Review Article

Recent Advances in Treatment for Tuberculosis: A Review

S.S Padgilwar[1], R.S. Manmode[2]*, A.Y. Sahare[3], Manish Kadam[1], J.V. Manwar[4], P.P. Warade[4], D.D. Kumbhar[4]

1. SGSPS Institute of Pharmacy, Kaulkhed, Akola 444 004, MS, India.
2. Genzyme Corporation, 500 Soldiers Field Road, Allston 02134, MA, USA.
3. Bajiraoji Karanjakar College of Pharmacy, Sakoli, Bhandara 441802, MS, India.
4. KYDSCT College of Pharmacy, Sakegaon (Bhusawal) 425 201, Dist. Jalgaon, MS, India.
*Corresponding author's E-mail: rahulmanmode@gmail.com

ABSTRACT

Tuberculosis (TB) is a scourge for human race for millennia with huge morbidity and mortality. Among the main obstacles to the global control of the disease are the HIV epidemic that has dramatically increased risk for developing active TB, the increasing emergence of multi-drug resistant TB (MDR-TB), Extensive drug resistant tuberculosis (XDR-TB) and the recalcitrance of persistent infections to treatment with conventional anti-TB drugs. Anti-tuberculosis drug resistance is a major public health problem that threatens the success of Directly Observed Treatment Short Course (DOTS), the WHO-recommended treatment strategy for detection and cure of TB, as well as global tuberculosis control. This made the currently available anti tubercular drugs into less effective and futile tools to control tuberculosis. Not only to radically transform the fight against tuberculosis but also to shortening the current six to eight-month treatment to two months or less. This in turn will improve patient adherence, increase cure rates, and lessen the likelihood of patients developing drug resistance. New drugs are also needed to benefit the growing number of people around the globe who are co-infected with TB and HIV, as well as those who have been exposed to TB but are not yet ill with disease i.e. latent TB.

Keywords: Tuberculosis, Therapy, Isoniazide, Fluoroquinolones.

INTRODUCTION

Tuberculosis (TB) is a potentially serious infectious disease that mainly affects your lungs. It is caused by Mycobacterium tuberculosis (see Fig 1). The bacteria that cause tuberculosis are spread from one person to another through tiny droplets released into the air via coughs and sneezes. Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV, the virus that causes AIDS. HIV weakens a person's immune system so it can't fight the TB germs. Many strains of tuberculosis resist the drugs most used to treat the disease. People with active tuberculosis must take several types of medications for many months to eradicate the infection and prevent development of antibiotic resistance.[1]

Figure 1: Scanning electron microscopy of Mycobacterium tuberculosis (www.google.com).

World health organization has given several regimens for successful treatment of tuberculosis. Although the current tuberculosis treatment regimens are highly effective but are far from being ideal and many times, it is associated with problems like patient non-adherence. Causes are long duration of treatment with use of multiple drugs like isoniazide, rifampin, pyrazinamide, ethambutal which are required to be taken simultaneously. Many patients experience unpleasant side effects due to simultaneous administration of multiple drugs. This leads to patient non adherence that in turn leads to increased mortality and drug-resistant cases. Treatment of MDR -TB (multiple drug resistance – tuberculosis) and XDR -TB (extensive drug resistance – Tuberculosis) may become the primary health scourge of the 21st century. In September 2006, the WHO announced a further worsening of the MDR- TB pandemic with multiple reports in all regions of the world. To assess the frequency and distribution of XDR TB, the WHO/CDC surveyed an international network of TB laboratories. The survey during 2000 to 2004, determined that among 17690 isolates from 49 countries, 20% were MDR -TB and 2% were XDR -TB. 2 The increasing emergence of MDR-TB along with HIV pandemic threatens disease control. Approximately one-third of the world's population are infected with the mycobacterium that causes TB, and each year 9 million people become ill with the disease, with 2 million deaths. The 40% of the cases are in Southeast Asia; India has 53.3% of those cases.[2] Currently available antitubercular drugs are not substantial to

combat MDR-TB and thus to prevent an emerging multidimensional problem. (The mode of transmission of tuberculosis is depicted in Fig. 2).
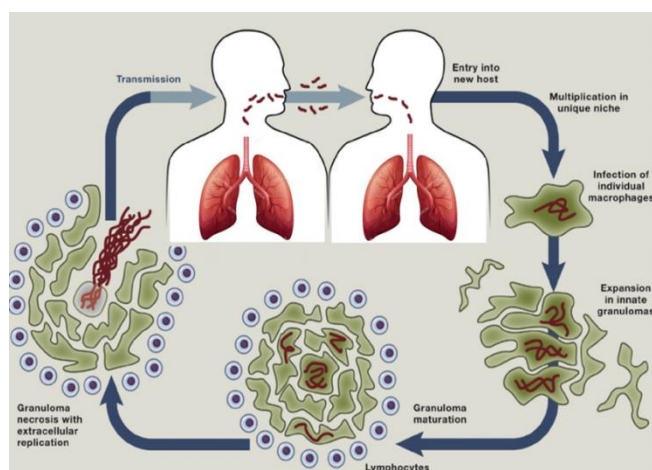

Figure 2: Transmission of tuberculosis.[3]

The current standard "short-course" treatment regimen requires at least 6 months which cannot be reduced using optimal combination of available antitubercular drugs. Because the existing anti TB drugs acts by the inhibition of cell processes such as cell wall biogenesis and DNA replication and thus can only kill actively growing bacteria. Actively growing mycobacteria are killed in initial period of therapy but it takes longer for the drugs to kill slowly growing or slowly metabolizing bacteria. This implies current TB chemotherapy is characterized by an efficient bactericidal activity but an extremely weak sterilizing activity, defined as the ability to kill the slowly growing or slowly metabolizing bacteria that persist after the growing bacteria have been killed by bactericidal drugs. Many *in vitro* studies showed that exponentially growing cultures of M. tubercle can be sterilized in a few days using combination of first line drugs like INH, RIF etc. But same combination of drugs takes much longer time to achieve sterilizing action in vivo. The cause for variation in sterilizing activity (in vivo v/s in vitro) was attributed to the heterogeneity of mycobacteria in human tissues. Based upon this the mycobacteria are classified into four different population viz., Bacteria that are actively growing, killed primarily by isoniazid (INH), Bacteria that have spurts of metabolism, killed by rifampicin (RIF), Bacteria that are characterized by low metabolic activity and reside in acid pH environment killed by pyrazinamide (PZA) and Bacteria that are "dormant" or "persisters", not killed by any current TB drug.

Treatment of HIV co-infection with tuberculosis, MDR-TB, XDR-TB, latent TB with currently available antitubercular drugs represents challenge. Hence newer antitubercular drugs are needed to simplify or reduce the necessary duration of treatment to 2 months or less; to effectively treat MDR-TB, XDR TB and mycobacteria in state of latency; to treat TB with HIV co infection effectively with least possible interaction between antitubercular and antiretroviral drugs. [4]

Most pharmaceutical companies were not interested to pursue tuberculosis research because of the high investment required to bring a product to market and the lack of likely commercial return. After decades of standstill in TB drug development, the drug pipeline has begun to fill up during the last 5 years. Established in 2000 and largely funded by the Bill & Melinda Gates Foundation, the Global Alliance for TB Drug Development (TB Alliance) has played a critical role in changing the TB research and development (R&D) landscape and is associated with approximately half of all compounds (or projects aimed to identify candidate compounds) in development.

