

DEVELOPMENT OF NEW DRUGS FOR TB CHEMOTHERAPY

Analysis of the current drug pipeline

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Author:

Martina Casenghi, PhD

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Summary

With approximately 9 million people developing active tuberculosis (TB) every year and 1.7 million deaths annually, TB is far from under control. Human immunodeficiency virus (HIV) infection dramatically increases the risk of developing active tuberculosis and is driving the TB epidemic in Africa. HIV renders tuberculosis more difficult to diagnose (due to higher incidence of sputum negative disease), and treat (due to interactions and side-effects). The increasing spread of multidrug-resistant TB (MDR-TB) and the recalcitrant nature of persistent infections pose additional challenges to treatment with currently available anti-TB drugs. The situation is exacerbated by the increasing emergence of extensively drug-resistant (XDR) TB. Resistance to at least two main first-line drugs and additionally to three or more of the six classes of second-line drugs makes this form of TB virtually untreatable with available drugs.

Although TB can be cured, current treatment is complex and long lasting, involving four drugs for 2 months and two drugs for at least another 4 months. Directly Observed Therapy (DOT), as promoted by the World Health Organisation (WHO) to improve compliance for the difficult and long-lasting regimen, is demanding for patients, labour intensive for health staff and is compromised in settings where health services are poorly accessible. MDR-TB is even more complex and expensive to treat, and in developing countries treatment is limited to a few projects with limited numbers of patients.

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 main criteria established by the TB Alliance to select drug candidates for further development are shortening of the current treatment, activity against MDR-TB and lack of interactions with antiretroviral drugs represent.

During the last years, increased public awareness of the lack of R&D for neglected diseases has led at least one pharmaceutical company to establish an institute undertaking R&D activities in tuberculosis on a 'no-profit-no-loss' basis. Other companies have engaged in tuberculosis R&D on for-profit basis, and with some success: three of the six anti-TB candidate drugs currently in clinical trials have been developed by for-profit companies.

Major advances have been also made in basic research. Modern molecular and genetic tools have become available for *Mycobacterium tuberculosis* (such as targeted mutagenesis, array-based analysis of mutant libraries, techniques for conditional gene silencing, and global gene expression profiling) and this has led to impressive improvements in the knowledge and understanding of the basic biology and physiology of *M. tuberculosis*. These progresses were

largely supported by major funding programmes from NIH/NIAID, the Wellcome Trust, and the EU during the 1990s.

Despite these positive changes there are still problems that need to be tackled. A critical question today is whether they are sufficient to bring improved treatment to patients in the next few years.

A first challenge concerns the sustainability of the current effort. As promising compounds move into expensive clinical trials, PDPs such as the TB Alliance face a significant funding gap. These initiatives are driven almost entirely by philanthropic effort, with governments only contributing a meagre 16% to PDPs engagement in drug development. Financial support will need to increase to ensure that the development of these promising new compounds is supported all the way to trials.

The next important question is whether there are a sufficient number of promising compounds in the TB pipeline for a broadly effective new treatment combination to be developed. Although different attrition rates might apply, the number of candidate compounds is still small compared to the drug pipelines for diseases of major concern to wealthy countries such as cancer or cardiovascular diseases (and the number of companies engaged in the latter is also greater).

Furthermore, many of the compounds in the pipeline are either derivatives of existing compounds or they target the same cellular processes as drugs currently in use. Whilst analogues and derivatives are far quicker to develop, they may be subject to cross-resistance, as has been the case with the new rifamycins and quinolones.

Modern technologies and rational approaches to drug design (such as creation of genomic libraries of *M. tuberculosis* conditional knock-out mutants for comprehensive target identification and validation, target-based drug discovery, or determination of three-dimensional crystal structure of molecular targets) are still weakly implemented in the field of drug discovery for tuberculosis. Even the more promising candidate compounds currently in clinical development were identified serendipitously in screenings that were not designed originally for activity against *M. tuberculosis*. There is consensus among the TB scientific community that in order to obtain a real breakthrough in TB therapy and drastically shorten treatment there is an urgent need for rational approaches aimed at tackling the problem of mycobacterial persistence. The adaptations that allow *M. tuberculosis* to persist in the host despite a vigorous adaptive immune response likely contribute to the difficulty in curing TB with current chemotherapy. Although drugs currently in the pipeline could significantly shorten treatment, it is likely to remain a matter of months rather than weeks or days.

There are two major roadblocks that hamper the implementation of rational drug design in TB drug discovery. The first is the lack of a comprehensive characterization of the fundamental biology of mycobacteria as they persist in human tissues, which prevents the identification and validation of potential targets that are relevant for the survival of the bacteria *in vivo*. The second is the weak engagement into early-stage drug discovery; as a consequence the advanced knowledge about *M.*

tuberculosis metabolism, physiology and genetics is not being translated into validated targets that can be used for screening of new lead compounds.

As part of the Grand Challenges in Global Health initiative the Gates Foundation is funding research into the molecular pathways of persistence, with the aim of novel target identification. In addition, the Gates Foundation recently announced a new initiative that specifically aims at accelerating drug discovery for tuberculosis. While acknowledging this significant contribution, it is important not to rely exclusively on a single initiative to address a complex scientific problem of such great importance. Much attention must be paid to these critical issues.

If faster progress is to be achieved in drug discovery for tuberculosis then the advanced knowledge about *M. tuberculosis* metabolism and physiology needs to be translated into validated targets that can be used for screening of new lead compounds. A key difficulty lies in securing sustained funding for translational research projects such as target validation and chemical genetics. Up to now the major funding bodies for basic research in TB – NIH/NIAID, the Wellcome Trust, and the EU – give priority to “blue sky” basic research projects and “hypothesis-driven” science. Rare exceptions are made for occasional grants based on request for application, but generally it is very difficult for academic labs to obtain funds for projects that fall between the areas of basic and applied research. The private sector for its part is reluctant to engage in early stage drug discovery projects; drug development is instead only embarked upon when rigorously validated targets are available or a lead compound has been already identified.

Real improvements in TB treatment will require substantial strengthening of early-stage discovery research to identify new compounds and targets. Without a thriving background of discovery-oriented translational research, which is largely dependent on public funding, drug development is destined to fail in terms of long-term goals for effective TB management. Existing modern technologies need to be urgently and more comprehensively applied to TB if the pipeline for drug R&D is to be filled. The reluctance of the pharmaceutical sector to invest in early-stage discovery research for neglected diseases exacerbates the pressing need to translate basic scientific knowledge into novel targets and fresh approaches towards improved therapies. Without proper public engagement in early stage drug discovery and implementation of rational approaches, progress in innovation will be severely hindered.

1. Introduction

Mycobacterium tuberculosis infects about 32% of the world's population. Every year, approximately 8 million of these infected people develop active tuberculosis (TB) and almost 2 million of these will die from the disease (WHO, 2005).

Despite 40 years of anti-TB chemotherapy, tuberculosis remains one of the leading infectious diseases worldwide. 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) and the recalcitrance of persistent infections to treatment with conventional anti-TB drugs (Corbett et al., 2003; Gomez and McKinney, 2004; Smith et al., 2003). The situation is exacerbated by the increasing emergence of extensively drug-resistant (XDR) TB (CDC, 2006). XDR-TB is characterized by resistance to at least the two first-line drugs rifampicin and isoniazid and additionally to a fluoroquinolone and an injectable drug (kanamycin, amikacin or capreomycin) among the second-line drugs (WHO Meeting of the Global XDR-TB Task Force). The extensive resistance makes this form of TB particularly cumbersome to treat with available drugs (ref). The current situation clearly demonstrates the need for a re-evaluation of our approach to treating TB. Drug development for tuberculosis and other neglected diseases has been at a virtual standstill for decades, but increased awareness and advocacy in recent years have led to new initiatives in TB drug development. Today, the TB drug pipeline is no longer empty as it was five years ago.

This report provides a detailed analysis of today's TB drug pipeline in an attempt to see whether current approaches are likely to provide truly effective new tools to treat tuberculosis.

2. Targets and mode of action of current TB drugs

Current chemotherapy for TB largely relies on drugs that inhibit bacterial metabolism with a heavy emphasis on inhibitors of the cell wall synthesis (for review see Zhang, 2005). According to their mode of action, first and second line TB drugs can be grouped as cell wall inhibitors (isoniazide, ethambutol, ethionamide, cycloserine), nucleic acid synthesis inhibitors (rifampicin, quinolones), protein synthesis inhibitors (streptomycin, kanamycin) and inhibitors of membrane energy metabolism (pyrazinamide). Targets and mechanisms of action of current TB drugs are summarized in Table 1.

Existing TB drugs are therefore only able to target actively growing bacteria through the inhibition of cell processes such as cell wall biogenesis and DNA replication. This implies that 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 metabolising bacteria that persist after the growing bacteria have been killed by bactericidal drugs (i.e.

persists; (Mitchison, 1980). Sterilizing activity also describes the ability to eliminate latent or “dormant” bacteria that survive inside the host macrophages. This bias is hardly surprising as anti-TB drugs have traditionally been identified by their ability to suppress or kill replicating cultures of bacteria *in vitro*.

The weak sterilizing property of available TB drugs is one of the major drawbacks for current TB chemotherapy. Although rifampicin (RIF) and pyrazinamide (PZA) are partially sterilizing drugs and play an important role in shortening the therapy from 12-18 months to 6 months, there are still populations of persisting bacteria that are not killed by RIF and PZA. Thus, although achieving a clinical cure, the current TB chemotherapy does not achieve a bacteriological cure since the therapy cannot completely eradicate all bacilli in the lesions (McCune and Tompsett, 1956).

Table 1. Commonly used TB drugs and their targets (adapted from Zhang, 2005)

Drug (year of discovery)	MIC ^a (µg/ml)	Effect on bacterial cell	Mechanisms of action	Targets	Genes involved in resistance
Isoniazid (1952)	0.01-0.2	Bactericidal	Inhibition of cell wall mycolic acid and other multiple effects on DNA, lipids, carbohydrates and NAD metabolism	Primarily acyl carrier protein reductase (InhA)	<i>katG^b</i> ; <i>InhA</i> ; <i>ndh</i>
Rifampin (1966)	0.05-0.5	Bactericidal	Inhibition of RNA synthesis	RNA polymerase β subunit	<i>rpoB</i>
Pyrazinamide (1952)	20-100 pH 5.5 or 6.0	Bactericidal	Disruption of membrane transport and energy depletion	Membrane energy metabolism	<i>pncA^b</i>
Ethambutol (1961)	1-5	Bacteriostatic/ Bactericidal	Inhibition of cell wall arabinogalactan synthesis	Arabinosyl transferase	<i>embCAB</i>
Streptomycin (1944)	2-8	Bacteriostatic	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRNA	<i>rpsL</i> ; <i>rrs</i> (operon)
Kanamycin (1957)	1-8	Bactericidal	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRNA	<i>rpsL</i> ; <i>rrs</i> (operon)
Quinolones (1963)	0.2-4	Bactericidal	Inhibition of DNA replication and transcription	DNA gyrase	<i>gyrA</i> ; <i>gyrB</i>
Ethionamide (1956)	0.6-2.5	Bacteriostatic	Inhibition of mycolic acid synthesis	Acyl carrier protein reductase (InhA)	<i>inhA</i> ; <i>etaA/ethA^b</i>
PAS (1946)	1-8	Bacteriostatic	Inhibition of folic acid and iron metabolism?	Unknown	Unknown
Cycloserine (1952)	5-20	Bacteriostatic	Inhibition of peptidoglycan synthesis	D-alanine racemase	<i>alrA</i> ; <i>Ddl^c</i>

^aMIC is based on Inderlied & Salfinger (Inderlied CB, Salfinger M. 1999. Antimycobacterila agents and susceptibility tests. In *Manual of Clinical Microbiology*, ed. PR Murray, EJ Baron, MA Tenover, FC Tenover, RH Tenover, pp1601-1623. Washington DC: ASM Press /1th ed.)

^bKatg, PncA and EtaA/EthA are enzymes involved in the activation of prodrugs INH, PZA and ETH, respectively.

^cIn fast growing *M. smegmatis*

3. Why new drugs are needed?

HIV has dramatically increased the risk of developing active tuberculosis and HIV co-infection makes tuberculosis more difficult to diagnose (due to more complicated presentations) and treat (due to interactions and side-effects). The increasing emergence of multi-drug resistant TB (MDR-

TB) and the recalcitrant nature of persistent infections pose additional challenges to treatment with conventional anti-TB drugs.

Although TB can be cured with current drugs treatment is complex and long-lasting, involving four drugs for two months and two drugs for at least another 4 months. This makes compliance difficult. Directly Observed Treatment (DOT) as promoted by the World Health Organisation (WHO) to improve compliance for the difficult and long regimen can improve cure rates but is demanding for patients and labour intensive for health staff (O'Brien and Nunn, 2001).

Why is the treatment for TB with conventional drugs so slow and difficult? In pioneering studies McDermott and colleagues showed that the efficacy of drugs against *M. tuberculosis in vitro* was not matched by their efficiency *in vivo* (McCune et al., 1956). The difference is striking; exponentially growing cultures of *M. tuberculosis* can be sterilized in a few days using frontline bactericidal drugs such as isoniazid and rifampicin, yet the same drug combination requires months to achieve similar effects against bacteria living in host tissues. Why? The most obvious explanation would be failure of drugs to achieve optimal levels within TB lesions, but there is evidence that drugs availability is not a limiting factor (Barclay et al., 1953; Clark, 1985). It has been proposed that persistence of tubercle bacilli in the face of chemotherapy might be attributable to physiologic heterogeneity of bacteria in the tissues (Mitchison, 1979). This idea was inspired and supported by the long-established observation that slow- and non-growing bacteria are phenotypically resistant or tolerant to killing by antimicrobials (Handwerger and Tomasz, 1985). According to Mitchison, tubercle bacilli in lesions consist of at least four different populations (see figure 1):

- 1) Bacteria that are actively growing, killed primarily by isoniazid (INH)
- 2) Bacteria that have spurts of metabolism, killed by rifampicin (RIF)
- 3) Bacteria that are characterized by low metabolic activity and reside in acid pH environment, killed by pyrazinamide (PZA)
- 4) Bacteria that are "dormant" or "persisters", not killed by any current TB drug.

During the initial phase of chemotherapy, which lasts for about 2 days, bacilli are killed exponentially at a rapid rate, followed by a further lengthy period of much slower exponential killing (Jindani et al., 2003). It is assumed that those bacilli killed in the first 2 days are actively multiplying, while those in the succeeding period are persisters killed by the slower sterilizing activities of the drugs. As mentioned in the previous section, drugs in the current regimen differ in their relative bactericidal activities, with the activity of isoniazid predominating during the initial phase, and in their subsequent sterilizing activity, with the activity of rifampicin and pyrazinamide predominating during the continuation phase (Dickinson and Mitchison, 1981; Jindani et al., 2003). In an *in vitro* model of drug action, a 30-day static culture has been extensively used for the last 60 years and has been taken to resemble the persister population in its response to drugs (Herbert et al., 1996; Mitchison, 1992; Mitchison and Selkon, 1956). The drugs added to this static culture have the

same slow sterilizing action that is responsible for the prolongation of therapy. Thus, evidence suggests that activity against the population of persistent bacilli ultimately determines the duration of therapy necessary for a given regimen to sterilize lesions and provide a stable cure of the host (Grosset and Ji, 1998). From this there is evidently an urgent need to develop new and more effective TB drugs that are not only active against MDR-TB but also shorten the length of treatment targeting non-replicating persistent bacilli.

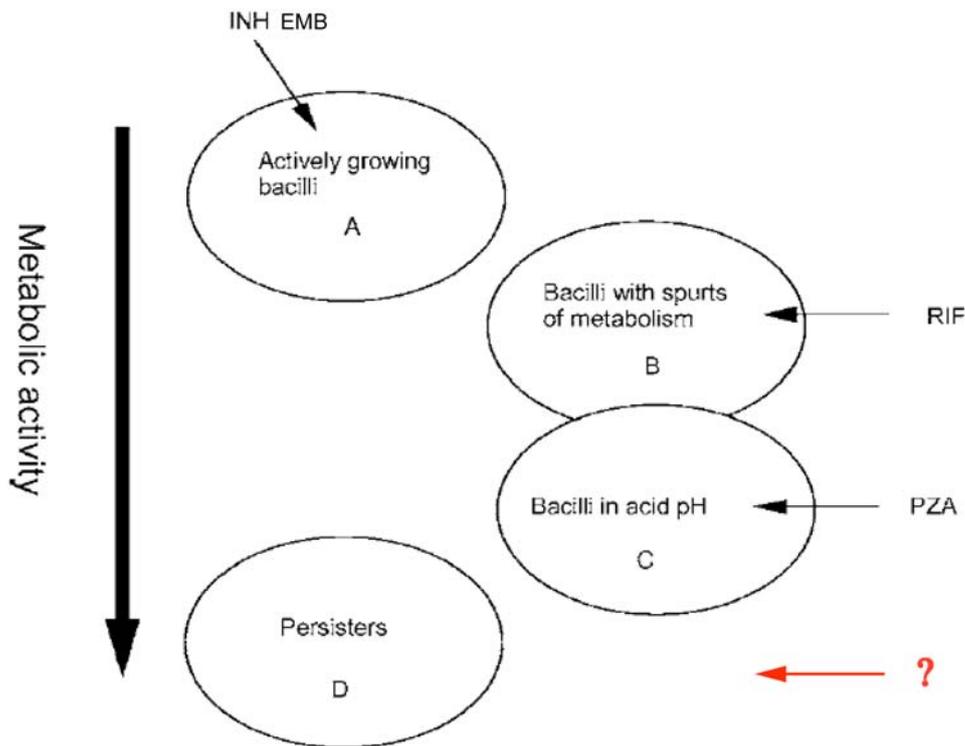


Figure 1. TB drugs targeting distinct *M. tuberculosis* subpopulation (adapted from Zhang, 2005)

As emphasized by the Guidelines for Tuberculosis drug development issued by the Global Alliance for TB Drug Development (TB Alliance) (Global Alliance for TB drug development, (2001); <http://www.tballiance.org/pdf/TB%20Scientific%20Blueprint%20Full.pdf>) a new TB treatment should offer at least one of the following three improvements over the existing regimens:

- 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

Shortening of the current treatment, activity against MDR-TB, and lack of liver enzyme induction and inhibition (to avoid interactions with antiretrovirals) are the main criteria the TB Alliance is using to select drug candidate that should be pursued for further development. Finding a treatment

for latent TB is currently not a strategic priority for the TB Alliance as it considers treatment of active TB as a more feasible achievement to be reached in a short-term perspective.

In order to shorten the development time for a new regimen, TB Alliance is working on both identifying individual novel compounds and developing new drug combinations. TB Alliance is currently engaged in discussions with regulatory authorities (FDA and EMEA) to see how they can test new compounds simultaneously rather than consecutively. Indeed, the conventional approach to drug development requires to substitute each drug in the current regimen singularly, only after each new drug has been approved. Considering that it takes on average 6 years for a new drug to be registered, the development of a completely new first line regimen could take approximately 24 years. TB Alliance's innovative proposal of testing new compounds simultaneously could drastically shorten the procedure, but ethical implications have to be taken in strong consideration in identifying a practical way to implement such clinical trial design.

4. The new TB drug pipeline

Drug development for tuberculosis and other neglected diseases has been at a standstill for decades. Today however, thanks also to the work of the Global Alliance for TB Drug Development (TB Alliance), the TB drug pipeline is richer than it has been in the last forty years. Created in 2000¹ and largely funded by the Bill & Melinda Gates Foundation, the TB Alliance is a product development partnership (PDP) that focuses on both pre-clinical and clinical development of candidate compounds for TB chemotherapy. The TB Alliance is associated with approximately half of all compounds (or projects aimed to identify candidate compounds) currently being developed.

