [51] Grosset J, Nuermberger E, Yoshimatsu T, et al. The nitroimidazopyran PA-824 has promising activity in the mouse model of TB. *Am J Respir Crit Care Med* 2004;169(Suppl):A260.
- [52] Diedda D, Lampis G, Fioravanti R, et al. Bactericidal activities of the pyrrole derivative BM212 against multidrug-resistant and intramacrophagic *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* 1998;42:3035–7.
- [53] Rastogi N, Goh KS, Berchel M, et al. In vitro activities of the ketolides telithromycin (HMR 3647) and HMR 3004 compared to those of clarithromycin against slowly growing mycobacteria at pHs 6.8 and 7.4. *Antimicrob Agents Chemother* 2000;44:2848–52.
- [54] Gregory WA, Brittelli DR, Wang C-LU, et al. Antibacterials, synthesis and structure—activity studies of 3-Aryl-2-oxooxazolidines. 2. The “A” Group. *J Med Chem* 1990;33:2569–78.
- [55] Gregory WA, Brittelli DR, Wang C-LJ, et al. Antibacterials, synthesis and structure-activity studies of 3-Aryl-2-oxooxazolidinones. 1. The “B” group. *J Med Chem* 1989;32:1673–81.
- [56] Birmingham MC, Rayner CR, Meagher AK, et al. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate use program. *Clin Infect Dis* 2003;36c:159–68.
- [57] Eustice DC, Feldman PA, Zajac PA, et al. Mechanism of action of DuP 721: inhibition of an early event during initiation of protein synthesis. *Antimicrob Agents Chemother* 1988;32:1218–22.
- [58] Slec AM, Wuonola MA, McRipley RJ, et al. Oxazolidinones, a new class of synthetic antibacterial agents: *in vitro* and *in vivo* activities of DuP 105 and DuP 721. *Antimicrob Agents Chemother* 1987;31:1791–7.
- [59] Cynamon MH, Kelmens SP, Sharpe CA, et al. Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. *Antimicrob Agents Chemother* 1999;43:1189–91.
- [60] Dworkin F, Winters SS, Munsiff C, et al. Use of linezolid in treating multidrug-resistant tuberculosis in New York City. *Am J Respir Crit Care Med* 2004;169(Suppl):A233.
- [61] Bressler AM, Zimmer SM, Gilmore JL, et al. Peripheral neuropathy associated with prolonged use of linezolid. *Lancet Infect Dis* 2004;4:528–31.
- [62] Lee R, Protopopova M, Crooks E, et al. Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. *J Comb Chem* 2003;5:172–87.
- [63] Boshoff HI, Myers TG, Copp BR, et al. The transcriptional responses of *M. tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J Biol Chem* 2004;279:40174–84.