The drug candidates in the portfolio originate from either analogue of existing molecules where innovative chemistry is used to help optimize compounds or novel chemical entities. Examples include novel macrolide, ethambutol, and isoniazid, fluoroquinolone (moxifloxacin and gatifloxacin). All these drugs show potent activity in vitro against M. tubercule. In novel chemical entities molecules like the nitroimidazole PA-824, the diarylquinoline TMC207, and the pyrrole LL-3858 are there.

1.1. Drug therapy

Fluoroquinolones are fluorine-containing nalidixic acid derivatives and few of them are recommended for use in MDR-TB, due to potent anti-Mycobacterium activity, good pharmacokinetics, in terms of tissue and cellular distribution, fewer adverse effects. 5 Although fluoroquinolones have been included in antitubercular regimens (particularly for MDR-TB) since the late 1980s but the role of fluoroquinolones in tuberculosis treatment still remains controversial. [6]

1.1.1. Older derivatives

Ciprofloxacin (CPFX), ofloxacin (OFLX), sparfloxacin (SPFX), and levofloxacin (LVFX) have good anti-Mycobacterium activity against experimental tuberculosis in animal models and are clinically effective in control of tuberculosis (including MDR-TB) when given in combination with other anti TB drugs. [7,8]

But in contrarily to above it was reported that "Substituting or adding fluoroquinolones to established first-line antitubercular drug regimens gives no additional benefit or risks. Fluoroquinolones have antitubercular activity, but are not one of the standard antitubercular medicines. Ciprofloxacin should not be used as a substitute drug in the standard antitubercular regimen, because of higher relapse rate in drug sensitive tubercular patients, longer duration of treatment, no added advantage in terms of therapeutic efficacy and safety over existing antitubercular agents. Sparfloxacin was no better than ofloxacin when added to antitubercular regimens in drug-resistant tuberculosis. [6]

1.1.2.   Novel derivatives

Novel fluoroquinolones like Moxifloxacin and gatifloxacin are of special interest in treatment of tuberculosis since they are considered to have highest activity than Ciprofloxacin, ofloxacin, levofloxacin, and sparfloxacin. It has reported that Ciprofloxacin had the least bactericidal activity, ofloxacin and levofloxacin had greater activities, and moxifloxacin and gatifloxacin had the greatest activities. 9 Superiority of moxifloxacin and gatifloxacin over older fluoroquinolones against mycobacterium have raised the hopes that these agents can be used in combination with other first line against to reduce the treatment duration. However, Burman et al has shown that substitution of moxifloxacin for ethambutal did not have an effect on 2-month sputum culture status but did result in a higher frequency of negative cultures at earlier time points in patients with smear-positive pulmonary tuberculosis. 10 Higher frequencies of negative cultures at earlier time points among patients with smear-positive pulmonary tuberculosis with moxifloxacin indicate that it may have the potential for treatment shortening.[11,12] The reason for decline in activity of moxifloxacin when it is used in combination with first line antitubercular drugs, demands further research.

The in vitro study by Tao Lu and Karl Drlica et al concluded that the combination of moxifloxacin and isoniazid was slightly more lethal to mycobacteria than either compound when used alone.[13] The same is true for combination of moxifloxacin and rifampicin at low rifampicin concentrations. However, when rifampicin concentration increased, lethality is reduced. Follow-up experiments with M. smegmatis revealed that bacteriostatic concentrations of rifampicin inhibit the bactericidal effects of moxifloxacin at high concentration. Interference with quinolone lethality by rifampicin is a well-known phenomenon with Escherichia coli. Presumably treatment with rifampicin, an inhibitor of RNA synthesis, or chloramphenicol, an inhibitor of protein synthesis, blocks the expression of a suicide protein involved in the lethal action of quinolones, and the ability of chloramphenicol to interfere with the lethal action of ciprofloxacin during treatment of Mycobacterium bovis BCG, support the idea that a similar phenomenon occurs in mycobacteria.

Nuremberger et al reported combination of moxifloxacin with rifampicin and pyrizinamide in place of isoniazid the anti-mycobacterial activity of regimen is much better in comparison with standard isoniazid regimen. The moxifloxacin with isoniazid in the standard regimen could relieve a possible antagonism among the currently used drugs. 14 Conflicting results have been reported about gatifloxacin's activity when added to isoniazid or rifampicin in cell cultures, with one study suggesting synergy but another reporting little additive effect (Paramasivan).[15] However, it is not clear how applicable these test tube studies are and what would happen in vivo.

1.1.2.1. Moxifloxacin

Moxifloxacin interferes with protein synthesis in slowly metabolizing bacteria through a mechanism which is different than rifampicin. In vitro, moxifloxacin appeared to kill a sub population of tubercle bacilli not killed by rifampicin, i.e. rifampicin-tolerant persisters, ciprofloxacin and ofloxacin did not have any such effect. 16 However, the characterization of the molecular mechanisms of such a bactericidal activity needs to be established. In Murine models the anti-tubercular activity of moxifloxacin was comparable to isoniazid.[17] Moxifloxacin appears to be a poor substrate for efflux pumps in other pathogens such as S. pneumonia. This might be due to the bulky C-7 substitute. The hydrophilic fluoroquinolones such as ciprofloxacin are good substrate and are easily effluxes out of bacteria. Inhibition of effluxes results in building of higher concentrations resulting in improved activity. [18-20]

1.1.2.2. Gatifloxacin

Gatifloxacin have demonstrated dramatically greater activity in preclinical studies and likely to be cross-resistant to moxifloxine. Early clinical indications of gatifloxacin's anti-TB activity have been found to be promising with identical activity to moxifloxacin and similar (though not quite as potent) EBA to isoniazid. The drug's toxicity and drug-drug interaction profile is similar to moxifloxacins. Likewise, the drug has not been adequately evaluated in children and pregnant or lactating women.

1.1.2.3. Gemifloxacin

Gemifloxacin is quinolone which has shown promising activity in various phase 3 trials. Drug now been submitted for approval in the USA for the treatment of respiratory infections. In healthy volunteers, oral bioavailability is approximately 70%; it is well tolerated and has a mean elimination half-life of 7.4 hrs. These characteristics, coupled with its potent antibacterial activity, suggest its suitability for a once-daily dosing regimen. [21] Two hundred fifty isolates of Mycobacterium tuberculosis were evaluated for susceptibility to ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, trovafloxacin, and gemifloxacin (SB-265805). Levofloxacin, ciprofloxacin, and grepafloxacin showed the greatest activity (MIC for 90% of strains tested [MIC90] 1 µg/ml), although ofloxacin also showed good activity, with an MIC90 of 2 µg/ml. Trovafloxacin and gemifloxacin showed lower in vitro activity, with MIC90s of 64 and 8 µg/ml, respectively. Extrapolating these data to serum concentrations seen in healthy volunteers suggests that, even at the highest non-toxic dose tested (800mg), gemifloxacin would not be effective for TB treatment. [22]

1.1.2.4. Sitafloxacin

Sitafloxacin is in Phase III trials in both Japan and the USA. Against broad range of bacteria sitafloxacin has outstanding activity. In comparison to other quinolones like - ciprofloxacin, trovafloxacin, clinafloxacin,

levofloxacin, gatifloxacin and moxifloxacin, sitafloxacin is having highest activity.[23] The reason for this higher activity and potency of sitafloxacin lies in its mechanism of action. Sitafloxacin has ability to equally inhibit both DNA gyrase and topoisomerase IV, and its IC50s against these enzymes were lowest amongst the quinolones.[24] Potency of sitafloxacin is found equal to that of gatifloxacin and sparfloxacin but exceeds to that of levofloxacin and ofloxacin when tested against strains of M. tuberculosis MIC90s (MICs at which 90% of strains of M. tuberculosis inhibited) were ~ 0.2µg/ml.[25,26] No data appear to have been published for the activity of the compound against M. tuberculosis in vivo.