In addition to this, increased public awareness on the lack of R&D for neglected diseases in recent years have led some multinational pharmaceutical companies to set up Research and Development (R&D) institutes or initiatives in drug development for tuberculosis, dengue, malaria and leishmaniasis on a 'no-profit-no-loss' basis. Among the multinational pharmaceutical companies currently involved in anti-TB drug R&D are Novartis, AstraZeneca and GlaxoSmithKline (GSK). Smaller pharmaceutical companies have also engaged in neglected disease R&D on a commercial basis (Moran et al., 2005), and with some success: two of the anti-TB candidate drugs currently in clinical trials have been developed by medium-size pharmaceuticals companies such as Lupin Limited (India) and Otsuka Pharmaceuticals (Japan).

The *global* TB drug pipeline as reported by the Stop TB partnership working group on new TB drugs

[http://www.stoptb.org/wg/new_drugs/assets/documents/WGND%20Strategic%20Plan%20\(final\).p](http://www.stoptb.org/wg/new_drugs/assets/documents/WGND%20Strategic%20Plan%20(final).p)

¹ MSF took an active role in the founding of the TB Alliance and former MSF president James Orbinski became the first president of the TB Alliance stakeholder Association right after he left MSF

df) is summarised in Table 2. This is an overview of all drug candidates in the pipeline, belonging to different entities and not only the TB Alliance. In order to analyse the pipeline it is useful to group drug candidates currently in two main categories:

- 1) Novel chemical entities
- 2) Compounds originating from existing families of drugs, where innovative chemistry is used to optimise the compounds

Table 2. Global TB drug pipeline March 2006 (kindly provided by Stop TB Partnership working group on new TB drugs)

<i>Discovery</i>		<i>Preclinical</i>	<i>Clinical</i>
Thiolactomycin Analogs NIAID, NIH	Nitrofuranylamides NIAID, University of Tennessee	Diamine SQ-109 Sequella Inc.	Diarylquinoline TMC207 Johnson & Johnson
Cell Wall Inhibitors Colorado State University, NIAID	Nitroimidazole Analogs NIAID, Novartis Institute for Tropical Diseases TR Alliance University of Auckland	Dipiperidines (SQ-609) Sequella Inc.	Gatifloxacin OFLOTUB Consortium, Lupin, NIAID TBRU, Tuberculosis Research Centre; WHO-TDR
Dihydroipoamide Acyltransferase Inhibitors Cornell University, NIAID	Focused Screening GlaxoSmithKline, TB Alliance	Nitroimidazo-oxazole Back-up Otsuka	Moxifloxacin Bayer Pharmaceuticals, CDC TBTC, Johns Hopkins University, NIAID TBRU, TB Alliance
InhA Inhibitors GlaxoSmithKline, TB Alliance	Picolinamide Imidazoles NIAID, TAACF	Synthase Inhibitor FAS20013 FASgen Inc.	Nitroimidazole PA-824 Chiron Corporation, TB Alliance
Isocitrate Lyase Inhibitors (ICL) GlaxoSmithKline, TB Alliance	Pleuromutilins GlaxoSmithKline, TB Alliance	Translocase I Inhibitors Sequella Inc., Sankyo	Nitroimidazo-oxazole OPC-67683 Otsuka
Macrolides TB Alliance, University of Illinois at Chicago	Quinolones KRICT/ Yonsei University, NIAID, TAACF, TB Alliance	Non-Fluorinated Quinolones TaiGen	Pyrrrole LL-3858 Lupin Limited
Methyltransferase Inhibitors Anacor Pharmaceuticals	Screening and Target Identification AstraZeneca		
Natural Products Exploration BIOTEC, California State University, ITR, NIAID, TAACF University of Auckland			

4.1 Novel chemical entities

Diarylquinoline TMC207 (Johnson & Johnson)

Diarylquinoline TMC207 is an extremely promising member of a new class of anti-mycobacterial agents. To date, 20 molecules of the diarylquinoline series have been shown to have a minimum inhibitory concentration below 0.5 µg/ml against *M. tuberculosis* H37Rv. Antimicrobial activity was confirmed *in vivo* for three of these compounds (Andries et al., 2005). The most active compound of the class is TMC207 and its spectrum is unique in its specificity to mycobacteria. The target and mechanism of action of diarylquinoline TMC207 is different from those of other anti-TB agents 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. Diarylquinoline TMC207 seems to act by inhibiting the ATP synthase (Andries et al., 2005; Petrella et al., 2006), leading to ATP depletion and pH imbalance. This new anti-mycobacterial compound

has potent early bactericidal activity in the non-established infection murine mouse model, matching or exceeding that of isoniazid. Moreover, diarylquinoline TMC207 has potent late bactericidal activity in the established infection in murine TB model. Substitution of rifampicin, isoniazid or pyrazinamide with diarylquinoline TMC207 accelerated activity leading to complete culture conversion 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. Diarylquinoline TMC207 has been also 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. Diarylquinoline containing regimen were more active than the current recommended regimen for MDR-TB amikacin-pyrazinamide-moxifloxacin-ethionamide and culture negativity of the both lungs and spleens was reached after 2 months of treatment in almost every case (Lounis et al., 2006). A thorough assessment of diarylquinoline activity against MDR-TB *in vivo* would however require testing of animal models infected with multi-drug resistant bacterial strains rather than with drug-susceptible strains.

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 (Andries et al., 2005).

Originally identified by Johnson&Johnson scientists diarylquinoline TMC207 has been transferred to Tibotec Pharmaceuticals Limited (a J&J subsidiary company) for further clinical development and it is now referred to as TMC207. Preliminary studies in mouse models indicate that diarylquinoline TMC207 has sterilizing activity *in vivo*. Studies in mice also showed potential reduction of treatment duration. Diarylquinoline TMC207 is currently in phase IIa clinical trials (Tibotec/Johnson & Johnson personal communication)

Nitroimidazole PA-824 (Chiron Corp.-TB Alliance)

Nitroimidazole PA-824 is a new nitroimidazole derivative developed by PathoGenesis-Chiron and currently being developed by the TB Alliance. The TB Alliance received worldwide exclusive rights to PA-824 and its analogs for the treatment of TB and Chiron pledged to sell it royalty-free for endemic countries. After activation by a mechanism dependent on *M. tuberculosis* F420 factor, PA-824 acts mainly by inhibiting the synthesis of cell wall components through molecular targets that are yet to be identified. *In vitro*, PA-824 showed high activity against drug-sensitive and drug-resistant *M. tuberculosis* strains, indicating that there is no cross-resistance with current TB drugs. Moreover, PA-824 exhibited bactericidal activity against both replicating and static bacteria *in vitro* (Stover et al., 2000). PA-824 bactericidal activity against non-replicating bacteria was comparable to that of RIF (Lenaerts et al., 2005). Experiments performed in mice showed that administration of

PA-824 at doses ranging from 25 to 100 mg/ml produced reductions in the bacterial burden in spleen and lungs that were comparable to that produced by INH at 25 mg/ml (Stover et al., 2000; Tyagi et al., 2005). In order to test for possible sterilizing activity the compound was tested in continuation phase in mouse models that had received RHZ for 2 months. Although PA-824 was significantly more efficient than isoniazid or moxifloxacin in clearing the infection during the continuation phase, it was not better than that of rifampicin+isoniazid combination (Tyagi et al., 2005). In long-term treatment experiments performed to determine its sterilizing capacity, administration of PA-824 as monotherapy in mice led to a decrease in bacterial counts in the lungs comparable to that obtained with rifampicin or isoniazid monotherapy. After 12 weeks of treatment with PA-824, rifampicin or isoniazid, a complete eradication of the bacterial load was not achieved in any of the treated mice (Lenaerts et al., 2005). When a 6-month treatment regimen containing PA-824 in combination with rifampicin, isoniazid and pyrazinamide was tested in mice, any of the PA-824 containing regimens resulted superior to the standard first line regimen in terms of more rapid reductions of the bacterial burden during treatment and lower rates of relapse after treatment (Nuemberger et al., 2006). Further investigations are required to assess the potentiality of PA-824 to improve the treatment of both drug-susceptible and multi-drug resistant tuberculosis when used in novel combinations with new drug candidates in addition to existing antituberculosis drugs. PA-824 entered phase I clinical trials in June 2005.

Nitroimidazole OPC-67683 (Otsuka Pharmaceuticals, Japan)

Little information about this compound is publicly available. It belongs to a subclass of mycolic acid inhibitors, thus it interferes with the biosynthesis of the mycobacteria cell wall. Minimum inhibitory concentrations (MICs) of this compound were determined using standard and clinically isolated *M. tuberculosis* strains, including multi-drug resistant strains. *In vitro*, OPC-67683 showed high activity against drug-sensitive as well drug-resistant strains with MICs ranging 6 - 24 ng/mL. No cross-resistance with any of the current first-line drugs was observed. Moreover, OPC-67683 showed strong intracellular activity against H37Rv strain of *M.tuberculosis* residing within human macrophages and type II pneumocytes. Studies in animal models showed that OPC-67683 is effective against sensitive (H37v) and MDR-TB strains *in vivo* starting from a concentration of 0.03125 mg/body. Furthermore, OPC showed *in vivo* efficacy against H37Rv strain even in SCID mice (affected by a severe immune deficiency and used as a model for AIDS), starting from a concentration of 0.00781 mg/body. When tested in mouse models for chronic tuberculosis, OPC-67683 showed a 6-7 fold higher activity compared to first-line drugs isoniazid and rifampicin. No antagonist activity could be observed when OPC-67683 was used in combination with currently used anti-TB drugs *in vivo*. Pharmacokinetics studies in mice, rats and dogs revealed that this compound is relatively well absorbed after oral dosing at 3 mg/kg. The bioavailability in each species was 35-60% with a concentration 3-7 times higher in the lung than in the plasma. The

compound was well distributed in most tissues (Abstracts submitted to 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Washington DC Dec 16-19/2005 <http://www.icaac.org/>). The TB Alliance is currently negotiating with Otsuka Pharmaceuticals concerning the further joint development of this compound and the project is in discussion.

Pyrrole LL- 3858 (Lupin Limited, India)

Very limited information on the development of pyrroles as anti-mycobacterial agents is currently available. Pyrroles derivatives were found to be active against standard and drug-sensitive *M. tuberculosis* strains in vitro (Deidda et al., 1998; Ragno et al., 2000) Lupin Limited reported the identification of a Pyrrole derivative (LL-3858) that showed higher bactericidal activity than Isoniazid when administered as monotherapy to infected mice. In mouse models, a 12 weeks treatment with LL-3858 plus isoniazid and rifampicin, or LL-3858 plus isoniazid-rifampicin-pyrazinamide, sterilized the lungs of all infected mice. 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. No information is available concerning the molecular mechanisms that mediate LL-3858's bactericidal activity (Abstract n.63 submitted to the American Chemical Society Meeting, Anaheim CA, March 28-April 01 2004; <http://wiz2.pharm.wayne.edu/mediabstracts2004.pdf>). Pyrrole LL3858 is currently in Phase I Clinical Trials (Lupin Limited, personal communication; Dr Federico Gomez de las Heras, GSK at TB Alliance stakeholder meeting, Paris 2005)

Pleuromutilins (GlaxoSmithKline-TB Alliance Partnership)

The pleuromutilins represent a novel class of antibiotics derived from a natural product. They interfere with protein synthesis by binding to the 23S rRNA and therefore inhibiting the peptide bond formation (Schlunzen et al., 2004). Despite the novelty of this class of compounds, recent studies have shown that cross-resistance might occur among pleuromutilins and oxazolidinones (Long et al., 2006). Pleuromutilins have been shown to inhibit the growth of *M. tuberculosis in vitro*. The goal of this project, launched in collaboration with GSK, is the identification of a pleuromutilin derivative that is active against MDR-TB and allows shortening of the treatment (GSK-TB Alliance Fact Sheet http://www.tballiance.org/specials/gsk/gsk-tba_fact_sheet.html; Global TB Alliance Annual report 2004-2005 http://www.tballiance.org/downloads/2005%20annual%2008_6b.pdf).

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 (<http://www.sequella.com/pipeline/SQ%20609.asp>; (Nikonenko et al., 2004); (Kelly et al., 1996)

ATP Synthase Inhibitor FAS20013 (FASgene)

FAS20013 is a novel compound identified by Fasgen. It belongs to the class of β -sulphonylcarboxamides. Fasgen claims that “FAS20013 will kill more organisms in a 4-hour exposure than isoniazid or rifampicin can during a 12- to 14-day exposure. The compound is very effective in killing MDR-TB organisms that are resistant to multiple drugs currently in use. A series of recent laboratory experiments indicates the superior effect of FAS20013 compared to current drugs in terms of its ability to "sterilize" TB lesions and kill latent TB. Therapeutic evaluation of FAS20013 has repeatedly shown its effectiveness in mice, and appears to have no serious side effects. The compound is up to 100% bioavailable when administered orally. To date no dose-limiting toxicity has been encountered, even when doses 10 times the effective dose were administered.” The compound is thought to act through inhibition of ATP synthase. However, available scientific publications assessing the efficacy of this compound are of poor quality. (Jones et al., 2000; Parrish et al., 2004)

Translocase I Inhibitor (Sequella Inc.)

Sequella is developing a series of translocase inhibitors for the potential treatment of tuberculosis. The compounds specifically inhibit mycobacterial translocase I, an enzyme required for bacterial cell wall synthesis. Preclinical evaluation of the compounds is planned. (<http://www.sequella.com/pipeline/translocaseinhibitor.asp>).

InhA Inhibitors (GlaxoSmithKline-TB Alliance)

InhA, the enoyl reductase enzyme from *Mycobacterium tuberculosis*, catalyses the last step in the fatty acid biosynthesis pathway (FAS II). Frontline anti-tuberculosis drugs such as isoniazid (INH) target this enzyme. Drug resistance to INH results primarily from mutations in KatG, the enzyme that activates INH. 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 (Banerjee et al., 1994) even if they occur less frequently than KatG mutations.

Isocitrate Lyase Inhibitors (GlaxoSmithKline-TB Alliance)

The isocitrate lyase (ICL) enzyme has been shown to be essential for long-term persistence of *M. tuberculosis* in mice, but not required for bacilli viability in normal culture or hypoxic conditions

(McKinney et al., 2000). McKinney and collaborators have recently shown that inhibition of ICL1 and ICL2, the two isoforms of isocitrate lyase present in *M. tuberculosis*, blocks growth and survival of *M. tuberculosis* bacteria in macrophages and in mice at early and late stage of infection (Munoz-Elias and McKinney, 2005). The absence of ICL orthologs in mammals should facilitate the development of glyoxylate cycle inhibitors as new drugs for the treatment for tuberculosis. Such a new drug is expected to be able to kill persistent bacteria and therefore have sterilizing activity and shorten treatment time. Guided by the three-dimensional structure of isocitrate lyase (Sharma et al., 2000), GSK launched in 2000 a screening to identify ICL inhibitors as potential therapeutic drugs. Up to now 900,000 compounds have been screened but no successful inhibitors have been identified. GSK is currently planning to screen additional 400,000 compounds (Global TB Alliance Annual report 2004-05; http://www.tballiance.org/downloads/2005%20annual%20008_6b.pdf). The structure of ICL active site is making the screening for inhibitors particularly lengthy and laborious. The active site of this enzyme, indeed, appears not to be easily and effectively reached by compounds (J McKinney personal communication).

4.2 Compounds originating from existing families of drugs

Using existing Fluoroquinolones for TB ?

Fluoroquinolones were introduced into clinical practice in the 1980s. Characterized by broad-spectrum antimicrobial activity, they are recommended and widely used for the treatment of bacterial infection of the respiratory, gastrointestinal and urinary tracts (Bartlett et al., 2000; Neu, 1987). Fluoroquinolones have been also found to have activity against *M. tuberculosis* (Grosset, 1992; Tsukamura et al., 1985) and are currently part of the recommended regimen as second-line drugs (Crofton, J., P. Chaulet, D. Maher, J. Grosset, W. Harris, N. Horne, M. Iseman, and B. Watt. 1997 Guidelines for the management of drug-resistant tuberculosis WHO, Switzerland). Since fluoroquinolones share the same molecular targets (for details refer to footnote)², it is highly

²Fluoroquinolones act by inhibiting DNA topoisomerase IV and DNA gyrase, enzymes that control DNA topology and are vital for cellular processes that involve duplex DNA, namely replication, recombination and transcription (Lewin CS et al., 1991; Willmott et al., 1994; for a review see Hooper, 2001). By inhibiting these enzymes, fluoroquinolones block DNA replication and induce DNA damage, triggering a set of still poorly defined events, which result in eventual cell death. Fluoroquinolone-dependent inhibition of RNA synthesis, and as a consequence protein synthesis, is also thought to contribute to the bactericidal activity of this class of drugs (Lewin et al., 1991; Willmott et al., 1994). Unlike most other bacterial species, *M. tuberculosis* lacks genes encoding for topoisomerase IV as revealed by the full genome sequence (Cole et al., 1998). Therefore, the main molecular target for fluoroquinolones in *M. tuberculosis* is the DNA gyrase (Onodera et al., 2001; Aubry et al., 2004). Consistently, resistance to fluoroquinolones in clinical isolates of *M. tuberculosis* occurs primarily due to mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene, which encodes for the A subunit of DNA gyrase (Sullivan et al., 1995; Kocagoz et al., 1996). Other mechanisms such as mutations in the B subunit of DNA gyrase, decreased cell permeability to the drug, and an active drug efflux pump mechanism could also be involved in triggering resistance. In particular, the expression or overexpression of energy-dependent efflux pumps that can actively remove antibacterial agents from the cell have been shown to play a role in determination of fluoroquinolone resistance (Li et al., 2004; Zhan et al., 2004; Brenwald et al., 1998; Colangeli et al., 2005)

probable that they will trigger the same mechanisms of resistance. Indeed, cross-resistance has been reported within the fluoroquinolone class such that reduced susceptibility to one fluoroquinolone likely confers reduced susceptibility to all fluoroquinolones (Alangaden et al., 1995; Ruiz-Serrano et al., 2000); for review see (Ginsburg et al., 2003a). The major concern is that widespread use of fluoroquinolones for treatment of other bacterial infections may select for resistant strains of *Mycobacterium tuberculosis*. Fluoroquinolone susceptibility is not routinely assessed in clinical isolates of tubercle bacilli, so there is not much information available about the prevalence of fluoroquinolone resistance in *M. tuberculosis*. 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 multi-drug resistant. The authors concluded that despite the widespread use of fluoroquinolones for treatment of common bacterial infection in USA and Canada, resistance to fluoroquinolones remains rare and occurs mainly in multi-drug resistant strains (Bozeman et al., 2005). In contrast, in a different study conducted by Ginsburg and collaborators at the John Hopkins Hospital (Baltimore) between 1998 and 2002, 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). Cross-resistance was observed among the different fluoroquinolones tested (ofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and ciprofloxacin) (Ginsburg et al., 2003b). In the Philippines, where fluoroquinolone use is poorly controlled, among 117 TB patients, fluoroquinolone resistance occurred in 53.4% of those with *M. tuberculosis* resistant to 4 or 5 drugs, in 23.3% with resistance to 3 drugs and in 18.2% with resistance to 2 drugs (Tupasi TE MDR in the Philippines 4th World Congress on TB, Washington DC2002). Because of the high prevalence of Tuberculosis in the Philippines, fluoroquinolones are not included in the clinical practice guidelines for the treatment of community-acquired pneumonia (The Philippine clinical practice guidelines on the diagnosis, empiric management and prevention of community-acquired pneumonia in immunocompetent adults. Report of the Task Force on Community Acquired Pneumonia. Manila: The Philippine Practice Guideline Group–Infectious Diseases, 1998. 1: 1–29). In conclusion, 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 ((1992)Hong Kong Chest Service /British Medical Research Council. A controlled study of rifabutin and an uncontrolled study of ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to isoniazid, streptomycin and rifampicin (Alangaden and Lerner, 1997; Yew et al., 1990). Moreover, the risk of selecting fluoroquinolone-resistant *M. tuberculosis* strains by empirically treating with fluoroquinolones other presumed infections before a diagnosis of tuberculosis is established is of great concern. For this reason some investigators in the TB field argue that the use of fluoroquinolones might be better reserved for specific serious

infection such as tuberculosis rather than becoming the workhorse of antimicrobial treatment; however, given the current widespread use of quinolones this might not be realistic.