1.2.   Fluoroquinolones and mycobactreial resistance

There are reasons for concerns about the rapid development of resistance particularly when fluoroquinolones are administered as the only active agent in a failing multi-drug regimen. In a study conducted in USA and Canada, among referral samples isolates between 1996 and 2000, resistance to ciprofloxacin was assessed and was found to occur in 1.8% (33/1852) of isolates. Of those, 75.8% (25/33) were also multidrug resistant. Despite of widespread use of fluoroquinolones for common bacterial infection the resistance to fluoroquinolones remains rare and occurs mainly in multi-drug resistant strains.[27] The study by Ginsburg et al reported the incidence of M. tuberculosis fluoroquinolone resistance in a small sample of patients (55) with newly diagnosed tuberculosis was found to be high among patients with prior fluoroquinolone exposure (2/19).[28] Cross-resistance was observed among the different fluoroquinolones tested (ofloxacin, levofloxacin, gatifloxacin moxifloxacin, and ciprofloxacin). For this reason only it is suggested that fluoroquinolones might be better reserved for specific serious infections like MDR TB.

1.3.   Safety of fluoroquinolones

According to Bayer, newer fluoroquinolones has been used in more than 35 million people, so the drug's safety profile is well characterized. In general, it seems much safer than isoniazid or rifampicin, however, there may be complications in patients with heart problems, and the drug has some rare CNS side-effects. The drug has been used widely in treatment of bacterial infections in people with HIV and has no drug interactions with antiretroviral. However, of crucial importance, the safety and effectiveness of moxifloxacin has yet to be established in pediatric patients, adolescents (less than 18 years of age), pregnant women, and lactating women. Thus rational behind the combination of newer fluoroquinolones with antitubercular drugs is not clear and demands further research.

1.7.   Newer rifamycin derivatives

1.7.1.   Rifabutine and rifapentine

As determined by MIC, rifabutine (RBT) is about 4 to 8 times more active than rifampin (RIF) against MTB and it possesses favorable pharmacokinetic features such as a long half-life and good tissue penetration. [29] Owing to the better pharmacokinetic profile and potency of RBT in comparison to RIF, Centers for Disease Control and Prevention (CDC) in the U.S. has recommended use of rifapentine (RBT) in place of RIF in multidrug regimens for the treatment of active TB in HIV patients. Prime reason for this is RBT can be co-administered with antiretroviral treatment that includes HIV protease inhibitors or nonnucleoside reverse transcriptase inhibitors.[30,31] Compare to RIF; RBT is very weak inducer of cytochrome P-450 isoenzymes which are responsible for metabolism of antiretroviral drugs. Thus in contrary to RIF. Co-administration of RBT with antiretroviral drugs does not substantially decrease blood levels of these antiretroviral drugs.[32]

RPT is 2 to 4 times more active than RIF against MTB and is having better pharmacokinetic profile than RIF.[33,34] Duration of action for RPT is much greater than those of other available rifamycin derivatives, owing to its high serum peak level and elimination half-life which   allows for its extended dosing intervals in TB patients.[35] In addition, INH in combination with RPT gives very long post antibiotic effect (PAE) (137 hrs) and this PAE is about 17 times of that of INH plus MXFX. Adverse events of RPT may occur less frequently at the currently recommended 600-mg dose as compared with RIF.[36] Although RPT induces CYP450 somewhat less than RIF, Cmax and AUC of a HIV protease inhibitor indinavir are strongly reduced, when RPT is co-administered with indinavir. [36] In any case, both RBT and RPT are now included in drug regimens for the treatment of TB and MAC infections, particularly in HIV patients.[37- 39]

1.7.2.   Rifametane

It is a new semi-synthetic rifamycin having bactericidal spectrum and potency similar to that of rifampicin. but rifametane is having much better with much better pharmacokinetic profile compared to rifampicin. [40] This is evident from the fact that when pharmacokinetics of rifamentane was studied in comparison with known rifamycin derivatives, like rifampicin although MIC90 values of the two compounds were the same against 20 strains of M. tuberculosis, in TB-infected mice rifametane proved to be the more effective orally.[41] In healthy male volunteers, the pharmacokinetics and safety of a 300 mg single oral dose of rifametane in comparison to a 300 mg dose of rifampicin.[42] Clearly showed that the pharmacokinetic profile of rifametane is significantly more favourable than that of rifampicin. Thus, the elimination half-life for rifametane was 10.58 hrs compared with 1.89 hrs for rifampicin, and the mean residence time was 18.05 hrs for rifametane and 3.93 hrs for rifampicin. The drug was well tolerated and no changes clinically or statistically in laboratory parameters were found. In another Phase 1 trial carried out in

collaboration with Glaxo India, a single oral dose of 150mg was administered and the half-life and area under curve (AUC) were some 6 or 7-fold that of rifampicin. Encouragingly, serum drug levels above the MIC for M. tuberculosis were maintained for up to 48 hrs after drug administration.[43]

1.7.3. Rifalazil

Rifalazil, a new semi synthetic rifamycin, is characterized by a long half-life and is more active than rifampicin and rifabutin against M. tuberculosis both in vitro and in vivo.[44] However, high level rifampicin– resistant strains present cross-resistance to all rifamycins. [45] However, due to severe side-effects in the four-day Phase II trial, the development of rifalazil has been terminated.

1.7.4. Mikasome

Amikacin is an aminoglycoside used as a second-line anti-TB drug. [46] The anti-mycobacterial activity of liposome-encapsulated drug, MiKasome (Gilliad Sciences), has been found to be effective against Mycobacterium avium infections in vitro and in animal models. In preclinical studies, 48 hr after delivery to the lungs via liposome, over half of the antibiotic remained in this tissue. [47] In animals, pharmacokinetic data showed that MiKasome produced 7-fold higher peak plasma levels compared to free drug (amikacin) administered intravenously (iv). Additionally, the AUC was 150-fold higher with the liposomal material and a single dose of liposomal amikacin produced therapeutic levels of antibiotic for more than 72 hrs. In a preliminary clinical study, a patient with tuberculosis treated with MiKasome for 49 days exhibited negative culture. [48] Once weekly dosing maintained constant amikacin dose levels. Pilot Phase II studies showed that MiKasome was able to resolve M. tuberculosis infections in adults and children who had failed conventional therapies.[47]

1.7.5. Aconiazide

Aconiazide is a pro-drug of isoniazid which was designed to be less toxic than the parent drug lacks carcinogenicity. [49] Isoniazid is metabolized to hydrazine and acetylhydrazine, which are considered to be responsible in the toxicity of isoniazid. Since aconiazide is converted to isoniazid and 2-formylphenoxyacetic acid, it was expected that the acid would bind to the isoniazid metabolites and so lower toxicity.[49] In healthy patients it was found to produce proportionately lower levels of isoniazid in serum than the parent molecule itself. [50] For some time, further progression of the compound was delayed due to problems with its commercial synthesis. However, additional toxicology data are now being submitted to the FDA by Lincoln Diagnostics in order to conduct clinical trials in tuberculosis patients, and the compound has been granted Orphan Drug status in the US.

1.7.6. Macrolides

Clarithromycin (CAM) is effective against MTB only at very high concentrations in vitro (MIC50 and MIC90 are 64 and >128 mg/ml, respectively), which are significantly greater than its Cmax values in the serum and lung tissue in humans and thus cannot be achieved clinically. [51] However, a recent study by Bosne-David et al. shown that the intrinsic resistance of MTB to CAM is reversed when inhibitors of bacterial cell wall synthesis, especially bacitracin and vancomycin (at subinhibitory concentration) are co-administerwed with CAM. This indicates that the natural resistance of MTB to macrolides is attributable to the cell wall structures of MTB as a drug permeability barrier.[52] They also found that EMB reversed CAM resistance in all of the test MTB strains regardless of their baseline susceptibility to CAM. It is also found that CAM or its metabolite, 14-hydroxyclarithromycin, when co-administered with anti tubercular drug like INH, RIF and EMB results in 4- to 32-fold reduction in MICs of these drugs and made drug-resistant strains susceptible. [53] Thus CAM may be concurrently given with first line antitubercular drugs to improve the therapeutic efficaciousness of these drugs in treating MDR-TB.

1.7.7. Clofazimine

Clofazimine (CFZ), a riminophenazine antimycobacterial agent, is mainly used for the treatment of leprosy. CFZ shows substantial activity against MTB both in vitro and in vivo in preclinical studies. The MICs of CFZ against MTB is 1 mg/ml this can be easily achievable clinically after oral administration. [54] Because of good activity against MTB it is often empirically included in regimens for treatment of MDR-MTB. However, oral CFZ was found inactive against intracellular MTB when it was used alone or co-administered with other drugs (such as PZA plus EMB) for the treatment of MDR-TB.[55] Long-term CFZ therapy is associated with dermal side effects like dark brown pigmentation, ceroid lipofuscinosis. [56] Thus, justification of the meaning of empirical use of CFZ in multidrug regimens for MDR-MTB requires more detailed and systematic experimentation in future. Novel a riminophenazines example-B746 and B4157 have shown better activity against MTB compared to CFZ in vitro studies and are devoid of side effects on skin.[57]

1.7.8. Capuramycin

Capuramycin analogs possess potent antimycobacterial activities in vitro and in vivo through the inhibition of enzyme phospho-MurNAc-pentapeptide translocase and thereby inhibit the peptidoglycan assembly of mycobacteria.[58,59] Few of the potent cupuramycin analogues were not considered for further development owing to the lack of desirable pharmacokinetic characteristics .example is RS-118641 one of the most potent anti-MTB capuramycin analogs, had its MIC50 and MIC90 for drug susceptible MTB strains 1 and 2 mg/ml respectively, while its MIC50 and MIC90 for MDR-MTB strains were 0.5 and 2 mg/ml, respectively.[59] However,

this agent had very poor solubility and was not consider suitable for further development. Other capuramycin analogs, RS-112997 and RS-124922 in in-vivo experiments using a murine experimental TB model, exhibited appreciable levels of therapeutic efficacy against MTB infection in mice, causing about 3-log unit-reductions of bacterial loads in the lungs, when given to mice at doses of 0.1 or 1 mg/mouse daily during the early phase of infection and the MIC50s against MDR-MTB strains were 16 and 4 mg/ml, respectively. 59 Capuramycin analogs may be considered for further evaluation in the treatment of MDRTB patients, since these agents act on unique and selective targets in the cell wall synthesis of TB bacilli.

1.7.9. *Linezolid*

Linezolid an oxazolidinone which acts by inhibiting protein synthesis and is active against multi drug-resistant strains mycobacterium tuberculosis. (1) Linezolid inhibits ribosomal protein synthesis by interfering with initiation complex formation, resulting in inhibition of protein synthesis at a very early stage and a lacks cross resistance to existing antimicrobial agents, (2) a spectrum of activity that includes a number of important bacterial species, (3) superior oral and parental bioavailability,(4) a low emergence rate of resistant mutants, and (5) totally synthetic compound, and there is no natural reservoir of resistance.[60,61] Linezolid lacks cross resistance to other existing antimicrobial agents and it has been confirmed by recent studies using 117.

Clinical isolates of MTB, including organisms susceptible to first-line drugs, resistant to one first-line drug and resistant to multiple first-line drugs (MIC50=0.5 mg/ml, MIC90=0.5 to 1.0 mg/ml), and a total of 234 strains of drug-susceptible or MDR-MTB.[62,63] Synergism was found between Linezolid and RIF in 30% of drug-susceptible MTB strains. 64 In experimental studies with the murine model of tuberculosis, oxazolidinones -linezolid have shown an activity similar to isoniazid. However, clinical experience with Linezolid in tuberculosis is scarce. In addition to the lack of information on the efficacy of linezolid in the treatment of tuberculosis, toxicity is a matter of concern when the drug has to be used for long periods. Clinical trials have shown that linezolid (600 mg twice daily in adults) is safe and generally well tolerated for courses of therapy of <28 days, but long-term linezolid use has been associated with reversible haematopoietic suppression, primarily thrombocytopenia and neuropathy.[65] Moreover reduction in dose of linezolid to half of the acceptable dose although does not interfere with its antimycobacterial activity but long-term use of the drug can cause side effects, such as peripheral and optic neuropathy.[66]

1.7.9. *Phenothiazines*

Phenothiazines are derivatives of methylene blue, which was described by Paul Ehrlich in the 19th century as rendering bacteria immobile. Since then, phenothiazines have been shown to be active against a wide variety of viruses, bacteria, mycobacteria and protozoa. The phenothiazines chlorpromazine (CPZ) and thioridazine (TZ) have equal in vitro activities against antibiotic-sensitive and resistant mycobacterium tuberculosis. Phenothiazines are calmodulin antagonist and antitubercular activity of these agents is due to presence of calmodulin-like protein in mycobacteria.[67] These compounds have not been sufficiently explored for their antimycobacterial activity because in-vitro activities take place at concentrations which are beyond those that are clinically achievable.[68] However pulmonary macrophages concentrate chlorpromazine 10-100 folds above the concentration found in plasma and has activity against mycobacteria that have been phagocytocyzed by these cells. Since chlorpromazine has antimycobacterial activity within the macrophage, it is likely that thioridazine exerts a similar activity with concentrations at the level of 1 mg/L.[69] In comparison to CPZ, TZ is very mild anti-psychotic agent whose most common side effect is drowsiness, and its use for a limited period of 2–3 months should not produce side effects that are more severe than simple drowsiness. It has equal anti-tuberculosis properties in vitro to chlorpromazine.[70] CPZ & TZ found to interacts synergistically with antimicrobial agents who include streptomycin, erythromycin, oleandomycin, spectinomycin, levofloxacin, azithromycin, and amoxicillin-clavulanic acid. The MICs of these antibiotics were reduced as much as 8,000-fold in the presence of the CPZ & TZ.[71] Hence human studies are required to evaluate efficacy of CPZ & TZ as alternative/adjuvant in treatment of tuberculosis.