Lately, the interest on fluoroquinolones as antituberculosis agent has focused on the new fluoroquinolones moxifloxacin (MXF) and gatifloxacin (GTF). Despite a lack of a comprehensive work comparing the activities of old and new classes of Fluoroquinolones in *M. tuberculosis*, what can be inferred from published results is that moxifloxacin and gatifloxacin are characterized by a higher activity against *M. tuberculosis in vitro* when compared to the old fluoroquinolones ofloxacin and ciprofloxacin (Hu et al., 2003; Paramasivan et al., 2005; Rodriguez et al., 2001; Sulochana et al., 2005). These new compounds are currently taken in consideration as anti-tuberculosis first-line drugs. A more detailed analysis of the properties of the new fluoroquinolones moxifloxacin and gatifloxacin will follow in the next paragraphs.

Gatifloxacin. Marketed in the U.S. by Bristol-Myers Squibb as Tequin, Gatifloxacin (GAT) has been found to have *in vitro* and *in vivo* bactericidal activity against *M. tuberculosis* ((Hu et al., 2003);(Alvarez-Freites et al., 2002)). In an *in vitro* study using stationary-phase mycobacterial culture, gatifloxacin (4 µg/ml) showed the highest bactericidal activity during the first 2 days but not thereafter (Paramasivan et al., 2005). Similar results were obtained when gatifloxacin was used in combination with isoniazid or rifampicin: gatifloxacin was able to slightly increase the bactericidal activity of INH or RIF only during the first 2 days (Paramasivan et al., 2005). This is in contrast with other studies showing that gatifloxacin and moxifloxacin had similar bactericidal activity on a stationary-phase culture of *M. tuberculosis* and comparable to the bactericidal activity of rifampicin (Hu et al., 2003; Lenaerts et al., 2005). One paper reported that when tested in mice in combination with Ethionamide and Pyrazinamide (high doses: 450 mg/kg, 5 days per week) gatifloxacin was able to clear the lungs of infected animals after 2 months of treatment (Cynamon and Sklaney, 2003). Thus, currently available data on gatifloxacin do not support the hypothesis that introduction of gatifloxacin in first-line regimen will impressively contribute to shorten TB treatment. Further investigation should be addressed to properly assess the activity of gatifloxacin *in vitro* and in mouse models.

Nevertheless, gatifloxacin is currently in Phase III Clinical Trials, conducted under the supervision of the European Commission Oflo tub Consortium, WHO-TDR, NIAID TBRU, Tuberculosis Research Centre. The aim of the trial is to evaluate the efficacy and safety of a four months gatifloxacin-containing regimen for the treatment of pulmonary tuberculosis.

Moxifloxacin. Produced by Bayer Pharmaceuticals and marketed as Avelox in the USA, moxifloxacin is the most promising of the new fluoroquinolones being tested against *M. tuberculosis*.

In vitro, moxifloxacin appeared to kill a subpopulation of tubercle bacilli not killed by rifampicin, i.e. rifampicin-tolerant persisters, while the older fluoroquinolones ciprofloxacin and ofloxacin did not have any significant bactericidal effect on the same subpopulation (Hu et al., 2003). One possibility is that moxifloxacin interferes with protein synthesis in slowly metabolising bacteria through a mechanism that differs from that used by rifampicin. However, the molecular mechanisms beyond such a bactericidal activity still await further characterization. (Refer to footnote for other advantages at molecular level of moxifloxacin in comparison with older fluoroquinolones, i.e. ciprofloxacin and ofloxacin)³.

In mouse models the activity of moxifloxacin against tubercle bacilli was comparable to that of isoniazid (Miyazaki et al., 1999). Moreover, when used in combination with moxifloxacin and pyrazinamide, moxifloxacin has been reported to kill the bacilli more effectively than the isoniazid+rifampicin+ pyrazinamide combination. Indeed, cultures from lungs of mice treated with rifampicin-moxifloxacin-pyrazinamide for 2 months followed by rifampicin-moxifloxacin resulted negative upon 4 months of treatment, while mice that received rifampicin-isoniazid-pyrazinamide/rifampicin-isoniazid showed complete culture conversion after 6 months of treatment (Nuermberger et al., 2004a). Furthermore, no relapse was observed in mice treated for at least 4 months with the combination rifampicin-moxifloxacin-pyrazinamide, while mice treated with rifampicin-isoniazidpyrazinamide required 6 months of treatment before no relapse could be detected (Nuermberger et al., 2004a); (Nuermberger et al., 2004b). The authors explain the better activity of the rifampicin-moxifloxacin-pyrazinamide combination over the rifampicin-INH-pyrazinamide combination as the consequence of a possible synergism in the anti-tuberculosis activity of the three drugs rifampicin, moxifloxacin and pyrazinamide. Alternatively, substitution of moxifloxacin with isoniazid in the standard regimen could relieve a possible antagonism among the currently used drugs (Grosset et al., 1992).

To summarise, results obtained so far in *in vitro* and *in vivo* studies suggest that moxifloxacin might be a promising candidate drug to shorten TB treatment. At the molecular levels, the reason for its improved efficiency is mainly a consequence of its poor susceptibility to active efflux that ensures the maintenance of high intracellular concentration. Shortening of therapy in mouse models seems to be mainly due to a released of antagonism among the drugs in the regimen. There is a possibility that moxifloxacin might be active against slowly metabolising bacteria by

³ -Moxifloxacin appears to be a poor substrate for efflux pumps in other pathogens such as *S. pneumoniae*. This might be due to the bulky C-7 substituent that characterizes this compound. In contrast older and more hydrophilic fluoroquinolones such as ciprofloxacin are more susceptible to be actively exported outside the bacterial cell (Daporta et al., 2004);(Coyle et al., 2001),(Pestova et al., 2000)

-*In vitro*, moxifloxacin inhibitory activity of DNA gyrase is higher than ofloxacin but comparable to ciprofloxacin (Aubry et al., 2004).

-In *S.pneumoniae*, moxifloxacin maintained clinically useful level of activity against bacterial strains that bore mutations in the QRDR region of *gyrA* genes, suggesting that MXF could target other key domains in the DNA-gyrase enzyme (Pestova et al., 2000)

inhibiting DNA transcription and, consequently, mRNA and protein synthesis, therefore having a mild sterilizing activity. However, this still needs to be rigorously proven.

As far as emergence of resistance is concerned, in vitro studies in *S.pneumoniae* revealed that moxifloxacin, used at concentration above the minimal inhibitory concentration (MIC), is less prone to select first-step mutants when compared to the fluoroquinolone sparfloxacin. However, moxifloxacin monotherapy in mice models showed that resistance to moxifloxacin might rapidly emerge (Ginsburg et al., 2005).

Moxifloxacin is currently in Phase II Clinical Trials. A trial substituting ethambutol with moxifloxacin during intensive phase (TBTC 27) was initiated before above cited animal studies had been conducted and showed no advantage over ethambutol. Preliminary results of this study have been published and showed that moxifloxacin containing regimen did not present increased sterilizing activity (measured as the ability to induce sputum culture conversion upon 2 month of treatment) over the standard regimen. However moxifloxacin-containing regimen did show increased activity at earlier time points (Burman et al., 2006). A new trial (TBTC 28), substituting moxifloxacin with isoniazid in intensive phase to compare culture conversion rate will be carried out by CDC in cooperation between TB Alliance and Bayer in several sites in the US as well as sites in Spain, South Africa and Uganda. At time of writing this report the trial is recruiting. (<http://clinicaltrials.gov/ct/gui/show/NCT00144417?order=4>; http://www.tballiance.org/bayer/docs/english-TB_Alliance_Bayer_Release.pdf)..

New Quinolones

In 2003 the TB Alliance launched a project in collaboration with the Korean Research Institute of Chemical Technology (KRICT) and the Yonsei University aimed to synthesize and evaluate novel and more effective quinolone compounds that could shorten first-line treatment. To date, 450 compounds have been synthesized and tested for their anti-TB activity. During this work, the sub-class termed 2-pyridones has been identified as the one showing most potent activity against *M. tuberculosis* in both its growing and persistent state. This sub-class of compounds was already identified by Abbott in 1998 and found to have activity against drug-susceptible and drug resistant *M. tuberculosis* (Oleksijew et al., 1998). In June 2005, the TB Alliance contacted Abbott and was granted rights to develop for TB indication this class of DNA gyrase inhibitors which is otherwise protected by certain Abbott patents. As fluoroquinolones, 2-pyridones are inhibitors of DNA gyrase (Flamm et al., 1995). Current work is focused on the modification of a position which influences activity, pharmacokinetics and safety profile. The lead compounds identified so far showed better activity than gatifloxacin and moxifloxacin. At present, the project is in the lead optimisation stage and aims to obtain a final candidate by the end of 2006 (TB Alliance Annual report 2004/2005, http://www.tballiance.org/downloads/2005%20annual%20008_6b.pdf)

Non-fluorinated quinolones

Recently, a series of 8-methoxy non-fluorinated quinolones (NFQs) have been developed by Procter & Gamble. NFQs lack a 6-fluorine in their quinolone nucleus differentiating them from fluorinated quinolones such as gatifloxacin and moxifloxacin. NFQs target a broad spectrum of bacteria and they seem to act preferentially through inhibition of DNA gyrase (Barry et al., 2001; Jones et al., 2002). NFQs are currently being tested against *M. tuberculosis*.

Diamine SQ-109

Diamine SQ-109 has been identified in a screening performed by Sequella Inc. using a combinatorial library based on the pharmacophore of ethambutol. The aim was to develop a second-generation agent from the first line drug ethambutol. 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 (Protopopova et al., 2005). Protopopova and collaborators claim that SQ-109 is effective against drug-resistant strains of *M. tuberculosis*, including those that are ethambutol-resistant, and that it targets different intracellular target(s). For this reason it can be considered as a new TB drug and not simply as an ethambutol analogue.

Macrolides

The aim of this project, launched by the TB Alliance in collaboration with the Institute for TB research of the University of Illinois in Chicago, is to optimise the anti-TB activity of the macrolide antibiotics through the synthesis of additional chemically modified derivatives of erythromycin. More than 200 derivatives have been synthesized and three series were identified as having anti-tuberculosis activity superior to that of the benchmark clarythromycin. Members of these series have exhibited potent anaerobic activity and appears to be safe in use with ARVs (TB Alliance Annual report 2004/2005, http://www.tballiance.org/downloads/2005%20annual%20008_6b.pdf).

Thiolactomycin analogs

Thiolactomicin (TLM) was the first example of naturally occurring thiolactone to exhibit antibiotic activity. The compound has moderate *in vitro* activity against a broad spectrum of pathogens, including Gram-positive and Gram-negative bacteria and *M. tuberculosis*. Analogs of thiolactomicin have been synthesized and found to have enhanced activity against whole cells of pathogenic strain of *M. tuberculosis* (Douglas et al., 2002). TLM analogs seem to act through the inhibition of the mycolate synthase, an enzyme involved in the biosynthesis of the cell wall.

Nitrofuranylamides

M. tuberculosis is quite susceptible to Nitro-containing compounds (Sun and Zhang, 1999) (Murugasu-Oei and Dick, 2000). Nitrofuranylamide was identified in a screening for UDP-Gal mutase inhibition. An expanded set of nitrofuranylamides was synthesized and tested for antimicrobial activity. This led to the identification of a number of nitrofuranylamides with activity against *M. tuberculosis*. However, the further investigation has revealed that the primary target for nitrofuranylamides antimicrobial activity is not the UDP-gal mutase. Four compounds of the nitrofuranylamides class showed significant activity in mouse models for TB infection (Tangallapally et al., 2004)

Nitroimidazole Analogs

While pursuing PA-824's remaining development activities, the TB Alliance in collaboration with the Novartis Institute for Tropical Diseases initiated in 2004 a back-up program to maximize the potential of this class of compounds by identifying superior compounds and improving on PA-824's properties as a drug.

Moreover, a number of drug screenings and target identification screenings are underway at several research institutes and pharmaceutical companies, notably GlaxoSmithKline (Tres Cantos, Spain) and AstraZeneca (Bangalore, India).

Several public research institutes (University of Auckland, National Institute for Allergy and Infectious diseases, California State University, Institute for tuberculosis Research-University of Illinois) are screening libraries of natural products (plant and bacterial products) with the hope to identify compounds that have anti-tubercular activity.

Dr Carl Nathan at the Cornell University Weill Medical College received a \$3.5 million grant from the National Institute of Allergy and Infectious Diseases (September 15, 2004 through August 31, 2007) to carry out a project aimed to the characterization of the *M. tuberculosis* enzyme Dihydrolipoamide Acyltransferase as a potential target for chemotherapy of TB (http://www.med.cornell.edu/news/dean/2005/01_05_05/article7.html; http://www3.niaid.nih.gov/Biodefense/Research/2004awards/03016_awards.htm). *M. tuberculosis* Dihydrolipoamide Acyltransferase (dlaT) is a component of two important multi-subunit complexes: pyruvate dehydrogenase, the enzyme that catalyses the synthesis of Acetyl Coenzyme A, and peroxynitrite reductase, a defence against oxidative/nitrosative stress (Tian et al., 2005); (Shi and Ehrt, 2006). DlaT has been shown to be required for full virulence *in vivo* in mice, while in *in vitro* experiments mouse macrophages can readily kill intracellular *M.tuberculosis* mutants lacking *dlaT* (Shi and Ehrt, 2006).

GSK and the TB Alliance are developing several projects under the general category of "Focused screening". The aim is to identify compounds that are active against specific distinct

molecular targets, including inhibitors of DNA gyrase (the target of fluoroquinolones), peptide deformylase (PDF) inhibitors and analogs of quinolone electron transport inhibitors.

Bacterial peptide deformylase belongs to a subfamily of metalloproteases catalysing the removal of the *N*-terminal formyl group from newly synthesized proteins. PDF is essential for bacterial growth but is not required by mammalian cells, so represents a promising target for the development of a new generation of broad-spectrum antibacterial agents. Two PDF inhibitors, VIC-104959 (LBM415) and BB-83698, have progressed to Phase I clinical trials (Jain et al., 2005). The PDF inhibitor BB-3497 was recently found to have potent *in vitro* activity against *M. tuberculosis* (Cynamon et al., 2004). This finding suggests that PDF inhibitors can find application in TB treatment. Beside the project jointly launched by GSK and the TB Alliance, researcher at the Novartis Institute for tropical Disease (NITD) are also working on identification of PDF inhibitors for TB treatment (NITD Symposium on Tuberculosis, October 17-20 2005, http://www.nitd.novartis.com/includes/teasers/teaser_attaches/tb_program_final.pdf).

Inhibition of electron transport can lead to ATP depletion and decline in intracellular redox potential. Recently, anti-tubercular drugs targeting ATP synthesis (i.e. diarylquinoline) have been shown to be particularly effective, even against non-replicating bacteria. Therefore, identification of compounds able to inhibit the electron transport process could lead to the development of more effective drugs active against both replicating and non-replicating bacilli.

Others drugs candidate that could find an application in TB treatment are:

New rifamycin derivatives

Rifalazil, a new semisynthetic 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* (Hirata et al., 1995; Shoen et al., 2000). However, high level rifampicin -resistant strains present cross-resistance to all rifamycins (Moghazeh et al., 1996).

Oxazolidinones (Linezolid)

Oxazolidinones are a new class of broad-spectrum antibiotics developed by Pharmacia. They inhibit protein synthesis by binding to the 50S subunit of ribosomes. Oxazolidinones had significant activities against *M. tuberculosis in vitro* and in mice (Cynamon et al., 1999; Zurenko et al., 1996). However, oxazolidinones are seen as less promising due to their toxicity and high price.

4.3 Summary of the drug pipeline.

Table 3 provides an overview of available information on the main properties of the drugs in the pipeline. (The overall strengths and weaknesses of the current pipeline are further discussed in section 6).

Table 3. Main properties of candidate anti-TB drugs

	DRUG	Effect on bacteria cells	Mechanism of action	Targets	Activity against MDR-TB
Clinical	Dyarylquinoline TCM207	Bactericidal Potentially sterilizing	ATP depletion and imbalance in pH omeostasis	AtpE, component of ATP synthase	Active against MDR-TB NO cross-resistance with current TB drugs
	Gatifloxacin	Bactericidal	Inhibition DNA replication and transcription	DNA gyrase	Active against isoniazid and rifampicin resistant strains (weak data)
	Moxifloxacin	Bactericidal	Inhibition DNA replication and transcription	DNA gyrase	Active against MDR-TB in combination with ethionammide (ETH)
	Nitroimidazole PA-824	Sterilizing <i>in vitro</i> Bactericidal <i>in vivo</i>	Inhibition of protein synthesis Inhibition of cell wall lipids synthesis	No data available	Active against MDR-TB NO cross-resistance
	Pyrrole LL-358	Bactericidal/ Sterilizing(?)	No data available	No data available	Active against MDR-TB
	Nitroimidazo-oxazole OPC-67683	Bactericidal	Inhibition of cell wall biosynthesis	No data available	Active against MDR-TB
	Diamine SQ-109	Bactericidal(?)	Inhibition cell wall biosynthesis	No data available	Effective against ethambutol-resistant strains(?)
	Dypiperidines (SQ-609)	Bactericidal(?)	Inhibition of cell wall biosynthesis	No data available	No data available
Preclinical	ATP Synthase Inhibitor FAS20013	No data available	ATP depletion	ATP synthase (?)	<i>In vitro</i> activity against MDR-TB strains
	Translocase I Inhibitor	Bactericidal (?)	Inhibitor of cell wall biosynthesis	Translocase I	No data available
	Non-Fluorinated Quinolones	Bactericidal	Inhibition DNA replication	DNA gyrase	No data available
Discovery	Nitrofuranylamides	No data available	No data available	No data available	No data available
	Picolinamide Imidazoles	No data available	No data available	No data available	No data available
	Pleuromutilins	Bactericidal/ sterilizing?	Inhibition of protein synthesis	Large subunit of ribosome	Active against MDR-TB

Thiolactomycin Analogs	Bactericidal?	Inhibition of cell wall biosynthesis	Mycolate shynthase	No data available
Dihydrolipoamide Acyltransferase Inhibitors	No data available	Inhibition of basic metabolism and oxidative/nitrosative stress response	Dihydrolipoamide Acyltransferase	No data available
InhA inhibitors	Bactericidal (?)	Inhibition of cell wall biosynthesis	Enoyl ACP reductase	Prone to cross-resistance with INH
Isocitrate lyase Inhibitors (ICL)	Expected to be sterilizing	Inhibition of glyoxylate cycle	Isocitrate lyase	No data available
Methyltransferas inhibitors	No data available	No data available	No data available	No data available
Quinolones	No data available-expected bactericidal	Inhibition DNA replication and transcription	DNA gyrase	No data available-prone to cross resistance with fluoroquinolones

5. Expected timelines towards approval for new candidate drugs

The expected time schedule towards approval for drugs that are currently in clinical development is summarised in Figure 2.