1.7.10. *5-Nitroimidazole*

In many individuals, M. tuberculosis infection takes the form of a latent disease state. At some time the disease may reactivate and give rise to an active and often fatal infection. In some patients, this may occur even after the individual has been given prolonged chemotherapy. A key problem in TB control is the persistence of organism

Significantly the dormant form of the mycobacterium was found to be resistant to the standard anti-mycobacterial agents like isoniazide & rifampicin.[72] M. tubercle is an obligate aerobe that is capable of long-term persistence under conditions of low oxygen tension. Mycobacteria encounter hypoxic environments invivo which cause it to shift down from active replication to dormancy i.e. nonreplicating (NR) state.[73,74] Metronidazole is the first drug which has shown activity against dormant tubercle. Metronidazole acts as a pro-drug as it undergoes reduction at low redox potential in susceptible micro-organism to form intermediate nitro-group which in turn damages DNA and cause sub-sequent cell death.[75] Significant synergistic activities were observed in combination with rifampin and metronidazole.[74,76] In the single blind study, patients receiving metronidazole showed clinical improvements on the basis of improved rates of reduction of sputum

quantity and greater radiographic improvements.[77] Thus, it may be valuable to carry out further clinical trials of metronidazole.

1.7.11. Nitroimidazopyran PA-824

PA-824, a lead compound of series of nitroimidazopyrans synthesized on the basis of 5-nitroimidazole CGI 17341 is highly active against MDR-MTB and exhibits bactericidal action against dormant MTB.[78] In addition, PA- 824 has much reduced mutagenicity compared to that of CGI 17341.[79] PA-824 shows potent bactericidal activity against MTB mainly due to its inhibitory activity against both protein and lipid synthesis by MTB organisms after activation by a mechanism dependent on MTB F-420 cofactor.[79] Notably, in contrast to current antitubercular drugs, nitroimidazopyrans exhibited bactericidal activity against both replicating and static MTB. It lack the cross resistance to other first line antitubercular drugs.

1.7.12. Diarylquinoline TMC207 (Johnson & Johnson)

Diarylquinoline TMC207 is currently in phase IIa clinical trials and has shown promising therapeutic activity against mycobacterium tuberculi in preclinical studies. In preclinical studies it has showed potential for reduction of treatment duration. Diarylquinoline TMC207 is most active compound with unique spectrum and specificity to mycobacteria with minimum inhibitory concentration below 0.5 g/ml. Antimicrobial activity of TMC207 was confirmed in in-vivo studies.[80] Pharmacokinetic and pharmacodynamic studies in mice showed long plasma half-life, high tissue penetration and long tissue half-life. These are all attributes that are valuable for treatment of chronic infections and may also be important for development of simpler dosing regimens. The target and mechanism of action of diarylquinoline TMC207 is different from those of other anti-TB agents it acts by inhibiting the ATP synthase.[80,81] leading to ATP depletion and pH imbalance implying low probability of cross-resistance with existing-TB drugs. This is further suggested by the fact that diarylquinoline TMC207 is able to inhibit bacterial growth when tested on MDR-TB isolates.TMC207 has potent early bactericidal activity matching or exceeding that of isoniazid. When diarylquinoline TMC07 is used as substition for drug used in existing first line drug combination to treat TB it is observed that culture conversion occurs after 2 months of treatment in some combinations. In particular, the diarylquinoline-isoniazid-pyrazinamide and diarylquinoline-rifampicin-pyrazinamide combinations cleared the lungs of TB in all the mice after two months. When diarylquinoline TMC207 was tested in various combination with the second line drugs amikacin, pyrazinamide, moxifloxacin and ethionamide in mice (infected with the drug-susceptible virulent M. tuberculosis strain H37Rv) the diarylquinoline containing regimen was found to be more active than the current recommended regimen for MDR-TB. The diarylquinoline containing regimen attains the culture negativity of the

both lungs and spleens after 2 months of treatment in almost every case. [82]

1.7.12. Pyrrole LL- 3858 (Lupin Limited, India)

Lupin Limited reported the identification of Pyrrole derivative (LL-3858) that showed antimycobacterial activity in preclinical studies. When pyrrol LL-3858 was administered as monotherapy in infected mice it was found to be more active than Isoniazid. Combination of LL-3858 with currently existing first line drugs isoniazid-rifampicin, pyrazinamide sterilized the lungs of all infected mice within the period of less than 2 months. Experiments conducted in mice and dogs showed that the compound is well absorbed, with levels in serum above the MIC and better half-life and Cmax than those showed by isoniazid.[83] No information is available concerning the molecular mechanisms that mediate LL-3858's bactericidal activity. Pyrrole LL3858 is currently in Phase I Clinical Trials.

1.7.13. Pleuromutilins

Pleuromutilins have been shown to inhibit the growth of M. tuberculosis and acts by interfering with mycobacterial protein synthesis by binding to the 23S r RNA and thus inhibits the peptide bond formation.[84] However the current studies revealed that there exists the cross resistance amongs pleromutilins and oxazolidinones. [85] Pleuromutilins is derived from natural source and represents noval class of antibiotic. Currently GSK-TB Alliance is working on Pleuromutilins for the identification of pleuromutilins derivative that is active against MDR-TB and efficient enough in shortening the duration of existing duration of tubercular treatment.

1.7.14. Dipiperidine SQ-609 (Sequella Inc.)

Dipiperidine SQ-609 is a novel compound structurally unrelated to existing anti-TB drugs. It kills M. tuberculosis by interfering with cell wall biosynthesis (precise mechanism unknown). Anti-microbial activity has been demonstrated in vivo in mouse models. When tested in mice using a low-dose infection model of TB, SQ-109 at 1 mg/kg was as effective as ethambutol at 100mg/kg. However SQ-109 did not show improved effectiveness at higher doses (10mg/kg; 25mg/kg) and was clearly less effective than isoniazid. [86]

1.7.15. ATP Synthase Inhibitor

1.7.15.1. FAS20013 (FASgene)

It is a novel compound identified by Fasgen belonging to the class Sulphonylcarboxamides. In many preclinical studies the compounds have shown the therapeutic effectiveness against M. tuberculi. The compound is thought to act through inhibition of ATP synthase. The compound is very effective in killing MDR-TB organisms that are resistant to multiple drugs currently in use. The compound FAS20013 was proved to be superior in its ability to sterilize the TB lesions and kill latent TB compared to the all existing antitubercular drugs in a

series of recent laboratory experiments. The compound has good pharmacokinetic characteristics and is 100% bioavailable on oral administration. To date no dose limiting toxicity has been encountered, even when doses 10 times the effective dose were administered. [87,88]

1.7.15.2. InhA Inhibitors (GlaxoSmithKline-TB Alliance)

Isoniazid is considered to be first line drug in tuberculosis treatment. It requires activation in which Inheritant protective enzyme of mycobacteria - Kat G plays vital role. Isoniazid resistance to tuberculosis develops primarily by mutations in Kat G. InhA, the enoyl reductase enzyme from M. tuberculosis, catalyses the last step in the fatty acid biosynthesis pathway (FAS II). Active form of isoniazid inhibits this step. Consequently, InhA inhibitors that do not require activation by KatG are attractive candidates for drug discovery. The main purpose for this screen is therefore to bypass the activation step and directly inhibit InhA. A possible limitation for this kind of compounds is that cross-resistance with isoniazid may easily occur. Indeed, mutations in InhA encoding gene have been already identified in INH-resistance strains even if they occur less frequently than KatG mutations. [89]

CONCLUSION

The current antitubercular drugs offer treatment which demands continuous administration of drugs for six months. The patient non adherence along with HIV co-infection, emergence of MDR TB, XDR TB and recalcitrance of mycobacterium has pushed alarm for the need of effective molecules with better therapeutic outcomes. Newer drugs are needed to shorten the total duration of treatment and/or significantly reduce the number of doses needed to be taken under DOT, improve the treatment of MDR-TB, provide a more effective treatment of latent TB infection, provide more effective treatment of TB associated with HIV with minimal or no interaction with antiretroviral drugs. Established in 2000 and largely funded by the Bill & Melinda Gates Foundation, the Global Alliance for TB Drug Development (TB Alliance) has played a critical role in changing the TB research and development (R&D) landscape. It is evident that although candidates in TB pipeline have shown therapeutic promise in reducing the treatment duration, the number of such candidates available in TB pipeline is few.

Many of the candidates are analogues of drugs with known antitubercular activity hence possibility of cross resistance cannot be neglected. Better understanding the clinicopathogenesis of mycobacteria in human host is required in order to discover novel targets. Rational approaches for drug discovery should be implemented The most promising novel drug candidates currently in clinical stage were identified serendipitously in screenings and it were not designed originally for activity against M. tuberculosis. There is an urgent need to identify compounds acting on key targets that are essential for mycobacterium persistence. An example is the search for inhibitors of the isocitrate lyase, an enzyme that has been proven to be involved in the "dormancy" response (compounds able to inhibit this enzyme are expected to kill persistent bacteria). It is necessary to re-think the traditional roles played by academia and pharmaceutical industry in drug discovery and development when it comes to drugs for diseases like TB that do not represent an interesting market for the multinational Pharmaceutical industry. Without proper public sector engagement into translational research and implementation of rational drug design, fast progresses will be severely hampered.

REFERENCES

1. Pratt RJ. Extensively drug-resistant (XDR) tuberculosis: a new threat to global public health. British J Infect Control. 8, 2007, 20-22.

2. Rattan A, Kalia A, Nishat A, Multidrug-Resistant Mycobacterium tuberculosis: Molecular Perspectives. Emerging Infectious Diseases, 4(2), 1998, 195.

3. Cambier CJ, Falkow S, Ramakrishnan L, Host Evasion and Exploitation Schemes of Mycobacterium tuberculosis, Cell, 159 (7), 2014, 1497-509.

4. Orme I, Search for New Drugs for Treatment of Tuberculosis Antimicrobial agents and chemotherapy, 45(7), 2001, 1943–1946.

5. Alangaden GJ, Lerner SA, The clinical use of fluoroquinolones for the treatment of mycobacterial diseases. Clin Infect Dis, 25, 1997, 1213–1221.

6. Ziganshina LE, Vizel AA, Squire SB, Fluoroquinolones for treating tuberculosis. In Cochrane Database of Systematic Reviews, 3, 2005, Art.   No.:   CD004795. http://dx.doi.org/10.1002/14651858.CD004795.pub2

7. Ji B, Lounis N, Truffot-Pernot C, Grosset J, In vitro and in vivo activities of levofloxacin against Mycobacterium tuberculosis. Antimicrob Agents chemother, 39, 1995, 1341–1344.

8. Yew WW, Au KF, Lee J, Chau CH, Levofloxacin in treatment of drug-resistant tuberculosis, Int J Tuberc Lung Dis., 1, 1997, 89.

9. Yanmin Hu, Anthony RM, Coates, Denis M, Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of mycobacterium tuberculosis, Antimicrobial agents and chemotherapy, 47(2), 2003, 653–657.

10. Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, Choudhri S, Daley CL, Munsiff SS, Zhao Z, Vernon A, Chaisson RE, Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis, Am J Respir Crit Care Med, 174, 2006, 331–338.

11. El-Sadr W, Perlman DC, Matts JP, Nelson ET, Cohn DL, Salomon N, Olibrice M, Medard F, Chirgwin KD, Mildvan D, et al. Evaluation of an intensive intermittent-induction regimen and short course duration of treatment for HIV-related pulmonary tuberculosis. Clin Infect Dis., l26, 1998, 1148–1158.

12. Kohno S, Koga H, Kaku M, Maesaki S, Hara K. Prospective comparative study of ofloxacin or ethambutal for the treatment of pulmonary tuberculosis, Chest, 102, 1992, 1815–1818.

13. Lu T, Drlica K, In vitro activity of C-8-methoxy fluoroquinolones against mycobacteria when combined with anti-tuberculosis agents, J Antimicrobial Chemotherapy, 52, 2003, 1025-1028.

14. Grosset J, Truffot-Pernot C, Lacroix C, Ji B, Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice, Antimicrob Agents Chemother, 36, 1992, 548-551.

15. Paramasivan CN, Sulochana S, Kubendiran G, Venkatesan P, Mitchison DA, Bactericidal action of gatifloxacin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of Mycobacterium tuberculosis. Antimicrob Agents Chemother, 49, 2005, 627-631.

16. Hu Y, Coates AR, Mitchison DA, Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2003, 47: 653-657

17. Miyazaki E, Miyazaki M, Chen JM, Chaisson RE, Bishai WR, Moxifloxacin (BAY12-8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. Antimicrob Agents Chemother, 43, 1999, 85-89.

18. Pestova E, Millichap JJ, Noskin GA, Peterson LR, Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother, 45, 2000, 583- 590.

19. Coyle EA, Kaatz GW, Rybak MJ, Activities of newer fluoroquinolones against ciprofloxacin-resistant Streptococcus pneumonia, Antimicrob Agents Chemother, 45, 2001, 1654-1659.

20. Daporta MT, Munoz Bellido JL, Guirao GY, Hernandez MS, Garcia-Rodriguez JA, In vitro activity of older and newer fluoroquinolones against efflux-mediated high-level ciprofloxacin resistant Streptococcus pneumonia, Int J Antimicrob Agents, 24, 2004, 185-187.

21. Allen A, Bygate E, Oliver S, Johnson M, Ward C, Cheon AJ, Choo YS, Kim IC, Pharmacokinetics and tolerability of gemifloxacin (SB-265805) after administration of single oral doses to healthy volunteers. Antimicrobial Agents Chemother, 44(6), 2000, 1604-1608;

22. Ruiz-Serrano MJ, Alcalá L, Martínez L, Díaz M, Marín M, González-Abad MJ, Bouza E, In vitro activities of six fluoroquinolones against 250 clinical isolates of mycobacterium tuberculosis susceptible or resistant to first-line antituberculosis drugs. Antimicrobial Agents Chemother, 44(9), 2000, 2567-2568.

23. Milatovic D, Schmitz FJ, Brisse S, Verhoef J, Fluit AC, In vitro activities of sitafloxacin (DU-6859a) and six other fluoroquinolones against 8,796 clinical bacterial isolates. Antimicrobial Agents Chemother, 44(4), 2000, 1102-1107.

24. Tomioka H, Sato K, Akaki T, Kajitani H, Kawahara S, Sakatani M.Comparative in vitro antimicrobial activities of the newly synthesized quinolone HSR-903, sitafloxacin (DU-6859a), gatifloxacin(AM-1155) and levofloxacin against Mycobacteria tuberculosis and Mycobacterium avium complex. Antimicrob Agents Chemother, 43(12), 1999, 3001-3004.