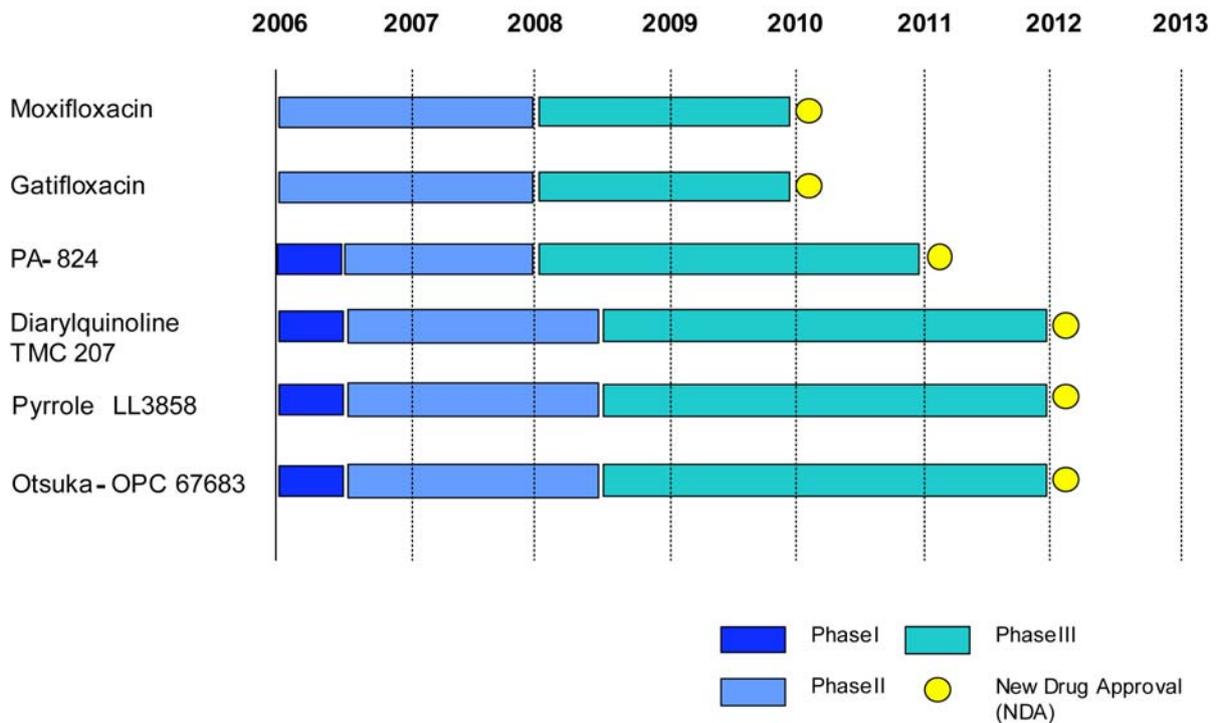


Figure 2. Expected timelines towards approval of candidate drugs currently in clinical stage of development (Sources: Global TB Alliance Annual report 2004-2005; Stop TB Partnership Working Group on New Drugs for TB. Strategic Plan 2006-2015)

Clinical trials to register a TB drug represent a lengthy and expensive process that can take a minimum of six years, generally longer than for other infectious diseases. The greatest challenge in the design of TB clinical trials is in Phase III Trials. These trials are usually large scale, randomised clinical trials designed to show improvement or equivalent efficacy compared to the standard regimen among diseased patients. Efficacy evaluation requires measurements of relapse rate during a 1-2 years follow-up after completion of the already lengthy 6 months treatment regimen. Relapse rate after chemotherapy is commonly accepted as the endpoint to determine the efficacy of a new therapy and to assess whether a new drug can improve sterilising activity. Since relapse rates under random clinical trial conditions are often 3% or less, large numbers of patients are needed to demonstrate an improvement in relapse rate. This results in high drug development costs and long delays in introducing new medicines.

Validated surrogate markers of relapse would provide evidence on the efficacy and the sterilising activity of a drug/regimen without requiring large numbers of patients in a conventional clinical trials and with great savings in development time and cost. The best validated surrogate marker for relapse is the proportion of sputum cultures that remain positive after about 2 months of chemotherapy. This method has been shown to associate with the fall in relapse rates in 8 clinical trials (Mitchison, 1993; Mitchison, 1996). Less well validated procedures that require further studies and validation are the rate of sputum conversion (Durban Immunotherapy Trial Group, 1999), the measurement of the 85B (alpha) antigen of *M. tuberculosis* in sputum (Desjardin et al., 1999) and the extended studies (beyond 2 days) of early bactericidal activity (EBA) (Sirgel et al., 2000). However, regulatory agencies still require that drug's efficacy is demonstrated during phase III trials through a combination of traditional and surrogate markers for activity.

Since the identification of biomarkers could significantly streamline and accelerate clinical development, the TB Alliance has recently established a collaboration with BG Medicine Inc. to identify biomarkers for drug efficacy in TB treatment. A biomarker is a quantifiable biochemical characteristic (such as a metabolite, hormone or enzyme) that is measured and evaluated as a pharmacologic response to chemotherapy. The TB Alliance/BG Medicine project will aim to identify biomarkers for two purposes: (i) to provide an early indication of drug's ability to shorten treatment during Phase II testing; (ii) to act as a surrogate marker of treatment efficacy and sterilizing activity that could shorten Phase III trials and eliminate the need for the 2 years follow-up to determine relapse rates (<http://www.tballiance.org/pdf/PRESS%20Release%20TB%20Alliance-BG%20Medicine%20FINAL.pdf>).

The current need for large number of patients recruited for Phase III clinical trials implies the need to conduct trials in countries with high TB incidence rates in order to ensure that a sufficient study population can be obtained. One of the major challenges that the TB Alliance and Pharma companies are facing while embarking in clinical development concern the lack of clinical trial capacity in endemic countries, Phase III infrastructure is in particular poorly developed and might

require additional coordination, regulatory support and specific funding to overcome gaps. In order to be considered as potential sites to run clinical trials for drug approval Good Clinical Practice standards (GCP) need to be implemented. The TB alliance faces also difficulties with regulatory issues because regulatory authorities (FDA, EMEA) have no experience any longer in approving new TB drugs (TB Alliance personal communications).

6. A crucial gap: lack of early stage drug discovery

As described in Appendix A, several genes that could be important for survival of *M. tuberculosis in vivo* have been identified and characterized during the last years. But validation of those potential drug targets through genetic or chemical inactivation is still largely missing. As pointed out by Dr Valerie Mizrahi (University of the Witwatersrand, Johannesburg) “there is an urgent need to put in place the advanced knowledge that we gained about *M. tuberculosis* metabolism and physiology and think how to translate it into validated target that can be used for screening of new drugs”.

Why is this not happening? Scientists working in the TB field have identified primarily two kind of obstacles that are hindering the drug discovery process. On one side there are “scientific obstacles” primarily represented by the current inadequate characterization of TB lesions in human host. On the other side there are “financial obstacles” mainly represented by the lack of interest by current industry initiatives, lack of capacity by the TB Alliance to enter into this field, and lack of sustained funding for academic laboratories to run research projects that fall in the borderline between basic and applied science

A systematic characterization of the heterogeneity of TB lesions in patients aimed to get a clearer picture of the different microenvironments that bacteria have to adapt to in order to survive and persist in human hosts, is still largely missing. Similarly, the understanding of the critical mechanisms that underlie survival of *M. tuberculosis* during the extended periods of chemotherapy is still rather limited. These gaps in our knowledge of *M. tuberculosis* biology are making the identification and validation of potential targets that are relevant *in vivo* in the human host still a rather difficult task. The “biological uncertainties” about *M. tuberculosis* also represent one of the reasons why pharmaceutical companies consider anti-TB drug discovery and development as particularly risky ground and are therefore generally reluctant to embark on this kind of projects. These critical open questions will be partially addressed by a research project coordinated by Dr Douglas Young (Imperial College London) and funded by the Gates Foundation as part of the Grand Challenges in Global health initiative (Grand Challenge #11, June 2005; <http://www.gcgh.org/subcontent.aspx?SecID=403>). In an effort to develop drugs for latent TB, this international collaboration is aiming to identify the molecular pathways essential for the bacteria to

survive inside human tissues. The purpose is to select targets that are essential for viability of "persistent" bacteria that are relatively tolerant to conventional drugs.

Another "scientific obstacle" that is currently under debate is the lack of adequate animal models truly representative of human latent tuberculosis. Although there is a consensus that mice, rabbits, guinea pigs and non-human primates infected with *M. tuberculosis* can model overlapping characteristics of human TB, some scientists strongly advocate for the use of non-human primates as the animal model of choice (Boshoff and Barry, 2005; Flynn and Chan, 2001). Currently, the most popular model is the murine model due to low cost, availability of genetically defined strains and comprehensive characterization of mouse immunology. While there are similarities in the immune control of TB in mice and humans, the progression of the disease is markedly different. In particular the features of chronic infections in mice (high bacterial titre in the lungs and spleen, absence of necrotic lesions, precursors of cavities) do not reflect the situation of advanced TB in humans (Gupta and Katoch, 2005; McMurray et al., 1996). However, the choice of the "right" animal model requires a previous systematic characterization of TB lesions in the human hosts. As far as this characterization will be largely missing it might be premature and is not really evidence-based to advocate for one or another animal model.

Lack of sustained funding for early stage drug discovery projects is the other barrier in the process of anti-TB drug discovery. Examples are target validation projects or projects in the field of chemical genetics. Up to now major funding bodies for TB research are represented by organisms such as NIH/NIAID, Wellcome Trust and EU. These funding organisms and the peer review system that they use to select grant recipients traditionally give priority to "blue-skies" basic research projects and "hypothesis-driven" science. Several experts in the field point out that exceptions made for specific grants based on request for application (RFA), it is pretty difficult for academic labs to obtain funds for projects that fall in between basic and applied research.

The fact that this kind of projects falls into cracks is creating a serious gap in the drug discovery process. The pharmaceutical industry considers it economically risky to develop drugs for diseases such as TB that are endemic in developing countries and do not offer exciting market perspectives. While pharmaceutical companies actively scout for advances in basic science and are ready to invest in early stage drug discovery in areas such as cancer drug development, the new TB programs by pharmaceutical companies are not doing this and are still too timid and risk averse. As pharmaceutical companies want their risk to be reduced to minimum levels when they embark on projects aiming to develop drugs for neglected diseases, they are more likely to become interested and accept to run a drug development project when rigorously validated targets are available or when a lead compound has been already identified. "As a consequence, more effort is required to academic labs working on TB, that are asked to provide target validation and/or drug leads" said Dr Guilhot (CNRS, Toulouse) and Dr Sherman (University of Washington, Seattle). So far, the TB Alliance, despite all its pioneering work, has not entered the area of translational research either,

likely because of financial reasons as they are still working to even secure funding for their existing projects.

What happens then if early stage drug discovery projects cannot be carried out in academic laboratories? “In TB field, target identification and target validation projects are not carried out by biotech or pharmaceutical companies due to lack of interest and lack of expertise - there is little capacity and expertise on TB among pharmaceutical companies since there have not been ongoing TB development projects. If this work cannot be done by academic laboratories, the process gets stuck” said Dr John McKinney (Rockefeller University, New York). It is worth noting that a research project aimed to target validation through genetic knockout is an extremely focused project. In an academic lab that has well-established expertises and facilities, the work can be done relatively quickly and with a limited amount of money. If faster progress is to be achieved “there is the need to enable the academic sector to go beyond the proof of principle that traditionally is the final aim”, said Dr Mizrahi. As pointed out by Dr Nathan, “it is necessary to re-think the traditional roles played by academia and pharmaceutical industry in drug discovery and development and push academia into fields that are traditionally ground for industry when it comes to drugs for diseases that do not ensure appealing market perspectives”. For this to happen, focused funding streams need to be established for translational research projects.

NIH/NIAID tried to make things move in this direction by establishing and funding facilities such as TARGET (Tuberculosis Animal Research and Gene Evaluation Taskforce) and TAACF (Tuberculosis Antimicrobial Acquisition and Coordinating Facility) that offer services respectively for testing of *M. tuberculosis* mutants in animal models and for high-throughput screening of large compound libraries against validated target. However this strategy does not look to be extremely efficient and successful so far. While this is a step in the right direction, it is far from satisfactory. It is likely that pull programs, which support projects on the basis of achievement rather than push programs supporting classical types of proposals, would be better suited to achieve this goal.

Progress in the field of drug development arising from close collaborations between the academic sector and pharma companies is promising. Despite the current industry stagnation in antibiotic R&D, recent research by two industry–academic collaborative groups has resulted in the discovery of two new classes of antimicrobial peptides for treating Gram-positive bacterial infections (Brotz-Oesterhelt et al., 2005; Mygind et al., 2005). So far, the only example of such kind of collaboration in the field of TB known to us is represented by the work done on the *M. tuberculosis* enzyme isocitrate lyase (ICL). This work has seen a coordinated collaboration among the laboratories of Dr McKinney (Albert Einstein College of Medicine, Rockefeller University), Dr Sacchettini (Texas A&M University), Dr Russell (Washington University School of Medicine), and GlaxoSmithKline. In this example the academic sector provided a package that comprised target validation (Dr McKinney), enzyme crystal structure (Dr Sacchettini) and establishment of the biochemical assay needed for the high-throughput screening (Dr Russell). GSK is now performing

the high-throughput screening for the identification of ICL inhibitors. More recently, this project has seen the added involvement of the TB Alliance and is now being directed under the collaboration between GSK and the TB Alliance. The work in academic laboratories was funded by GSK, through a grant of the “TB Action Initiative” that currently has been completed. While such academic-industry cooperation can be successful scientifically, it is important to follow through to ensure that the fruits of such research lead to products, even if these should be less profitable, and that equitable access to the product will be guaranteed.

7. Discussion and conclusions

As new models of drug development are being established, major advances have been achieved in the basic research field. Modern molecular and genetic tools have become available for *M. tuberculosis* (i.e. targeted mutagenesis, array-based analysis of mutant libraries, techniques for conditional gene silencing) and this has led to impressive improvements in the knowledge and understanding of the fundamental biology and physiology of *M. tuberculosis* (Jansen and Yu, 2006; Kana and Mizrahi, 2004; Kaufmann et al., 2005; please refer to Appendix A for a detailed review of major advances achieved in the basic research field). This was made possible by funding programmes supportive for research on *M. tuberculosis* lunched by major research funding organisms (NIH/NIAID, Wellcome Trust, EU) during the 1990s. However, as testified by the academic community, the situation is changing again and there are serious concerns that such a sustained funding availability will not last for long.

Today the TB drug pipeline is richer than it has been in the last forty years. This is also thanks to the work carried-out by the Global Alliance for TB Drug Development which is associated with approximately half of all compounds (or projects aimed to identify candidate compounds) currently being developed. Increased public awareness on the lack of R&D for neglected diseases in recent years has also led some multinational pharmaceutical companies to invest in TB drug development on a ‘no-profit-no-loss’ basis (namely, Novartis, AstraZeneca and GlaxoSmithKline). Some pharmaceutical companies have engaged in tuberculosis R&D on a commercial basis, and with some success: three of the six anti-TB candidate drugs currently in clinical trials have been developed for profit.

Pressing needs still remain

Despite the positive changes occurred in the last years, there are still problems that need to be tackled and major roadblocks still exist that are hindering the implementation of rational drug design and a fast progress in anti-TB drug R&D.

A first important question is if there are enough promising compounds in the TB pipeline for a comprehensive new TB treatment to be developed (Glickman et al., 2006). Although different

attrition rates might apply, the number of candidate compounds is still small if compared to drug pipeline for diseases that principally affects wealthy countries. This is reflected by the limited number of biotech and pharmaceutical companies working on TB (see Figure 3). The ambition of the TB Alliance and its partners is to register an improved, faster acting regimen by 2010 and a regimen containing completely novel drugs by 2015 (TB Alliance Annual report 2004-2005 http://www.tballiance.org/downloads/2005%20annual%20008_6b.pdf). It is not possible to say today if the current pipeline will permit the attainment of this goal.

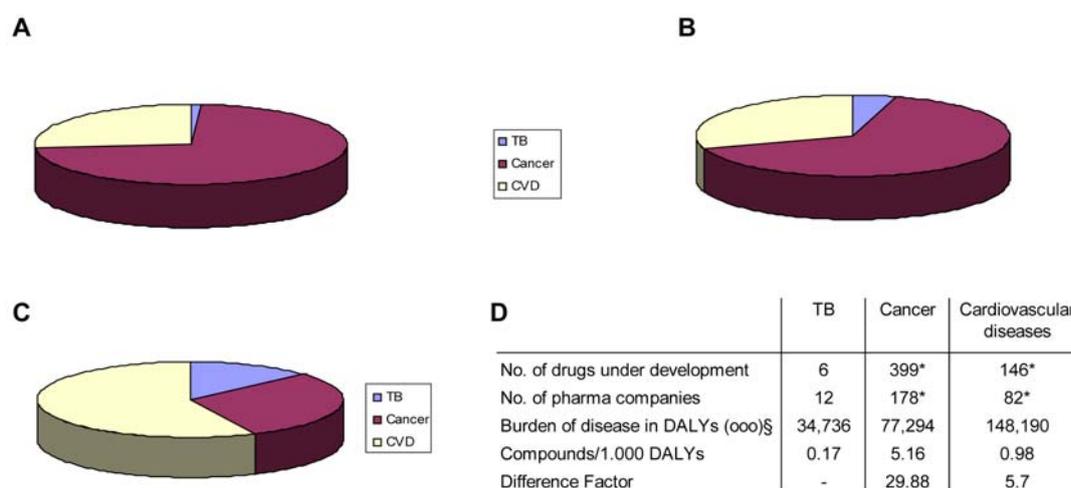


Figure 3. Comparison of drug pipelines for TB and for “more profitable” diseases.

Graphs represent the number of drugs in clinical stage of development (A) and number of pharmaceutical and biotech companies involved in drugs development projects (B) for Tuberculosis, Cancer and Cardiovascular diseases. Source: Pharmaceutical Research and manufacturers of America (PhRMA) Survey (<http://www.phrma.org>). (C) Comparison of worldwide burden of disease in DALY (Disability Adjusted Life Years) for Tuberculosis, cancer and cardiovascular diseases. Source: WHO, World Health Report 2004 (http://www.who.int/whr/2004/en/09_annexes_en.pdf). (E) Table summarizing the data plotted in the graphs.*Source: PhRMA; §Source: WHO)

Another important issue concerns characteristics of candidate compounds currently in development. Table 4 summarizes the main properties in terms of mechanism of action of the candidate drugs that are in the pipeline (for more detailed information on single compounds see also table 3).

It is evident that many of the candidate drugs are either derivatives of existing compounds or target the same cellular processes as drugs currently in use. While analogs and derivatives are far quicker to develop, agents identified by this approach may have cross-resistance problem, as seen for the new rifamycins or quinolones (Ginsburg et al., 2003a; Moghazeh et al., 1996).

Fresh approaches and novel sets of microbial targets need to be taken in consideration. One example of a promising new compound is diarylquinoline TMC-207 (currently in phase IIa clinical trials) which acts through a novel molecular mechanism, most probably by inhibiting the ATPase

synthase, leading to ATP depletion and pH imbalance. Preliminary results in animal models indicate that it has the potential to shorten the treatment to 2 months (Andries et al., 2005).

What also comes to light from a critical analysis of the drug pipeline is that rational approaches are weakly implemented in anti-TB drug discovery and development. Even the most promising novel drug candidates currently in clinical stage were identified serendipitously in screenings that were not designed originally for activity against *M. tuberculosis*. Moreover these compounds were selected for their ability to kill actively growing bacteria (Andries et al., 2005; Stover et al., 2000). There is consensus among the TB community that in order to obtain a real breakthrough in TB therapy and drastically shorten the treatment, there is an urgent need to identify compounds acting on key targets that are essential for mycobacterial persistence. There is a growing awareness that different subpopulations of bacteria that vary for their metabolism and growing rate can co-exist in an infected patient. Novel and more effective drugs should be rationally designed to interfere with metabolic and physiological strategies used by the bacteria to survive to host immune defences. 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. However, most of the compounds in the current pipeline target actively growing bacteria and so have bactericidal but not sterilizing activity. Therefore, while drugs currently in the pipeline could significantly shorten the treatment to two to three months they are unlikely to lead to a major breakthrough and reduce the treatment to a matter of weeks or days.

Table 4. Adapted from STOP TB Working Group on New drugs. Compounds have been categorized by the author considering their novelty in structure and mechanism of action

<i>Discovery</i>		<i>Preclinical</i>	<i>Clinical Testing</i>
Thiolactomycin Analogs NIAID, NIH	Nitrofuranylamides NIAID, University of Tennessee	Diamine SQ 109 Sequella Inc.	Diarylquinoline TMC207 Johnson & Johnson
Cell Wall Inhibitors Colorado State University, NIAID	Nitroimidazole Analogs NIAID, Novartis Institute for Tropical Diseases, TB Alliance, University of Auckland	Dipiperidines (SQ 609) Sequella Inc.	Gatifloxacin OFLOTUB Consortium, Lupin, NIAID TBRU, Tuberculosis Research Centre WHO TDR
Dihydroipoamide Acyltransferase Inhibitors Cornell University, NIAID	Focused Screening GlaxoSmithKline, TB Alliance	Nitroimidazo oxazole Back-up Otsuka	Moxifloxacin Bayer Pharmaceuticals, CDC TBTC, Johns Hopkins University, NIAID TBRU, TB Alliance
InhA Inhibitors GlaxoSmithKline, TB Alliance	Picolinamide Imidazoles NIAID, TAACF	Synthase Inhibitor FAS20013 FASgen Inc.	Nitroimidazole PA-824 Chiron Corporation, TB Alliance
Isocitrate Lyase Inhibitors (ICL) GlaxoSmithKline, TB Alliance	Pleuromutilins GlaxoSmithKline, TB Alliance	Translocase I Inhibitors Sequella Inc., Sankyo	Nitroimidazo - oxazole OPC67683 Otsuka
Macrolides TB Alliance, University of Illinois at Chicago	Quinolones KRICI, Yonsei University, NIAID, TAACF, TB Alliance		Pyrrrole LL 3858 Lupin Limited
Methyltransferase Inhibitors Anacor Pharmaceuticals	Screening and Target Identification AstraZeneca		
Natural Products Exploration BIOTEC, California State University, ITR, NIAID, TAACF, University of Auckland			

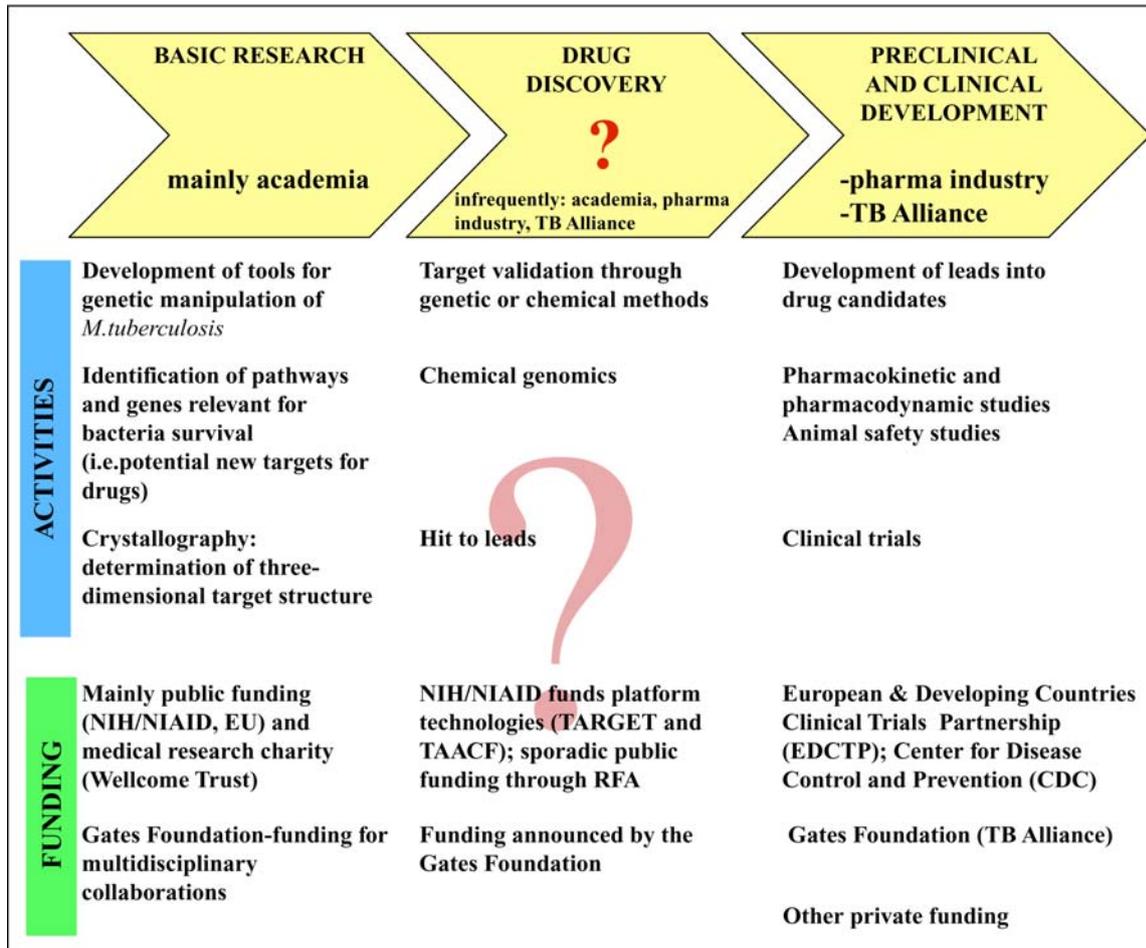
	Novel compound new mechanism of action		Novel compound old mechanism of action
	Novel compound unknown mechanism of action		Analogs and derivatives of existing compound
	Screening		No info available

A critical obstacle to such rational design is the lack of a comprehensive characterization of the fundamental biology of mycobacteria as they persist in human tissues. Thus, the identification and validation of potential targets that are relevant for the survival of the bacteria *in vivo* still represent a difficult task. This high degree of uncertainty about biochemical processes and molecular targets that can be potential target for effective new drugs renders the whole drug R&D process risky and, therefore, even less attractive for pharma investments. There is urgent need for a better characterization of heterogeneity of TB lesions to obtain a clearer picture of the different microenvironments in which *M. tuberculosis* persists. Moreover, there is still a pressing need to decrease the degree of uncertainty about the critical metabolic processes that drugs should target to achieve sterile mycobacterial elimination. As part of the Grand Challenges in Global Health initiative (<http://www.gcgh.org/subcontent.aspx?SecID=403>) the Bill and Melinda Gates Foundation (Gates Foundation) is funding research into the molecular pathways of persistence, aimed at identifying novel targets and subsequently run target-based drug discovery programs.

A second critical obstacle to the implementation of rational drug design is the lack of well-validated drug targets. The fundamental genetics of *M. tuberculosis* growth and persistence in animal models are slowly being unravelled. Several enzymes involved in alternative metabolic pathways, energy generation, micronutrient acquisition, and survival in activated macrophages as well as in patient lesions have recently been identified as new sets of potential anti-microbial targets (Shi et al., 2005; Darwin et al., 2003, Sasseti et al., 2003; Schnappinger et al., 2003; Rachman et al., 2006), but validation of these potential targets through genetic or chemical inactivation is largely missing. This creates a critical gap in early stage drug discovery research (figure 4). There is an urgent need to translate this advanced knowledge about *M. tuberculosis* metabolism and physiology into validated targets that can be used for screening of new lead compounds. A key difficulty lies in securing sustained funding for research projects that fall into the area of target validation and chemical genetics.

The Gates Foundation recently announced a new initiative that specifically aims at accelerating drug discovery for tuberculosis (the “TB drug accelerator” initiative, http://www.gatesfoundation.org/GlobalHealth/Pri_Diseases/Tuberculosis/default.htm). While acknowledging these important contributions, it must be questioned whether the isolated effort of the Gates Foundation will be sufficient to promptly address such a broad and important public health problem. Much greater public leadership is needed.

Figure 4. Main players in the anti-TB drug discovery and development process



Time to sow new seeds now as all the low-hanging fruit have been eaten

The product-development partnership (PDP) model has considerably contributed to a burst of activities in R&D for TB and other neglected diseases (Moran et al., 2005), mainly by testing and reformulating existing drugs already used for other indications and pushing into pre-clinical and clinical development existing drug leads that would have been otherwise forgotten in laboratory drawers for lack of an industry sponsor. Since improved therapies are urgently required the strategy of adopting a development-oriented portfolio has probably been a sensible short-term perspective. The critical question is whether this strategy will be successful in the long run. There are concerns that most of the “low-hanging fruit” have been already used up, and real breakthroughs will require a strengthening of early-stage discovery research to identify new compounds and targets. Without a thriving background of discovery-oriented translational research, itself largely dependent on public funding, the PDP model is destined to fail in a longer-term perspective. The renewed and welcome engagement of some pharmaceutical companies in TB drug development is so far still too modest and risk (cost) averse to tackle this problem.

Another point that deserves attention is the lack of rational approaches in the discovery process of compounds currently being developed. Serendipity will only get us so far. In the market-driven

pharmaceutical sector, advances in genomic technologies, high-throughput screenings and X-ray crystallography are facilitating both the understanding of infectious organisms and approaches to rational drug design. These technologies need to be urgently and more comprehensively applied to neglected diseases if the pipeline for drug discovery and development is to remain full of real promise. The reluctance of the pharmaceutical sector to invest in early-stage discovery research for neglected diseases is leaving the pressing need to translate basic scientific knowledge into novel targets and new therapies unmet. 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.

Appendix A: Promises from the basic research field

In 1979 Mitchison introduced the influential concept of distinct *M. tuberculosis* populations characterized by distinct metabolic rate and therefore drug susceptibility, coexisting in an infected individual. Studies of surgically removed infected lung tissue clearly showed that infections in individual patients are highly heterogeneous and comprise pathologically discrete lesions, including open cavities, closed cavities and end-stage cavities (Kennedy et al., 1957; Kennedy et al., 1958; Vandiviere et al., 1956). The only lesion in which abundant bacterial growth has been observed is on the inner surface of open cavities, a site that is insulated from the immune system. In this location, most bacteria are found inside macrophages (Kaplan et al., 2003). Closed lesions instead typically contained caseum, a wet/semi-dry chalky paste that results from tissue necrosis in granulomas, and were not connected to the bronchi of the host. Clinical evidence supporting the Mitchison hypothesis came from a study in which pulmonary lesions were resected from TB patients after prolonged drug therapy (Vandiviere et al., 1956). Lesions classified as open and active yielded colonies of predominantly drug-resistant tubercle bacilli in the normal timeframe (3–8 weeks). In contrast, lesions that were closed and inactive gave colonies only with prolonged (3–10 months) incubation. Surprisingly, the bacteria isolated from these lesions were almost uniformly drug sensitive. Therefore, different types of lesions are present at the same time in an actively infected TB patient, and can be seen as a wide range of distinct microenvironments that the bacteria have to adapt to in order to survive inside the host organism. Adaptation to those distinct microenvironments most likely requires a fine-tuning of bacterial physiology and metabolism, leading to metabolically distinct bacteria subpopulations co-existing in an actively infected patient. Current multidrug therapy in TB patients leads to a relatively rapid sterilization of bacteria in the sputum: typically sputum from most patients fails to yield bacteria in cultures within eight weeks (Garay, 2004). Considering that sputum-borne bacteria represent those that originate from macrophages at the immediate cavity surface, the remaining 16 weeks of conventional therapy are likely to be required primarily to eradicate bacteria from other types of lesions, possibly those located in the necrotic or caseous regions that are remote from the cavity surface and are probably characterized by a limited availability of oxygen and nutrients (Dannenbergh and Rock, 1994; Saunders et al., 1999). The microenvironment in which these treatment-refractory bacteria are found in actively infected patients is potentially very similar to the microenvironment of the bacteria in patients with latent disease.

It is therefore clear that a successful search for better, faster and cheaper drugs urgently requires an improved understanding and consideration of the physiology and metabolism of bacteria *in vivo*.

Although this idea is not new and pioneering studies aimed to highlight differences among *in vitro* and *in vivo* metabolic strategies of *M. tuberculosis* were already performed in the 1950s (Bloch and Segal, 1956), significant advances have been made only recently thanks to the advent

of molecular and genetics tools for mycobacteria and the availability of the full genome sequence (Cole et al., 1998). The application of genome-based biology, including array-based analysis of mutant libraries and expression profiling, is allowing dissection of the fundamental genetics of *M. tuberculosis* growth and persistence in animal models (Lamichhane et al., 2005; Sasseti and Rubin, 2003; Shi et al., 2005); for review see (Manabe and Bishai, 2000; McKinney, 2000). More recently, crucial advances have been made in developing technologies that allow conditional gene silencing in *M. tuberculosis* (Blokpoel et al., 2005; Ehrt et al., 2005). The successful application of these systems for inducible gene silencing *in vitro* make the TB scientific community believe that it will be finally possible to silence *M. tuberculosis* genes at specific stage of infection in animal models. This would allow investigators to turn a gene off at a particular time after infection has begun, and thereby model the effect of treating the infection with an antibiotic directed against the gene product. Furthermore, the introduction of conditional gene inactivation will allow the characterization *in vivo* of genes that once inactivated preclude growth *in vitro*. Elucidation of the genetic requirements for *in vivo* growth will not only lead to the identification of potential drug targets that traditional screens would miss, but will also allow the refinement and improvement of *in vitro* screens to mimic the environmental conditions that characterize *in vivo* growth.

Genes involved in energy metabolism and response to oxygen limitation

Oxygen limitation during granuloma development has been proposed to be one of the main signals that result in a change in the metabolic state of bacteria. An *in vitro* model for the metabolic alterations that are associated with oxygen depletion has shown that replication is dependent on the presence of oxygen (Wayne and Hayes, 1996; Wayne and Sohaskey, 2001). *In vivo*, the number of bacilli in a lesion generally correlates well with the degree of oxygen (Canetti, 1955), suggesting that oxygen supply might limit *M. tuberculosis* growth during infection. Moreover, when an appropriate dose of tubercle bacilli is used to infect mice the resulting lung infection typically exhibit an acute phase (~20 days of exponential bacterial growth) and a subsequent chronic phase during which bacterial counts reach a plateau (~10⁵-10⁶ CFU/lung) (for a review see Orme and Collins, 1994). The arrest of bacterial growth during chronic phase is thought to be the result of the expression of host adaptive T helper 1-mediated immunity and the consequent activation of infected macrophages that produce inducible nitric oxide (NO) synthase which catalyses the production of NO (for a review see (Flynn and Chan, 2001). NO acts as a potent oxidant and inhibitor of cellular respiration (MacMicking et al., 1997). Persistent bacilli are therefore thought to be exposed *in vivo* to an environment which is poor in O₂ and rich in NO (for a review see (Boshoff and Barry, 2005). Detailed analysis of the response of *M. tuberculosis* to hypoxia or exposure to NO using microarray-based whole genome profiling has identified a set of genes that are rapidly up-regulated in these conditions (Sherman et al., 2001; Voskuil et al., 2003). Among those genes is the putative two-component regulatory system ***dosR-dosS/dosT*** (also called *devR-devS*,

Rv3133c/Rv3132c). In bacteria two-component response regulator systems are an important means by which a variety of environmental signals are transduced into a phenotypic response. These systems typically consist of a membrane-bound sensor kinase and a soluble response regulator that is activated by a histidine-aspartate phosphorelay to bind upstream of specific genes and regulate their expression (Hoch and Silhavy, 1995). In the DosR-DosS/DosT two component system dosS and dosT encode for sensor kinases while DosR encodes for a transcription factor. *M. tuberculosis* mutants lacking DosS and DosT as well as the transcription factor DosR can no longer activate DosR-dependent gene expression in response to reduced oxygen tension (Park et al., 2003; Roberts et al., 2004). *In vitro* data suggest that the components of the DosR-DosS/DosT could be good targets to develop drugs against persister bacteria. However, currently available data does not allow ruling out the precise role of the DosR-DosS/DosT *in vivo*. Indeed, while DosR mutants are not attenuated for growth and survival in mouse tissue (Parish et al., 2003), DosR is required for virulence in guinea pigs (Malhotra et al., 2004). Future investigations should be aimed at better assessing the role of these candidate drug targets in animal models.

In a recent study, transcription profiling of genes encoding components of the respiratory chain and ATP synthesizing apparatus of *M. tuberculosis* was performed *in vivo* in infected mice (Shi et al., 2005). This study revealed that adaptation of *M. tuberculosis* to host immunity requires an exchange in enzymes involved in respiratory pathways. While strong downregulation of proton-pumping type I NADH dehydrogenase and aa3-type cytochrome c oxidase was observed during chronic phase of infection, the less energy-efficient cytochrome bd oxidase and the nitrate transporter narK2 were clearly upregulated. These findings identify additional potential targets for drugs directed against persisters bacilli and interest in this class of enzymes has been sparked by the discovery of efficient new antitubercular drugs that target respiratory components and ATP synthesis (Andries et al., 2005). Validation of cytochrome bd oxidase or narK2 as potential drug targets requires a detailed characterization of *M. tuberculosis* mutants lacking these enzymes and their ability to establish infection *in vivo*.

Genes encoding enzymes of the glyoxylate shunt

The strategy of survival of *M. tuberculosis* during chronic stages of infection is thought to involve a metabolic shift in the bacteria's carbon source. Several lines of evidence suggest that pathogenic mycobacteria primarily use fatty acid rather than carbohydrates as carbon substrate during infection. Under these conditions, glycolysis is decreased and the glyoxylate shunt is significantly upregulated (Wayne, 1994); (Gupta and Katoch, 1997). Isocitrate lyase (ICL) and malate synthase are the two enzymes of this pathway, which has been described in various eubacteria, fungi, and plants. In *Mycobacterium tuberculosis*, isocitrate lyase activity is increased when the bacilli are in an environment of low oxygen tension or in a transition from an actively replicating to a non-replicating state (Kannan et al., 1985); (Wayne and Lin, 1982). ICL plays a crucial role for survival

of *M. tuberculosis* bacteria in activated macrophages. It has also been shown that the gene encoding ICL (*icl*) is important for survival of *M. tuberculosis* in the lungs of mice during the persistent phase of infection (2-16 weeks), but is not essential during the acute phase (0-2 weeks). These findings emphasize the importance of this pathway to the bacteria in sustaining a chronic infection (McKinney et al., 2000). A single malate synthase gene called *glcB* (also referred to as *aceB* in *M. tuberculosis* CDC1551; MT1885; see www.tigr.org) has been identified in *M. tuberculosis* encoding a malate synthase G (MSG) (Rv1837c; see (Cole et al., 1998). Since malate synthase and ICL are part of the same metabolic pathway, inactivation of malate synthase is expected to result in survival defects phenotypically similar to that observed in *icl* mutants. Validation of malate synthase as a potential drug target awaits characterization of the *in vivo* phenotype for bacteria lacking this enzyme. The crystal structure of malate synthase has been solved and available data can facilitate development of specific inhibitors through structure-based drug design (Smith et al., 2003)

Genes involved in the response to nutrients limitation

Rel_{Mtb}. Intact granulomas formation are believed to be characterized by nutrient deprivation in the form of amino acid and carbohydrate depletion in a process that is thought to be essential for curtailing growth of microorganisms (Betts et al., 2002). When microorganisms encounter a nutrient limited environment they slow down their growth rate dramatically and reduce levels of rRNA, tRNA and protein synthesis. Often RNA polymerase activities are modified, the activity of transport systems is reduced and metabolism of carbohydrates, amino acids, and phospholipids is decreased. Known as the stringent response, this broad alteration in metabolism is mediated by the accumulation of hyperphosphorylated guanine nucleotides (p)ppGpp. The stringent response is reversed when environmental conditions become favourable and (p)ppGpp levels decrease. *Mycobacterium tuberculosis* has one dual function enzyme, Rel_{Mtb}, for the synthesis and hydrolysis of (p)ppGpp (Avarbock et al., 1999). In *in vitro* studies, *rel_{Mtb}* mutants displayed a significant lower aerobic growth rate than the wild-type bacterial strain. Moreover, long term survival of *rel_{Mtb}* mutants during *in vitro* starvation or nutrient run out in normal media was significantly impaired compared to that of the wild-type strain (Primm et al., 2000). In mouse models of infection, *rel_{Mtb}* mutants presented normal initial bacterial growth but their ability to sustain chronic infection was severely impaired (Dahl et al., 2003). More recently, *rel_{Mtb}* was identified as one of the gene required for short-term survival in mouse tissues in a microarray-based screen of *M. tuberculosis* mutants suggesting that in addition to having a role in long-term persistence, *rel_{Mtb}* may also be important for short term survival in certain *in vivo* situations (Lamichhane et al., 2005).