25. Saito H, Tomioka H, Sato K, S Dekio, In vitro and in vivo antimycobacterial activities of a new quinolone, DU-6859a. Antimicrobial Agents Chemother, 38(12), 1994, 2877-2882.

26. Chan J, Flynn J. The immunological aspects of latency in tuberculosis. Clin Immunol, 110, 2004, 2-12.

27. Bozeman L, Burman W, Metchock B, Welch L, Weiner M, Fluoroquinolone susceptibility among mycobacterium tuberculosis isolates from the United States and Canada. Clin Infect Dis, 40, 2005, 386-391.

28. Ginsburg AS, Hooper N, Parrish N, Dooley KE, Dorman SE, Booth J, Diener-West M, Merz WG, Bishai WR, Sterling TR, Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. Clin Infect Dis, 37, 2003, 1448-1452.

29. Brogden RN, Rifabutin FA, A review of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. Drugs, 47, 1994, 983-1009.

30. Centers for Disease Control and Prevention (CDC), 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. MMWR Morb Mortal Wkly Rep, 49, 1999, 185-189.

31. Centers for Disease Control and Prevention (CDC), Prevention and treatment of tuberculosis among patients with human immunodeficiency virus: principles of therapy and revised recommendations.Centers for Disease Control and Prevention. MMWR Recomm Rep, 47, 1998, 1-58

32. Weber A, Kaplan M, Chughtai SA, Cohn LA, Smith AL, Unadkat JD, CYP3A inductive potential of the rifamycins, rifabutin and rifampin, in the rabbit,Biopharm Drug Dispos, 22, 2001, 157-168.

33. Bemer-Melchior P, Bryskier A, Drugeon HB, Comparison of the in vitro activities of rifapentine and rifampicin against Mycobacterium tuberculosis complex, J Antimicrob Chemother, 46, 2000, 571-575.

34. Mor N, Simon B, Mezo N, Heifets L, Comparison of activities of rifapentine and rifampin against Mycobacterium tuberculosis residing in human macrophages, Antimicrob Agents Chemother, 39, 1995, 2073-2077.

35. Keung ACF, Eller MG, McKenzie KA, Weir SJ, Single and multiple dose pharmacokinetics of rifapentine in man: part II, Int J Tuberc Lung Dis, 3, 1999, 437-44.

36. Temple ME, Nahata MC, Rifapentine: its role in the treatment of tuberculosis, Ann Pharmacother, 33, 1999, 1203-1210.

37. O'Brien RJ, Lyle MA, Snider DE Jr, Rifabutin (ansamycin LM 427): a new rifamycin-S derivative for the treatment of mycobacterial diseases, Rev Infect Dis, 9, 1987, 519-530.

38. Sullam PM, Rifabutin therapy for disseminated Mycobacterium avium complex infection, Clin Infect Dis, 22 (Suppl 1), 1996, S37-S41.

39. Grassi C, Peona V, Use of rifabutin in the treatment of pulmonary tuberculosis, Clin Infect Dis, 22 (Suppl 1), 1996, S50-S54.

40. Bruzzese T et al. Pharmacokinetics and tissue distribution of rifametane, a new 3-azinomethylrifamycin derivative, in several animal species. Arzneimittel-Forschung, 50(1), 2000, 60-71.

41. Strippoli V, Bruzzese T, Galli R, Simonetti N, Tronci N, The antibacterial activity of a new 3-azinomethylrifamycin. Il Farmaco, Edizione Scientifica 43(7-8), 1988, 619-625.

42. Potkar C, Gogtay N, Gokhale P, Kshirsagar NA, Ajay S, Cooverji ND, Bruzzese T, Phase I pharmacokinetic study of a new 3-azinomethyl-rifamycin (rifametane) as compared to rifampicin. Chemotherapy, 45(3), 1999, 147-153.

43. Kelkar MS, Saraf AP, Bakhle DS, Nazare V, Ajay S, Hegde S, Lal HM, Cooverji ND, Bruzzese T, Pharmacokinetic profile of a new 3-azinomethyl rifamycin (SPA-S-565) in volunteers as compared with conventional rifampicin, Int J Clin Pharmacol Res, 18(3), 1998, 137-143.

44. Shoen CM, DeStefano MS, Cynamon MH, Durable cure for tuberculosis: rifalazil in combination with isoniazid in a murine model of Mycobacterium tuberculosis infection, Clin Infect Dis, 30 (Suppl 3), 2000, 288-290.

45. Moghazeh SL, Pan XS, Arain TM, Stover CK, Musser JM, Comparative antimycrobial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant Mycobacterium tuberculosis isolates with known rpoB mutations, Antimicrob Agents Chemother, 40, 1996, 2655-2657.

46. Allen BW, Mitchison DA, Chan YC, Yew WW, Allan WG, Girling DJ. Amikacin in the treatment of pulmonary tuberculosis, Tubercle, 64, 1983, 111-118.

47. Davis KK, Kao PN, Jacobs SS, Ruoss SJ, Aerosolized amikacin for treatment of pulmonary Mycobacterium avium infections: an observational case series BMC Pulm Med, 7, 2007, 2.

48. Whitehead TC, Lovering AM, Cropley IM, Wade P, Davidson RN, Kinetics and toxicity of liposomal and conventional amikacin in a

patient with multidrug-resistant tuberculosis, European J Clin Microbiol Infectious Diseases, 17(11), 1998, 794-797.

49. Held HR, Landi S, Binding of toxic metabolites of isoniazid by aconiazide. J Pharm Sci, 69(11), 1980, 1284-1287.

50. Peloquin CA, James GT, Craig LD, Kim M, McCarthy EA, Iklé D, Iseman MD, Pharmacokinetic evaluation of aconiazide, a potentially less toxic isoniazid prodrug, Pharmacotherapy, 14(4), 1994, 415-423.

51. Truffot-Pernot C, Lounis N, Grosset JH, Ji B, Clarithromycin is inactive against Mycobacterium tuberculosis, Antimicrob Agents Chemother, 39, 1995, 2827-2828.

52. Bosne-David S, Barros V, Verde SC, Portugal C, David HL, Intrinsic resistance of Mycobacterium tuberculosis to clarithromycin is effectively reversed by subinhibitory concentrations of cell wall inhibitors. J Antimicrob Chemother, 46, 2000, 391-395.

53. Cavalieri SJ, Biehle JR, Sanders WE J, Synergistic activities of clarithromycin and antitubercular drugs against multidrug resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother, 39, 1995, 1542-1545.

54. Klemens SP, DeStefano MS, Cynamon MH, Therapy of multidrugresistant tuberculosis: lessons from studies with mice, Antimicrob Agents Chemother, 37, 1993, 2344-2347.

55. Janulionis E, Sofer C, Song HY, Wallis RS, Lack of activity of orally administered clofazimine against intracellular Mycobacterium tuberculosis in whole-blood culture, Antimicrob Agents Chemother, 48, 2004, 3133-3135.

56. Job CK, Yoder L, Jacobson RR, Hastings RC, Skin pigmentation from clofazimine therapy in leprosy patients: a reappraisal, J Am Acad Dermatol, 23, 1990, 236-241.

57. Jagannath C, Reddy VM, Kailasam S, O'Sullivan JF, Gangadharam PRJ, Chemotherapeutic activity of clofazimine and its analogues against Mycobacterium tuberculosis. In vitro, intracellular, and in vivo studies. Am Rev Respir Crit Care Med, 151, 1995, 1083-1086.