Genes involved in cell wall and membrane metabolism

PcaA. Another mutant impaired in late-stage survival was discovered by Glickmann and collaborators *pcaA* (Glickman et al., 2000), who identified a gene called *pcaA* encoding a cyclopropane synthetase, a methyl transferase involved in the modification (cyclopropanation) of the acyl chain of mycolic acids in mycobacterial cell wall. In mouse models, mutants lacking the *pcaA* gene (*pcaA*) showed no defects for early growth. However, at later stage of infection the ability of the *pcaA* mutants to maintain a stable peak was impaired. Although the effect of *pcaA* deletion on bacterial persistence was subtle, inactivation of *pcaA* clearly decreased lung damage and mortality from chronic *M. tuberculosis* infection. This suggests the possibility that cyclopropanated mycolic acid can contribute to TB pathogenesis, either directly by inducing tissue damage or indirectly by stimulating tissue-damaging immune responses. If this is true, drugs targeting *pcaA* might offer the additional benefit of reducing tissue pathology.

At least two other cyclopropane synthetases (*CmaA1*, *CmaA2*) are involved in the site-specific modification of mycolic acids (Glickman et al., 2001; Yuan et al., 1995). Crystal structure of these enzymes has been determined and would be therefore available for structure-based inhibitor design. The similarity of these enzymes in their active site suggests the possibility that one inhibitor may be effective against multiple targets, reducing the potential for drug resistance (Huang et al., 2002). Whether the inactivation of the three genes encoding the cyclopropane synthetases (*CmaA1*, *CmaA2* and *pcaA*) would more drastically affect bacterial persistence and pathogenesis in animal models remains an open question that deserves further investigation.

PE-PGRS. The PE-PGRS protein family counts approximately 60 members and is unique to mycobacteria. The function of PE-PGRS family members is unknown. There is evidence that some are cell-wall associated and may play a role in modifying host immune responses (Banu et al., 2002; Brennan et al., 2001). Mutation of two PE-PGRS genes in *M. marinum* produced strains unable to replicate in macrophages and with impaired persistence in granulomas (Ramakrishnan et al., 2000). A recent study using transposon mutagenesis identified the PE-PGRS as a family enriched in essential genes (Lamichhane et al., 2003). Although some members of the PE-PGRS family present interesting features, further investigations about the role of PE-PGRS proteins during infection in mouse models is required before they can be taken in consideration as potential drug targets. Moreover, development of drugs targeting PE-PGRS proteins might be complicated due to the redundancy existing among family members.

MmpL. Members of the mycobacterial membrane large protein family (*MmpL*) are involved in the transport of a range of substrates across the membrane. The first member of this protein family that was found to play a role in *M. tuberculosis* survival *in vivo* is *MmpL7*. *M. tuberculosis* mutants lacking *MmpL7* activity are severely attenuated for growth in the lungs but not in the spleen and

liver of animal models (Camacho et al., 2001; Cox et al., 1999). Several independent studies have identified the *MmpL7* gene as part of the *M. tuberculosis fadD26-mmpL7* (Rv2930-Rv2942) locus that plays a major role in biosynthesis and secretion of phthiocerol dimycocerosate (PDIM), a cell wall associated lipid necessary for *M. tuberculosis* virulence (Camacho et al., 1999; Cox et al., 1999; Pinto et al., 2004). In particular, PDIM seems to have a role in protecting *M. tuberculosis* bacteria from the toxic action of nitric oxide released by activated macrophages (Rousseau et al., 2004).

Recent studies have showed that, with the exception of *mmpL3* that is apparently essential for viability, members of the MmpL protein family are not required for growth *in vitro* but they seem to play a crucial role for survival of *M. tuberculosis in vivo* in mouse models (Domenech et al., 2005; Lamichhane et al., 2005). When assessed for growth kinetics and lethality in a murine model of tuberculosis, mutants with inactivation in *mmpL8* and *MmpL 11* were able to establish a normal infection but they were significantly attenuated for lethality in time-to-death studies. On the other hand, bacterial strains carrying mutation in *MmpL4* and *MmpL7* were found to have impaired growth kinetics and impaired lethality (Domenech et al., 2005). In another independent study, preliminary results showed that mutants with disruption in *mmpL4*, *mmpL5*, *mmpL7*, *mmpL8*, *mmpL10*, *mmpL11* showed significant attenuation for growth in the lungs, while only *mmpL4* showed a survival defects in the spleen (Lamichhane et al., 2005). Further investigation should aim to identify the substrates that are transported across the membrane by the MmpL family members. In particular it would be interesting to identify *MmpL4*'s substrates, since bacteria lacking *MmpL4* showed impaired survival in lungs and spleen in mouse models. Thus, drugs targeting MmpL proteins or enzymes involved in the biosynthesis of the substrates transported by MmpL proteins across the membrane could effectively impair *in vivo* survival of *M. tuberculosis*.

Genes involved in transcriptional regulation.

Sig F. RNA polymerase σ -factors are transcription factors that are upregulated under specific conditions and direct the transcription machinery to a specific group of genes necessary for the survival of the bacterium under those environmental conditions. One of these σ -factors, Sig F, is expressed during stationary phase, when bacterial growth and division are slowed, and is virtually undetectable when cells are dividing rapidly (DeMaio et al., 1996). A strain of *M. tuberculosis* in which the gene *SigF* was inactivated has been constructed and tested in mouse models for TB infection. While loss of *SigF* did not prevent the mutant strain from producing lethal infection, death was significantly delayed in mice infected with the mutant strain. More interestingly, *in vitro* experiments showed that *SigF* mutants were more susceptible to rifamycin drugs (Chen et al., 2000). It is interesting to speculate that SigF could be taken in consideration as a target for development of a “drug-enhancing drug” that would not have intrinsic bactericidal activity and yet it would accelerate treatment by promoting the action of conventional drugs (McKinney, 2000).

Genes involved in promoting M. tuberculosis survival inside macrophages.

M. tuberculosis is an obligate pathogen whose primary target cells are macrophages. The successful parasitization of macrophages by pathogenic mycobacteria involves the inhibition of several host-cell processes. Host processes inhibited by pathogenic mycobacteria include the fusion of phagosomes with lysosomes, antigen presentation, apoptosis and the stimulation of bactericidal responses due to the activation of pathways involving mitogen-activated protein kinases (MAPKs), interferon- γ (IFN- γ) and calcium (Ca²⁺) signalling (for a review see (Koul et al., 2004; Nguyen and Pieters, 2005).

Inhibition of phagosome maturation

One of the strategies by which pathogenic mycobacteria survive the bactericidal milieu of macrophages is the inhibition of fusion of their intracellular niche (the phagosome) with late endosomes and lysosomes ((Armstrong and Hart, 1971; Russell, 2001). Phagosomes are vesicles that are formed by invagination of the plasma membrane during endocytosis and subsequently fuse with primary lysosomes to degrade engulfed material, a processes also termed as “phagosome maturation”. Lysosomes are membrane-limited cellular organelles with a low internal pH and contain enzymes for degradation of polymers such as proteins, DNA, RNA polysaccharides and lipids. Normally, phagocytosed microorganisms are rapidly transferred from phagosomes to lysosomes and are then digested and degraded by hydrolytic enzymes. However, pathogenic mycobacteria are directed to phagosomes that subsequently fail to fuse to lysosomes (Armstrong and Hart, 1975).

The *M. tuberculosis* cell wall component phosphatidylinositol-3-phosphate (PI3P) analog glycosylated lipoarabinomannan (**Man-LAM**) has been reported to block phagosome maturation by inhibiting a signalling cascade that consist of Ca²⁺, calmodulin and phosphatidylinositol-3-kinase (PI3K) (Fratti et al., 2003; Rojas et al., 2000). The arrest of phagosome maturation by Man-LAM represents an effective mechanism that is used by mycobacteria for long term survival in host cell. In view of the central role played by Man-LAM in mediating intracellular survival of *M. tuberculosis*, genes involved in the biosynthetic pathway of Man-LAM represent potential targets for novel anti-TB drug.

In addition to cell wall lipids, the serine/threonine protein kinase G (**PknG**) was found to play a role in the arrest of phagosome maturation (Pethe et al., 2004; Walburger et al., 2004). When the gene encoding PknG was disrupted in *M. bovis* BCG, the resulting mutant mycobacterial strain was immediately transferred to lysosomes and was not able to survive inside macrophages (Walburger et al., 2004). The ability of PknG to block lysosomal delivery suggests that this kinase might be valuable target in the development of drugs that could induce mycobacterial death inside the macrophages. A screen for PknG inhibitors identified a tetrahydrobenzothipene that specifically

inhibits the PknG kinase activity. When added to infected macrophages, this compound induces the fusion of phagosomes to lysosomes and mediates killing of mycobacteria inside macrophages (Walburger et al., 2004). Preliminary subcellular localization studies suggest that PknG is secreted into the phagosomal lumen and cytosol of infected macrophages. If this is proven to be the case it would represent a crucial advantage in terms of drug delivery. By targeting a secreted molecule such as PknG indeed, transport of anti-microbial agents through the extremely impermeable mycobacterial cell wall can be circumvented, greatly improving the accessibility of the compounds to their target. Further investigations should be aimed to confirm the role of PknG in *M. tuberculosis* species. Validation of PknG as a potential drug target requires analysis of *M. tuberculosis* mutants lacking PknG and assessment of their ability to survive and successfully establish infection *in vivo*.

Resistance to nitric oxide stress

It has been showed that production of reactive nitrogen intermediates by induction of nitric oxide synthase (iNOS) in activated macrophages is necessary to protect against tuberculosis (Chan et al., 1992). Macrophages activation in the response to *M. tuberculosis* is mainly mediated by IFN- γ . Consistently, mice deficient in the gene for IFN- γ or in the gene for iNOS succumb rapidly to *M. tuberculosis* (Dalton et al., 1993; Flynn et al., 1993; MacMicking et al., 1997). A recent study identified the genes that are required by *M. tuberculosis* to resist to the harmful effect of reactive nitrogen intermediates (Darwin et al., 2003). These genes encode for the subunits of the mycobacterial proteasome. The proteasome is a multisubunit molecular machine that is highly conserved from archibacteria to humans and is responsible for the proteolysis of cellular proteins. *M. tuberculosis* has adapted the proteasome machinery to protect itself from the killing effect of nitric oxide and deletion of genes that encode proteins involved in the formation of proteasome causes hypersensitivity of the bacilli to nitric oxide (Darwin et al., 2003; Pieters and Ploegh, 2003). In an independent study, a comparison of the intraphagosomal gene expression profile of *M. tuberculosis* in both resting and activated macrophages identified several genes that are involved in induction of persistence, fatty-acid metabolism and resistance to nitric oxide (Schnappinger et al., 2003). Further characterization of these gene products will provide information about the survival strategies of this pathogen and also help to identify new targets.

Appendix B: Update on compounds in the pipeline

By the time this report has reached its final format, few new compounds have entered the pipeline under the discovery phase. Hereafter we briefly review the compounds for which information were publicly available.

Malate Synthase Inhibitors (GSK, Rockefeller University, Texas A&M)

As the isocitrate lyase (ICL), the malate synthase is an enzyme of the glyoxylate shunt, a metabolic pathway of *M. tuberculosis* that appears to be upregulated during the chronic stage of infection. Since malate synthase and ICL are part of the same metabolic pathway, inactivation of malate synthase is expected to result in survival defects phenotypically similar to that observed in *icl* mutants (see Appendix A). Identification of inhibitors for ICL has proven lengthy and laborious due to the conformation of the enzyme active side (see paragraph 3.1). Therefore, efforts are currently being focused on identifying inhibitors of the second identified component of the glyoxylate shunt, the malate synthase.

Riminophenazines (Institutes of Materia Medica/BRTI)

Riminophenazines were specifically developed as drugs active against *M. tuberculosis* but these compounds are also active against many other mycobacterial infections, particularly those caused by *M. leprae* and the *M. avium* complex (MAC). Clofazimine, is the lead compound in this series. With the introduction of rifampicin and later pyrazinamide for the treatment of tuberculosis, interest in riminophenazines abated, mainly because the drug is not as powerful as either rifampicin or isoniazid (Reddy et al., 1999). With emergence of multidrug-resistant (MDR) tuberculosis, the interest in riminophenazines was renewed. Clofazimine has indeed showed activity against MDR strains of *M. tuberculosis* in animal models (Klemens et al., 1993). This drug is currently included in the list of the second line drugs recommended by WHO (http://whqlibdoc.who.int/publications/2006/9241546956_eng.pdf).

Investigation of new analogues of clofazimine could lead to the identification of compounds that increased activity and cause less pigmentation of internal organs and fatty tissues (Jagannath et al., 1995)

Capuramycins (Sankyo/Sequella)

Capuramycin analogues have been shown to have selective antibacterial activity against mycobacteria (Muramatsu et al., 2003). These compounds inhibit the phospho-N-acetylmuramyl-pentapeptide translocase and therefore interfere with the biosynthesis of mycobacterial the cell wall. When tested against *M. tuberculosis*, Capuramycins analogues appear to be equally active against drug-susceptible and drug resistant strains. However, the MIC range showed by these

compounds was significantly higher than the MIC for rifampicin or isoniazid when tested against drug-susceptible strains (Koga et al., 2004). Further studies are required to thoroughly assess the activity of the capuramycins analogues *in vivo*

Proteasome Inhibitors (Cornell University)

As described in Appendix A, the proteasome (i.e. the protein degradation machinery of the cell) represents an interesting potential new target for anti-tuberculosis drugs. The activity of *M. tuberculosis* proteasome, appears to be important for protecting the bacteria from the killing effect of the nitric oxide produced in activated macrophages. Deletion of genes that encode proteins involved in the formation of proteasome causes hypersensitivity of the bacilli to nitric oxide. Drugs targeting the proteasome are expected to be active against MDR *M. tuberculosis* strains as they would act through a completely novel mechanism.

Protease Inhibitors (Medivir)

Proteases are a special class of proteins, operating as extremely precise biological 'scissors' - cutting long protein chains, thereby increasing or decreasing a particular protein's activity.

Since 2001, Medivir has been in collaboration with scientists at Bart's, and the London School of Medicine, Queen Mary College, the University of London in the development of new antibiotics-an arrangement funded by the UK Department of Trade and Industry (in its Applied Genomics initiative), the UK's Medical Research Council and Medivir.

This project is founded on the expertise within Queen Mary Hospital in identifying and validating bacterial proteases as target enzymes for pharmaceuticals. Medivir is using its pharmaceuticals knowledge and technology platforms in the protease sphere to develop new antibiotics. As part of this project, the genome of *M. tuberculosis* is being examined to identify genes encoding for proteases of interest as drug targets, to the purpose of developing protease inhibitors as antibiotics against these bacteria (<http://www.medivir.se/v2/eng/news/show-news.asp?trigYear=2001&id=29>).

The following drug discovery projects are being carried out in collaboration with the TB Alliance.

Bifunctional Molecules (Cumbre- TB Alliance)

Cumbre Pharmaceuticals Inc and the TB Alliance have just launched a joint program to develop new anti-tuberculosis agents.

Under the terms of the agreement, the parties will work on the design, synthesis and optimization of two different classes of multi-functional antibiotics. The collaboration will utilize Cumbre's unique antibiotic discovery and development technical platform to identify and deliver novel antibiotic compounds. The TB Alliance will have exclusive rights to these compounds for the treatment of

tuberculosis and other neglected diseases, while Cumbre will retain the rights to pursue compounds for use in other infectious disease areas (http://www.tballiance.org/7_1_1GenericNewsArticles.asp?itemId=728).

Bacterial Topoisomerase Inhibitors (GlaxoSmithKline-TB Alliance)

The DNA Topoisomerases are enzymes that control DNA topology and are vital for cellular processes that involve duplex DNA, namely replication, recombination and transcription. DNA gyrase, the single type II topoisomerase of *M. tuberculosis*, is the molecular target of fluoroquinolones. Due to limited public information about this project, it is not known whether the new compounds under development will target DNA gyrase or DNA topoisomerase I

Appendix C: Extensive Drug Resistant Tuberculosis (XDR-TB)

Multi-drug resistant tuberculosis (MDR-TB) is a form of tuberculosis resistant to *at least* the two principal first-line drugs rifampicin and isoniazid. The Global XDR-TB Taskforce convened by the World Health Organization in October 2006 defined extensive drug-resistant tuberculosis (XDR-TB) as a form of tuberculosis resistant not only to rifampicin and isoniazid, but also to certain second-line drugs (at least one fluoroquinolone and one of the three injectable drugs kanamycin, amikacin or capreomycin).

XDR-TB in itself is not a new problem. The existence of XDR-TB strains has been known to practitioners working in Eastern European and Central Asian countries. What is alarming about the recent outbreak is that it is occurring in a country with very high HIV prevalence (South Africa), and risks spreading extremely rapidly amongst HIV positive people.

XDR-TB in KwaZulu Natal, South Africa – the spread of a new strain

A survey among suspected TB patients in the rural district of KwaZulu Natal between January 2005 and March 2006 revealed that 221 (41%) of 544 patients that tested culture positive for *M.tuberculosis* were infected by multi-drug resistant strains.

53 patients out of 221 (24% of MDR or 10% of all culture positive patients) were infected with XDR strains. 51% of the XDR patients had no prior TB treatment, suggesting that they had been newly infected by XDR-TB strains, and that resistance did not develop during treatment.

52 of the 53 XDR-TB patients died. The combination of XDR-TB and HIV infection leads patients to develop a highly aggressive form of tuberculosis that causes death in a very short time.

The emergence and rapid spread of XDR-TB in high HIV prevalence settings represent a major threat to global health. The phenomenon is a demonstration of the limitations of TB control programmes, which have been relying on outdated tools for TB diagnosis and treatment.

XDR's implications for the TB drug and diagnostics R&D pipelines

The immediate responses of the public health community must not focus solely on strengthening control programmes. It is also urgent to mobilise all necessary resources for the rapid delivery of new drugs and diagnostic tools.

Concerning drugs, it is crucial that the pipeline be filled with compounds that act through novel mechanisms which are able to target novel molecular targets, in order to avoid cross-resistance with drugs currently in use.

Currently, there are a few new promising candidate drugs in the clinical phase of development. These candidate drugs have been shown to be active against MDR-TB strains in vitro and therefore have the potential to be effective against MDR-TB in human patients.

There is an urgent need for innovative thinking in the field of clinical trials for new TB drugs, in order to speed up the development of these new drugs and accelerate their delivery to patients.

A major limitation currently is the difficulty of diagnosing patients with TB. This problem is even more acute in the case of XDR-TB because the disease is so rapidly fatal that most patients will die before the results of their diagnosis are available. Rapid, reliable and field adapted diagnostic tools for TB and drug resistant forms of TB are an integral part of treatment strategies and urgently need to be developed.

References

- Alangaden, G. J., and Lerner, S. A. (1997). The clinical use of fluoroquinolones for the treatment of mycobacterial diseases. *Clin Infect Dis* 25, 1213-1221.
- Alangaden, G. J., Manavathu, E. K., Vakulenko, S. B., Zvonok, N. M., and Lerner, S. A. (1995). Characterization of fluoroquinolone-resistant mutant strains of *Mycobacterium tuberculosis* selected in the laboratory and isolated from patients. *Antimicrob Agents Chemother* 39, 1700-1703.
- Alvarez-Freites, E. J., Carter, J. L., and Cynamon, M. H. (2002). In vitro and in vivo activities of gatifloxacin against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 46, 1022-1025.
- Andries, K., Verhasselt, P., Guillemont, J., Gohlmann, H. W., Neefs, J. M., Winkler, H., Van Gestel, J., Timmerman, P., Zhu, M., Lee, E., *et al.* (2005). A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 307, 223-227.
- Armstrong, J. A., and Hart, P. D. (1971). Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J Exp Med* 134, 713-740.
- Armstrong, J. A., and Hart, P. D. (1975). Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J Exp Med* 142, 1-16.
- Aubry, A., Pan, X. S., Fisher, L. M., Jarlier, V., and Cambau, E. (2004). *Mycobacterium tuberculosis* DNA gyrase: interaction with quinolones and correlation with antimycobacterial drug activity. *Antimicrob Agents Chemother* 48, 1281-1288.
- Avarbock, D., Salem, J., Li, L. S., Wang, Z. M., and Rubin, H. (1999). Cloning and characterization of a bifunctional RelA/SpoT homologue from *Mycobacterium tuberculosis*. *Gene* 233, 261-269.
- Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K. S., Wilson, T., Collins, D., de Lisle, G., and Jacobs, W. R., Jr. (1994). *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* 263, 227-230.
- Banu, S., Honore, N., Saint-Joanis, B., Philpott, D., Prevost, M. C., and Cole, S. T. (2002). Are the PE-PGRS proteins of *Mycobacterium tuberculosis* variable surface antigens? *Mol Microbiol* 44, 9-19.
- Barclay, W. R., Ebert, R. H., Le Roy, G. V., Manthei, R. W., and Roth, L. J. (1953). Distribution and excretion of radioactive isoniazid in tuberculous patients. *J Am Med Assoc* 151, 1384-1388.
- Bartlett, J. G., Dowell, S. F., Mandell, L. A., File Jr, T. M., Musher, D. M., and Fine, M. J. (2000). Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. *Clin Infect Dis* 31, 347-382.
- Betts, J. C., Lukey, P. T., Robb, L. C., McAdam, R. A., and Duncan, K. (2002). Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. *Mol Microbiol* 43, 717-731.

- Bloch, H., and Segal, W. (1956). Biochemical differentiation of *Mycobacterium tuberculosis* grown in vivo and in vitro. *J Bacteriol* 72, 132-141.
- Blokpoel, M. C., Murphy, H. N., O'Toole, R., Wiles, S., Runn, E. S., Stewart, G. R., Young, D. B., and Robertson, B. D. (2005). Tetracycline-inducible gene regulation in mycobacteria. *Nucleic Acids Res* 33, e22.
- Boshoff, H. I., and Barry, C. E., 3rd (2005). Tuberculosis - metabolism and respiration in the absence of growth. *Nat Rev Microbiol* 3, 70-80.
- Bozeman, L., Burman, W., Metchock, B., Welch, L., and Weiner, M. (2005). Fluoroquinolone Susceptibility among *Mycobacterium tuberculosis* Isolates from the United States and Canada. *Clin Infect Dis* 40, 386-391.
- Brennan, M. J., Delogu, G., Chen, Y., Bardarov, S., Kriakov, J., Alavi, M., and Jacobs, W. R., Jr. (2001). Evidence that mycobacterial PE_PGRS proteins are cell surface constituents that influence interactions with other cells. *Infect Immun* 69, 7326-7333.
- Brenwald, N. P., Gill, M. J., and Wise, R. (1998). Prevalence of a putative efflux mechanism among fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 42, 2032-2035.
- Brotz-Oesterhelt, H., Beyer, D., Kroll, H. P., Endermann, R., Ladel, C., Schroeder, W., Hinzen, B., Raddatz, S., Paulsen, H., Henninger, K., *et al.* (2005). Dysregulation of bacterial proteolytic machinery by a new class of antibiotics. *Nat Med* 11, 1082-1087.
- Burman, W. J., Goldberg, S., Johnson, J. L., Muzanye, G., Engle, M., Mosher, A. W., Choudhri, S., Daley, C. L., Munsiff, S. S., Zhao, Z., *et al.* (2006). Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. *Am J Respir Crit Care Med* 174, 331-338.
- Camacho, L. R., Constant, P., Raynaud, C., Laneelle, M. A., Triccas, J. A., Gicquel, B., Daffe, M., and Guilhot, C. (2001). Analysis of the phthiocerol dimycocerosate locus of *Mycobacterium tuberculosis*. Evidence that this lipid is involved in the cell wall permeability barrier. *J Biol Chem* 276, 19845-19854.
- Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B., and Guilhot, C. (1999). Identification of a virulence gene cluster of *Mycobacterium tuberculosis* by signature-tagged transposon mutagenesis. *Mol Microbiol* 34, 257-267.
- Canetti, G. (1955). Growth of the tubercle bacillus in the tuberculosis lesion, In *The Tubercle Bacillus in the Pulmonary Lesion of Man* (New York: Springer Publishing), pp. 111-126.
- CDC (2006). Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs--worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* 55, 301-305.
- Chan, J., Xing, Y., Magliozzo, R. S., and Bloom, B. R. (1992). Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J Exp Med* 175, 1111-1122.

- Chen, P., Ruiz, R. E., Li, Q., Silver, R. F., and Bishai, W. R. (2000). Construction and characterization of a *Mycobacterium tuberculosis* mutant lacking the alternate sigma factor gene, sigF. *Infect Immun* 68, 5575-5580.
- Clark, D. W. (1985). Genetically determined variability in acetylation and oxidation. Therapeutic implications. *Drugs* 29, 342-375.
- Colangeli, R., Helb, D., Sridharan, S., Sun, J., Varma-Basil, M., Hazbon, M. H., Harbacheuski, R., Megjugorac, N. J., Jacobs, W. R., Jr., Holzenburg, A., *et al.* (2005). The *Mycobacterium tuberculosis* *iniA* gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. *Mol Microbiol* 55, 1829-1840.
- Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, C. E., 3rd, *et al.* (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537-544.
- Corbett, E. L., Watt, C. J., Walker, N., Maher, D., Williams, B. G., Raviglione, M. C., and Dye, C. (2003). The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 163, 1009-1021.
- Cox, J. S., Chen, B., McNeil, M., and Jacobs, W. R., Jr. (1999). Complex lipid determines tissue-specific replication of *Mycobacterium tuberculosis* in mice. *Nature* 402, 79-83.
- Coyle, E. A., Kaatz, G. W., and Rybak, M. J. (2001). Activities of newer fluoroquinolones against ciprofloxacin-resistant *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 45, 1654-1659.
- Cynamon, M. H., Alvirez-Freites, E., and Yeo, A. E. (2004). BB-3497, a peptide deformylase inhibitor, is active against *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 53, 403-405.
- Cynamon, M. H., Klemens, S. P., Sharpe, C. A., and Chase, S. (1999). Activities of several novel oxazolidinones against *Mycobacterium tuberculosis* in a murine model. *Antimicrob Agents Chemother* 43, 1189-1191.
- Cynamon, M. H., and Sklaney, M. (2003). Gatifloxacin and ethionamide as the foundation for therapy of tuberculosis. *Antimicrob Agents Chemother* 47, 2442-2444.
- Dahl, J. L., Kraus, C. N., Boshoff, H. I., Doan, B., Foley, K., Avarbock, D., Kaplan, G., Mizrahi, V., Rubin, H., and Barry, C. E., 3rd (2003). The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of *Mycobacterium tuberculosis* in mice. *Proc Natl Acad Sci U S A* 100, 10026-10031.
- Dalton, D. K., Pitts-Meek, S., Keshav, S., Figari, I. S., Bradley, A., and Stewart, T. A. (1993). Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* 259, 1739-1742.
- Dannenbergh, A. M. J., and Rock, G. A. W. (1994). Pathogenesis of pulmonary tuberculosis: an interplay of tissue-damaging and macrophage-activating immune responses dual mechanisms that control bacillary multiplication, In *Tuberculosis, pathogenesis, protection, and control*, B. R. Bloom, ed. (Washington D.C. : American Society for Microbiology), pp. 459-483.

- Daporta, M. T., Munoz Bellido, J. L., Guirao, G. Y., Hernandez, M. S., and Garcia-Rodriguez, J. A. (2004). In vitro activity of older and newer fluoroquinolones against efflux-mediated high-level ciprofloxacin-resistant *Streptococcus pneumoniae*. *Int J Antimicrob Agents* 24, 185-187.
- Darwin, K. H., Ehrt, S., J.C., G.-R., Weich, N., and Nathan, C. F. (2003). The proteasom of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 302.
- Deidda, D., Lampis, G., Fioravanti, R., Biava, M., Porretta, G. C., Zanetti, S., and Pompei, R. (1998). Bactericidal activities of the pyrrole derivative BM212 against multidrug-resistant and intramacrophagic *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* 42, 3035-3037.
- DeMaio, J., Zhang, Y., Ko, C., Young, D. B., and Bishai, W. R. (1996). A stationary-phase stress-response sigma factor from *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 93, 2790-2794.
- Desjardin, L. E., Perkins, M. D., Wolski, K., Haun, S., Teixeira, L., Chen, Y., Johnson, J. L., Ellner, J. J., Dietze, R., Bates, J., *et al.* (1999). Measurement of sputum *Mycobacterium tuberculosis* messenger RNA as a surrogate for response to chemotherapy. *Am J Respir Crit Care Med* 160, 203-210.
- Dickinson, J. M., and Mitchison, D. A. (1981). Experimental models to explain the high sterilizing activity of rifampin in the chemotherapy of tuberculosis. *Am Rev Respir Dis* 123, 367-371.
- Domenech, P., Reed, M. B., and Barry, C. E., 3rd (2005). Contribution of the *Mycobacterium tuberculosis* MmpL protein family to virulence and drug resistance. *Infect Immun* 73, 3492-3501.
- Douglas, J. D., Senior, S. J., Morehouse, C., Phetsukiri, B., Campbell, I. B., Besra, G. S., and Minnikin, D. E. (2002). Analogues of thiolactomycin: potential drugs with enhanced anti-mycobacterial activity. *Microbiology* 148, 3101-3109.
- DurbanImmunotherapyTrialGroup (1999). Immunotherapy with *Mycobacterium vaccae* in patients with newly diagnosed pulmonary tuberculosis: a randomised controlled trial. Durban Immunotherapy Trial Group. *Lancet* 354, 116-119.
- Ehrt, S., Guo, X. V., Hickey, C. M., Ryou, M., Monteleone, M., Riley, L. W., and Schnappinger, D. (2005). Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. *Nucleic Acids Res* 33, e21.
- Flamm, R. K., Vojtko, C., Chu, D. T., Li, Q., Beyer, J., Hensey, D., Ramer, N., Clement, J. J., and Tanaka, S. K. (1995). In vitro evaluation of ABT-719, a novel DNA gyrase inhibitor. *Antimicrob Agents Chemother* 39, 964-970.
- Flynn, J. L., and Chan, J. (2001). Tuberculosis: latency and reactivation. *Infect Immun* 69, 4195-4201.
- Flynn, J. L., Chan, J., Triebold, K. J., Dalton, D. K., Stewart, T. A., and Bloom, B. R. (1993). An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* 178, 2249-2254.

- Fratti, R. A., Chua, J., Vergne, I., and Deretic, V. (2003). Mycobacterium tuberculosis glycosylated phosphatidylinositol causes phagosome maturation arrest. *Proc Natl Acad Sci U S A* 100, 5437-5442.
- Garay, S. M. (2004). In *Tuberculosis*, W. N. a. G. Rom, S.M., ed. (Philadelphia: Lippincott Williams & Wilkins), pp. 345-394.
- Ginsburg, A. S., Grosset, J. H., and Bishai, W. R. (2003a). Fluoroquinolones, tuberculosis, and resistance. *Lancet InfectDis* 3, 432-442.
- Ginsburg, A. S., Hooper, N., Parrish, N., Dooley, K. E., Dorman, S. E., Booth, J., Diener-West, M., Merz, W. G., Bishai, W. R., and Sterling, T. R. (2003b). Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. *Clin Infect Dis* 37, 1448-1452.
- Ginsburg, A. S., Sun, R., Calamita, H., Scott, C. P., Bishai, W. R., and Grosset, J. H. (2005). Emergence of fluoroquinolone resistance in Mycobacterium tuberculosis during continuously dosed moxifloxacin monotherapy in a mouse model. *Antimicrob Agents Chemother* 49, 3977-3979.
- Glickman, M. S., Cahill, S. M., and Jacobs, W. R., Jr. (2001). The Mycobacterium tuberculosis *cmaA2* gene encodes a mycolic acid trans-cyclopropane synthetase. *J Biol Chem* 276, 2228-2233.
- Glickman, M. S., Cox, J. S., and Jacobs, W. R., Jr. (2000). A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of Mycobacterium tuberculosis. *Mol Cell* 5, 717-727.
- Glickman, S. W., Rasiel, E. B., Hamilton, C. D., Kubataev, A., and Schulman, K. A. (2006). Medicine. A portfolio model of drug development for tuberculosis. *Science* 311, 1246-1247.
- Global Alliance for TBdrug development (2001). Tuberculosis. Scientific blueprint for tuberculosis drug development. *Tuberculosis (Edinb)* 81 Suppl 1, 1-52.
- Gomez, J. E., and McKinney, J. D. (2004). M. tuberculosis persistence, latency, and drug tolerance. *Tuberculosis (Edinb)* 84, 29-44.
- Grosset, J., and Ji, B. (1998). Experimental chemotherapy of mycobacterial diseases, In *Mycobacteria, II chemotherapy*, P. R. J. Gangadharam, and P. A. Jenkins, eds. (New York: Chapman & Hall), pp. 51-97.
- Grosset, J., Truffot-Pernot, C., Lacroix, C., and Ji, B. (1992). Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. *Antimicrob Agents Chemother* 36, 548-551.
- Grosset, J. H. (1992). Treatment of tuberculosis in HIV infection. *Tuber Lung Dis* 73, 378-383.
- Gupta, U. D., and Katoch, V. M. (1997). Understanding the phenomenon of persistence in mycobacterial infections. *Indian J Lepr* 69, 385-393.
- Gupta, U. D., and Katoch, V. M. (2005). Animal models of tuberculosis. *Tuberculosis (Edinb)* 85, 277-293.

- Handwerger, S., and Tomasz, A. (1985). Antibiotic tolerance among clinical isolates of bacteria. *Rev Infect Dis* 7, 368-386.
- Herbert, D., Paramasivan, C. N., Venkatesan, P., Kubendiran, G., Prabhakar, R., and Mitchison, D. A. (1996). Bactericidal action of ofloxacin, sulbactam-ampicillin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 40, 2296-2299.
- Hirata, T., Saito, H., Tomioka, H., Sato, K., Jidoi, J., Hosoe, K., and Hidaka, T. (1995). In vitro and in vivo activities of the benzoxazinorifamycin KRM-1648 against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 39, 2295-2303.
- Hoch, J. A., and Silhavy, T. J. (1995). Two components signal transduction (Washington D.C.: American Society Microbiology).
- Hong Kong Chest Service/British Medical Research Council (1992). A controlled study of rifabutin and an uncontrolled study of ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to isoniazid, streptomycin and rifampin. *Tuber Lung Dis* 73, 59-67.
- Hu, Y., Coates, A. R., and Mitchison, D. A. (2003). Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 47, 653-657.
- Huang, C. C., Smith, C. V., Glickman, M. S., Jacobs, W. R., Jr., and Sacchettini, J. C. (2002). Crystal structures of mycolic acid cyclopropane synthases from *Mycobacterium tuberculosis*. *J Biol Chem* 277, 11559-11569.
- Jagannath, C., Reddy, M. V., Kailasam, S., O'Sullivan, J. F., and Gangadharam, P. R. (1995). Chemotherapeutic activity of clofazimine and its analogues against *Mycobacterium tuberculosis*. In vitro, intracellular, and in vivo studies. *Am J Respir Crit Care Med* 151, 1083-1086.
- Jain, R., Chen, D., White, R. J., Patel, D. V., and Yuan, Z. (2005). Bacterial Peptide deformylase inhibitors: a new class of antibacterial agents. *Curr Med Chem* 12, 1607-1621.
- Jansen, A., and Yu, J. (2006). Differential gene expression of pathogens inside infected hosts. *Curr Opin Microbiol* 9, 138-142.
- Jindani, A., Dore, C. J., and Mitchison, D. A. (2003). Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *Am J Respir Crit Care Med* 167, 1348-1354.
- Jones, P. B., Parrish, N. M., Houston, T. A., Stapon, A., Bansal, N. P., Dick, J. D., and Townsend, C. A. (2000). A new class of antituberculosis agents. *J Med Chem* 43, 3304-3314.
- Kana, B. D., and Mizrahi, V. (2004). Molecular genetics of *Mycobacterium tuberculosis* in relation to the discovery of novel drugs and vaccines. *Tuberculosis (Edinb)* 84, 63-75.
- Kannan, K. B., Katoch, V. M., Bharadwaj, V. P., Sharma, V. D., Datta, A. K., and Shivannavar, C. T. (1985). Metabolic studies on mycobacteria--II. Glyoxylate by-pass (TCA cycle) enzymes of slow and fast growing mycobacteria. *Indian J Lepr* 57, 542-548.

- Kaplan, G., Post, F. A., Moreira, A. L., Wainwright, H., Kreiswirth, B. N., Tanverdi, M., Mathema, B., Ramaswamy, S. V., Walther, G., Steyn, L. M., *et al.* (2003). Mycobacterium tuberculosis growth at the cavity surface: a microenvironment with failed immunity. *Infect Immun* 71, 7099-7108.
- Kaufmann, S. H., Cole, S. T., Mizrahi, V., Rubin, E., and Nathan, C. (2005). Mycobacterium tuberculosis and the host response. *J Exp Med* 201, 1693-1697.
- Kelly, B. P., Furney, S. K., Jessen, M. T., and Orme, I. M. (1996). Low-dose aerosol infection model for testing drugs for efficacy against Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 40, 2809-2812.
- Kennedy, H. E., Vandiviere, H. M., Melvin, I. G., and Willis, H. S. (1957). The treated pulmonary lesion and its tubercle bacillus. III. Drug susceptibility studies. *Am J Med Sci* 233, 676-684.
- Kennedy, H. E., Vandiviere, H. M., and Willis, H. S. (1958). The effects of extended incubation on propagability of tubercle bacilli. *Am Rev Tuberc* 77, 802-814.
- Klemens, S. P., DeStefano, M. S., and Cynamon, M. H. (1993). Therapy of multidrug-resistant tuberculosis: lessons from studies with mice. *Antimicrob Agents Chemother* 37, 2344-2347.
- Kocagoz, T., Hackbarth, C. J., Unsal, I., Rosenberg, E. Y., Nikaido, H., and Chambers, H. F. (1996). Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra. *Antimicrob Agents Chemother* 40, 1768-1774.
- Koga, T., Fukuoka, T., Doi, N., Harasaki, T., Inoue, H., Hotoda, H., Kakuta, M., Muramatsu, Y., Yamamura, N., Hoshi, M., and Hirota, T. (2004). Activity of capuramycin analogues against Mycobacterium tuberculosis, Mycobacterium avium and Mycobacterium intracellulare in vitro and in vivo. *J Antimicrob Chemother* 54, 755-760.
- Koul, A., Herget, T., Klebl, B., and Ullrich, A. (2004). Interplay between mycobacteria and host signalling pathways. *Nat Rev Microbiol* 2, 189-202.
- Lamichhane, G., Tyagi, S., and Bishai, W. R. (2005). Designer arrays for defined mutant analysis to detect genes essential for survival of Mycobacterium tuberculosis in mouse lungs. *Infect Immun* 73, 2533-2540.
- Lamichhane, G., Zignol, M., Blades, N. J., Geiman, D. E., Dougherty, A., Grosset, J., Broman, K. W., and Bishai, W. R. (2003). A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: application to Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A* 100, 7213-7218.
- Lenaerts, A. J., Gruppo, V., Marietta, K. S., Johnson, C. M., Driscoll, D. K., Tompkins, N. M., Rose, J. D., Reynolds, R. C., and Orme, I. M. (2005). Preclinical testing of the nitroimidazopyran PA-824 for activity against Mycobacterium tuberculosis in a series of in vitro and in vivo models. *Antimicrob Agents Chemother* 49, 2294-2301.
- Lewin, C. S., Howard, B. M., and Smith, J. T. (1991). 4-Quinolone interactions with gyrase subunit B inhibitors. *J Med Microbiol* 35, 358-362.