58. Muramatsu Y, Ishii MM, Inukai M, Studies on novel bacterial translocase I inhibitors, A-500359s. II. Biological activities of A-500359 A, C, D and G, J Antibiot (Tokyo), 56, 2003, 253-258.

59. Koga T, Fukuoka T, Doi N, Harasaki T, Inoue H, Hotoda H, Kakuta M, Muramatsu Y, Yamamura N, Hoshi M, Hirota T. Activity of capuramycin analogues against Mycobacterium tuberculosis, Mycobacterium avium and Mycobacterium intracellulare in vitro and in vivo. J Antimicrob Chemother, 54, 2004, 755-760.

60. Moellering RC, Linezolid: the first oxazolidinone antimicrobial. Ann Intern Med, 138, 2003, 135-142.

61. Bozdogan B, Appelbaum PC, Oxazolidinones: activity, mode of action, and mechanism of resistance, Int J Antimicrob Agents, 3, 2004, 113-9.

62. Luis Alcalá, María Jesús Ruiz-Serrano, Cristina Pérez-Fernández Turégano, Darío García de Viedma, Marisol Díaz-Infantes, Mercedes Marín-Arriaza, Emilio Bouza, In vitro activities of linezolid against clinical isolates of Mycobacterium tuberculosis that are susceptible or resistant to first-line antitubercular drugs, Antimicrob Agents Chemother, 47,2003, 416-417.

63. Rodriguez JC, Ruiz M, Lopez M, Royo G, In vitro activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against Mycobacterium tuberculosis Int J Antimicrob Agents, 20, 2002, 464-467.

64. Rodriguez JC, Daiz JC, Ruiz M, Lopez M, Royo G, Synergic activity of fluoroquinolones and linezolid against Mycobacterium tuberculosis, Int J Antimicrob Agents, 21, 2003, 354-6.

65. Jesu´ s Fortu´n, Pilar Martı´n-Da´vila, Enrique Navas, Ma Jesu´s Pe´rez-Elı´as, Javier Cobo, Marta Tato, Elia Go´mez-G, De la Pedrosa, Enrique Go´mez-ampaso, Santiago Moreno, Linezolid for the

treatment of multidrug-resistant tuberculosis, J Antimicrob Chemother, 56, 2005, 180–185.

66. I-Nae Park, Sang-Bum Hong, Yeon-Mok Oh, Mi-Na Kim, Chae-Man Lim, Sang Do Lee, Younsuck Koh, Woo Sung Kim, Dong Soon Kim, Won Dong Kim, Tae Sun Shim, Efficacy and tolerability of daily-half dose linezolid in patients with intractable multidrug-resistant tuberculosis, J Antimicrob Chemother, 58, 2006, 701.

67. Ying zhang, The Magic bullets and tuberculuous drug targets, Annual review of pharmacology and toxicology; Pro-quest medical library, 45, 2005, 529.

68. Ordway D, Viveiros M, Leandro C, Rosa´rio Bettencourt, Almeida J, Martins M, Kristiansen JE, Molnar J, Amaral L, Clinical concentrations of thioridazine kill intracellular multidrug-resistant Mycobacterium tuberculosis, antimicrobial agents and chemotherapy, 47, 2003, 917–922.

69. Amaral L., Kristiansen JE, Abebe'f L.S, Millett W, Brief reports Inhibition of the respiration of multi-drug resistant clinical isolates of Mycobacterium tuberculosis by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis, J Antimicrob Chemother, 38, 2003, 1049-1053.

70. Amaral L, Kristiansen JE, Atouguia MVJ, Activity of phenothiazines against antibiotic-resistant Mycobacterium tuberculosis: a review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy, J Antimicrob Chemother, 47, 2001, 505–511.

71. Dr Adrian C Harrison, Guidelines for Tuberculosis Control in New Zealand, Chapter 16: Treatment of Tuberculosis by Thoracic Society of Australia and New Zealand. 25, 2003,

72. Dick T, Dormant tubercule bacilli: Key to more effective TB chemotherapy, J Antibact Chemother, 47, 2001, 117.

73. Wanye LG, Sramek Hilda AA, Metronidazole Is Bactericidal to Dormant Cells of Mycobacterium tuberculosis, Antimicrobial agents chemother, 38(9), 1994, 2054 .

74. Iona E, Giannoni F, Pardini M, Brunori L, Orefici G, Fattorini L, Metronidazole plus Rifampin Sterilizes Long-Term Dormant Mycobacterium tuberculosis antimicrobial agents and chemotherapy, Antimicrobial Agents Chemother, 51(4), 2007, 1537-1540.

75. Bernadette Murugasu-Oei, Thomas Dick, Bactericidal activity of nitrofurans against growing and dormant Mycobacterium bovis BCG, J Antimicrob Chemother, 46(6), 2000, 917-919.

76. Li Y, Franzblau SG, Microplate Assay for Testing Bactericidal Activity of Compounds against Non growing Mycobacterium tuberculosis, chemotherapy Intersci Conf, Antimicrob Agents Chemother, 39, 1999, 203 (abstract no. 862)

77. Desai CR, Heera S, Patel A, Babrekar AB, Mahashur AA, Kamat SR, Role of metronidazole in improving response and specific drug sensitivity in advanced pulmonary tuberculosis, J Assoc Physicians India, 37, 1989, 694-697.

78. Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barry CE, Baker WR, A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis, Nature, 405, 2000, 962-966.

79. Barry CE, Boshoff HI, Dowd CS. Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis, Curr Pharm Des, 10, 2004, 3239-62.

80. Andries K, Verhasselt P, Guillemont J, Göhlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science, 307, 2005, 223-227.

81. Petrella S, Cambau E, Chauffour A, Andries K, Jarlier V, Sougakoff W, Genetic basis for natural and acquired resistance to the

diarylquinoline R207910 in mycobacteria. Antimicrob Agents Chemother, 50, 2006, 2853-2856.

82. Lounis NN, Veziris A, Chauffour C, Truffot-Pernot K. Andries, Jarlier V, Combinations of R207910 with drugs used to treat multidrug-resistant tuberculosis have the potential to shorten treatment duration. Antimicrob Agents Chemother, 50, 2006, 3543-3547.

83. Abstract n.63 submitted to the American Chemical Society Meeting, Anheim CA, 2004; http://wiz2.pharm.wayne.edu/ mediabstracts2004.pdf

84. Schlunzen F, Pyetan E, Fucini P, Yonath A, Harms JM, Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from Deinococcus radiodurans in complex with tiamulin. Mol Microbiol, 54, 2004, 1287-1294.

85. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B, The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics, Antimicrob Agents Chemother, 50, 2006, 2500-2505.

86. Protopopova M, Hanrahan C, Nikonenko B, Samala R, Chen P, Gearhart J, Einck L, Nacy CA, Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. J Antimicrob Chemother, 56, 2005, 968-974.

87. Jones PB, Parrish NM, Houston TA, Stapon A, Bansal NP, Dick JD, Townsend CA, A new class of antituberculosis agents. J Med Chem; 43, 2000, 3304-3314.

88. Parrish NM, Ko CG, Hughes MA, Townsend CA, Dick JD, Effect of noctanesulphonylacetamide (OSA) on ATP and protein expression in Mycobacterium bovis BCG. J Antimicrob Chemother, 54, 2004, 722-729.

89. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G. Jacobs WR, Jr. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science, 263, 1994, 227-230.