- Li, X. Z., Zhang, L., and Nikaido, H. (2004). Efflux pump-mediated intrinsic drug resistance in *Mycobacterium smegmatis*. *Antimicrob Agents Chemother* 48, 2415-2423.
- Long, K. S., Poehlsgaard, J., Kehrenberg, C., Schwarz, S., and Vester, B. (2006). The Cfr rRNA methyltransferase confers resistance to Phenicol, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrob Agents Chemother* 50, 2500-2505.
- Lounis, N., Veziris, N., Chauffour, A., Truffot-Pernot, C., Andries, K., and Jarlier, V. (2006). Combinations of R207910 with drugs used to treat MDR-TB have the potential to shorten treatment duration. *Antimicrob Agents Chemother*.
- MacMicking, J., Xie, Q. W., and Nathan, C. (1997). Nitric oxide and macrophage function. *Annu Rev Immunol* 15, 323-350.
- Malhotra, V., Sharma, D., Ramanathan, V. D., Shakila, H., Saini, D. K., Chakravorty, S., Das, T. K., Li, Q., Silver, R. F., Narayanan, P. R., and Tyagi, J. S. (2004). Disruption of response regulator gene, *devR*, leads to attenuation in virulence of *Mycobacterium tuberculosis*. *FEMS Microbiol Lett* 231, 237-245.
- Manabe, Y. C., and Bishai, W. R. (2000). Latent *Mycobacterium tuberculosis*-persistence, patience, and winning by waiting. *Nat Med* 6, 1327-1329.
- McCune, R. M., Jr., McDermott, W., and Tompsett, R. (1956). The fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculous infection to the latent state by the administration of pyrazinamide and a companion drug. *J Exp Med* 104, 763-802.
- McCune, R. M., Jr., and Tompsett, R. (1956). Fate of *Mycobacterium tuberculosis* in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli in the tissues despite prolonged antimicrobial therapy. *J Exp Med* 104, 737-762.
- McKinney, J. D. (2000). In vivo veritas: the search for TB drug targets goes live. *Nat Med* 6, 1330-1333.
- McKinney, J. D., Honer zu Bentrup, K., Munoz-Elias, E. J., Miczak, A., Chen, B., Chan, W. T., Swenson, D., Sacchettini, J. C., Jacobs, W. R., Jr., and Russell, D. G. (2000). Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 406, 735-738.
- McMurray, D. N., Collins, F. M., Dannenberg, A. M., Jr., and Smith, D. W. (1996). Pathogenesis of experimental tuberculosis in animal models. *Curr Top Microbiol Immunol* 215, 157-179.
- Mitchison, D. A. (1979). Basic mechanisms of chemotherapy. *Chest* 76, 771-781.
- Mitchison, D. A. (1980). Treatment of tuberculosis. The Mitchell lecture 1979. *J R Coll Physicians Lond* 14, 91-95, 98-99.
- Mitchison, D. A. (1992). The Garrod Lecture. Understanding the chemotherapy of tuberculosis--current problems. *J Antimicrob Chemother* 29, 477-493.

Mitchison, D. A. (1993). Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months. *Am Rev Respir Dis* 147, 1062-1063.

Mitchison, D. A., and Selkon, J. B. (1956). The bactericidal activities of antituberculous drugs. *Am Rev Tuberc* 74, 109-116; discussion, 116-123.

Miyazaki, E., Miyazaki, M., Chen, J. M., Chaisson, R. E., and Bishai, W. R. (1999). Moxifloxacin (BAY12-8039), a new 8-methoxyquinolone, is active in a mouse model of tuberculosis. *Antimicrob Agents Chemother* 43, 85-89.

Moghazeh, S. L., Pan, X. S., Arain, T. M., Stover, C. K., and Musser, J. M. (1996). Comparative antimicrobial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Antimicrob Agents Chemother* 40, 2655-2657.

Moran, M., Ropars, A. L., Guzman, J., Diaz, J., and Garrison, C. (2005). The new landscape of neglected disease drug development (Wellcome Trust).

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

Murugasu-Oei, B., and Dick, T. (2000). Bactericidal activity of nitrofurans against growing and dormant *Mycobacterium bovis* BCG. *J Antimicrob Chemother* 46, 917-919.

Mygind, P. H., Fischer, R. L., Schnorr, K. M., Hansen, M. T., Sonksen, C. P., Ludvigsen, S., Raventos, D., Buskov, S., Christensen, B., De Maria, L., *et al.* (2005). Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature* 437, 975-980.

Neu, H. C. (1987). Clinical use of the quinolones. *Lancet* 2, 1319-1322.

Nguyen, L., and Pieters, J. (2005). The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends Cell Biol* 15, 269-276.

Nikonenko, B. V., Samala, R., Einck, L., and Nacy, C. A. (2004). Rapid, simple in vivo screen for new drugs active against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 48, 4550-4555.

Nuermberger, E. L., Yoshimatsu, T., Tyagi, S., O'Brien, R. J., Vernon, A. N., Chaisson, R. E., Bishai, W. R., and Grosset, J. H. (2004a). Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *AmJRespirCrit Care Med* 169, 421-426.

Nuermberger, E. L., Yoshimatsu, T., Tyagi, S., Williams, K., Rosenthal, I., O'Brien, R. J., Vernon, A. A., Chaisson, R. E., Bishai, W. R., and Grosset, J. H. (2004b). Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. *AmJRespirCrit Care Med* 170, 1131-1134.

O'Brien, R. J., and Nunn, P. P. (2001). The need for new drugs against tuberculosis. Obstacles, opportunities, and next steps. *Am J Respir Crit Care Med* 163, 1055-1058.

- Oleksijew, A., Meulbroek, J., Ewing, P., Jarvis, K., Mitten, M., Paige, L., Tovcimak, A., Nukkula, M., Chu, D., and Alder, J. D. (1998). In vivo efficacy of ABT-255 against drug-sensitive and -resistant *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* *42*, 2674-2677.
- Onodera, Y., Tanaka, M., and Sato, K. (2001). Inhibitory activity of quinolones against DNA gyrase of *Mycobacterium tuberculosis*. *JAntimicrobChemother* *47*, 447-450.
- Orme, I. M., and Collins, F. M. (1994). Mouse models of tuberculosis, In *Tuberculosis: pathogenesis, protection and control*, B. R. Bloom, ed. (Washington D.C.: American society of microbiology), pp. 113-134.
- Paramasivan, C. N., Sulochana, S., Kubendiran, G., Venkatesan, P., and Mitchison, D. A. (2005). Bactericidal action of gatifloxacin, rifampin, and isoniazid on logarithmic- and stationary-phase cultures of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* *49*, 627-631.
- Parish, T., Smith, D. A., Kendall, S., Casali, N., Bancroft, G. J., and Stoker, N. G. (2003). Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. *Infect Immun* *71*, 1134-1140.
- Park, H. D., Guinn, K. M., Harrell, M. I., Liao, R., Voskuil, M. I., Tompa, M., Schoolnik, G. K., and Sherman, D. R. (2003). Rv3133c/dosR is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. *Mol Microbiol* *48*, 833-843.
- Parrish, N. M., Ko, C. G., Hughes, M. A., Townsend, C. A., and Dick, J. D. (2004). Effect of n-octanesulphonylacetamide (OSA) on ATP and protein expression in *Mycobacterium bovis* BCG. *J Antimicrob Chemother* *54*, 722-729.
- Pestova, E., Millichap, J. J., Noskin, G. A., and Peterson, L. R. (2000). Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. *JAntimicrobChemother* *45*, 583-590.
- Pethe, K., Swenson, D. L., Alonso, S., Anderson, J., Wang, C., and Russell, D. G. (2004). Isolation of *Mycobacterium tuberculosis* mutants defective in the arrest of phagosome maturation. *Proc Natl Acad Sci U S A* *101*, 13642-13647.
- Petrella, S., Cambau, E., Chauffour, A., Andries, K., Jarlier, V., and Sougakoff, W. (2006). Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. *Antimicrob Agents Chemother* *50*, 2853-2856.
- Pieters, J., and Ploegh, H. (2003). Microbiology. Chemical warfare and mycobacterial defense. *Science* *302*, 1900-1902.
- Pinto, R., Saunders, B. M., Camacho, L. R., Britton, W. J., Gicquel, B., and Triccas, J. A. (2004). *Mycobacterium tuberculosis* defective in phthiocerol dimycocerosate translocation provides greater protective immunity against tuberculosis than the existing bacille Calmette-Guerin vaccine. *J Infect Dis* *189*, 105-112.
- Primm, T. P., Andersen, S. J., Mizrahi, V., Avarbock, D., Rubin, H., and Barry, C. E., 3rd (2000). The stringent response of *Mycobacterium tuberculosis* is required for long-term survival. *J Bacteriol* *182*, 4889-4898.

- Protopopova, M., Hanrahan, C., Nikonenko, B., Samala, R., Chen, P., Gearhart, J., Einck, L., and Nancy, C. A. (2005). Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. *J Antimicrob Chemother* 56, 968-974.
- Rachman H, Strong M, Ulrichs T, et al. (2006). Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis. *Infect Immun* 74(2),1233-42.
- Ragno, R., Marshall, G. R., Di Santo, R., Costi, R., Massa, S., Rompei, R., and Artico, M. (2000). Antimycobacterial pyrroles: synthesis, anti-*Mycobacterium tuberculosis* activity and QSAR studies. *Bioorg Med Chem* 8, 1423-1432.
- Ramakrishnan, L., Federspiel, N. A., and Falkow, S. (2000). Granuloma-specific expression of *Mycobacterium* virulence proteins from the glycine-rich PE-PGRS family. *Science* 288, 1436-1439.
- Reddy, V. M., O'Sullivan, J. F., and Gangadharam, P. R. (1999). Antimycobacterial activities of riminophenazines. *J Antimicrob Chemother* 43, 615-623.
- Roberts, D. M., Liao, R. P., Wisedchaisri, G., Hol, W. G., and Sherman, D. R. (2004). Two sensor kinases contribute to the hypoxic response of *Mycobacterium tuberculosis*. *J Biol Chem* 279, 23082-23087.
- Rodriguez, J. C., Ruiz, M., Climent, A., and Royo, G. (2001). In vitro activity of four fluoroquinolones against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 17, 229-231.
- Rojas, M., Garcia, L. F., Nigou, J., Puzo, G., and Olivier, M. (2000). Mannosylated lipoarabinomannan antagonizes *Mycobacterium tuberculosis*-induced macrophage apoptosis by altering Ca²⁺-dependent cell signaling. *J Infect Dis* 182, 240-251.
- Rousseau, C., Winter, N., Pivert, E., Bordat, Y., Neyrolles, O., Ave, P., Huerre, M., Gicquel, B., and Jackson, M. (2004). Production of phthiocerol dimycocerosates protects *Mycobacterium tuberculosis* from the cidal activity of reactive nitrogen intermediates produced by macrophages and modulates the early immune response to infection. *Cell Microbiol* 6, 277-287.
- Ruiz-Serrano, M. J., Alcalá, L., Martínez, L., Díaz, M., Marin, M., Gonzalez-Abad, M. J., and Bouza, E. (2000). In vitro activities of six fluoroquinolones against 250 clinical isolates of *Mycobacterium tuberculosis* susceptible or resistant to first-line antituberculosis drugs. *Antimicrob Agents Chemother* 44, 2567-2568.
- Russell, D. G. (2001). *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat Rev Mol Cell Biol* 2, 569-577.
- Sasseti, C. M., and Rubin, E. J. (2003). Genetic requirements for mycobacterial survival during infection. *PNAS* 100, 12989-12994.
- Saunders, B. M., Frank, A. A., and Orme, I. M. (1999). Granuloma formation is required to contain bacillus growth and delay mortality in mice chronically infected with *Mycobacterium tuberculosis*. *Immunology* 98, 324-328.

- Schlunzen, F., Pyetan, E., Fucini, P., Yonath, A., and Harms, J. M. (2004). Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with tiamulin. *Mol Microbiol* *54*, 1287-1294.
- Schnappinger, D., Ehrt, S., Voskuil, M. I., Liu, Y., Mangan, J. A., Monahan, M. I., Dolganov, G., Efron, B., P.D., B., Nathan, C., and Schoolnik, G. K. (2003). Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: Insights into phagosomal environment. *J Exp Med* *198*, 693-704.
- Sharma, V., Sharma, S., Hoener zu Bentrup, K., McKinney, J. D., Russell, D. G., Jacobs, W. R., Jr., and Sacchettini, J. C. (2000). Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. *Nat Struct Biol* *7*, 663-668.
- Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I., and Schoolnik, G. K. (2001). Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha-crystallin. *Proc Natl Acad Sci U S A* *98*, 7534-7539.
- Shi, L., Sohaskey, C. D., Kana, B. D., Dawes, S., North, J. R., Mizrahi, V., and Gennaro, M. L. (2005). Changes in energy metabolism of *Mycobacterium tuberculosis* in mouse lung and under in vitro conditions affecting aerobic respiration. *PNAS* *102*, 15629-15634.
- Shi, S., and Ehrt, S. (2006). Dihydrolipoamide acyltransferase is critical for *Mycobacterium tuberculosis* pathogenesis. *Infect Immun* *74*, 56-63.
- Shoen, C. M., DeStefano, M. S., and Cynamon, M. H. (2000). Durable cure for tuberculosis: rifalazil in combination with isoniazid in a murine model of *Mycobacterium tuberculosis* infection. *Clin Infect Dis* *30 Suppl 3*, S288-290.
- Sirgel, F. A., Donald, P. R., Odhiambo, J., Githui, W., Umapathy, K. C., Paramasivan, C. N., Tam, C. M., Kam, K. M., Lam, C. W., Sole, K. M., and Mitchison, D. A. (2000). A multicentre study of the early bactericidal activity of anti-tuberculosis drugs. *J Antimicrob Chemother* *45*, 859-870.
- Smith, C. V., Huang, C. C., Miczak, A., Russell, D. G., Sacchettini, J. C., and Honer zu Bentrup, K. (2003). Biochemical and structural studies of malate synthase from *Mycobacterium tuberculosis*. *J Biol Chem* *278*, 1735-1743.
- Stover, C. K., Warrenner, P., VanDevanter, D. R., Sherman, D. R., Arain, T. M., Langhorne, M. H., Anderson, S. W., Towell, J. A., Yuan, Y., McMurray, D. N., *et al.* (2000). A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* *405*, 962-966.
- Sullivan, E. A., Kreiswirth, B. N., Palumbo, L., Kapur, V., Musser, J. M., Ebrahimzadeh, A., and Frieden, T. R. (1995). Emergence of fluoroquinolone-resistant tuberculosis in New York City. *Lancet* *345*, 1148-1150.
- Sulochana, S., Rahman, F., and Paramasivan, C. N. (2005). In vitro activity of fluoroquinolones against *Mycobacterium tuberculosis*. *JChemother* *17*, 169-173.
- Sun, Z., and Zhang, Y. (1999). Antituberculosis activity of certain antifungal and antihelminthic drugs. *Tuber Lung Dis* *79*, 319-320.

- Tangallapally, R. P., Yendapally, R., Lee, R. E., Hevener, K., Jones, V. C., Lenaerts, A. J., McNeil, M. R., Wang, Y., Franzblau, S., and Lee, R. E. (2004). Synthesis and evaluation of nitrofuranylamides as novel antituberculosis agents. *J Med Chem* 47, 5276-5283.
- Tian, J., Bryk, R., Shi, S., Erdjument-Bromage, H., Tempst, P., and Nathan, C. (2005). *Mycobacterium tuberculosis* appears to lack alpha-ketoglutarate dehydrogenase and encodes pyruvate dehydrogenase in widely separated genes. *Mol Microbiol* 57, 859-868.
- Tsukamura, M., Nakamura, E., Yoshii, S., and Amano, H. (1985). Therapeutic effect of a new antibacterial substance ofloxacin (DL8280) on pulmonary tuberculosis. *Am Rev Respir Dis* 131, 352-356.
- Tyagi, S., Nuermberger, E., Yoshimatsu, T., Williams, K., Rosenthal, I., Lounis, N., Bishai, W., and Grosset, J. (2005). Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. *Antimicrob Agents Chemother* 49, 2289-2293.
- Vandiviere, H. M., Loring, W. E., Melvin, I., and Willis, S. (1956). The treated pulmonary lesion and its tubercle bacillus. II. The death and resurrection. *Am J Med Sci* 232, 30-37; passim.
- Voskuil, M. I., Schnappinger, D., Visconti, K. C., Harrell, M. I., Dolganov, G. M., Sherman, D. R., and Schoolnik, G. K. (2003). Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J Exp Med* 198, 705-713.
- Walburger, A., Koul, A., Ferrari, G., Nguyen, L., Prescianotto-Baschong, C., Huygen, K., Klebl, B., Thompson, C., Bacher, G., and Pieters, J. (2004). Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. *Science* 304, 1800-1804.
- Wayne, L. G. (1994). Dormancy of *Mycobacterium tuberculosis* and latency of disease. *Eur J Clin Microbiol Infect Dis* 13, 908-914.
- Wayne, L. G., and Hayes, L. G. (1996). An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect Immun* 64, 2062-2069.
- Wayne, L. G., and Lin, K. Y. (1982). Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions. *Infect Immun* 37, 1042-1049.
- Wayne, L. G., and Sohaskey, C. D. (2001). Nonreplicating persistence of mycobacterium tuberculosis. *Annu Rev Microbiol* 55, 139-163.
- WHO (2005). Global tuberculosis control-surveillance, planning, financing (Geneva: World Health Organization).
- Willmott, C. J., Critchlow, S. E., Eperon, I. C., and Maxwell, A. (1994). The complex of DNA gyrase and quinolone drugs with DNA forms a barrier to transcription by RNA polymerase. *J Mol Biol* 242, 351-363.
- Yew, W. W., Kwan, S. Y., Ma, W. K., Lui, K. S., and Suen, H. C. (1990). Ofloxacin therapy of *Mycobacterium fortuitum* infection: further experience. *J Antimicrob Chemother* 25, 880-881.

Yuan, Y., Lee, R. E., Besra, G. S., Belisle, J. T., and Barry, C. E., 3rd (1995). Identification of a gene involved in the biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 92, 6630-6634.

Zhanel, G. G., Hoban, D. J., Schurek, K., and Karlowsky, J. A. (2004). Role of efflux mechanisms on fluoroquinolone resistance in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 24, 529-535.

Zhang, Y. (2005). The magic bullets and tuberculosis drug targets. *Annu Rev Pharmacol Toxicol* 45, 529-564.

Zurenko, G. E., Yagi, B. H., Schaadt, R. D., Allison, J. W., Kilburn, J. O., Glickman, S. E., Hutchinson, D. K., Barbachyn, M. R., and Brickner, S. J. (1996). In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. *Antimicrob Agents Chemother* 40, 839-845.