Review Article

DRUG DISCOVERY IN TUBERCULOSIS: A MOLECULAR APPROACH

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Summary: Despite unquestionable success of the combination drug therapy, tuberculosis (TB) very recently has drawn major attention because of the global upsurge of MDR-TB, XDR –TB and HIV-TB co-infection cases. In the last four decades, only one compound is added to the treatment regimen leaving ample opportunities to find out a new generation of TB drugs. The modern concept of drug discovery utilizes the integrated knowledge of genomics, proteomics, molecular biology and systems biology to identify more specific targets. The purpose of this review is to revisit the field of tuberculosis drug discovery based on those new concepts to identify novel targets. **[*Indian J Tuberc 2012; 59:* 194-206]**

INTRODUCTION

TB had been considered as one of the most fatal diseases to the human race long before Robert Koch discovered the staining technique to detect the responsible bacilli *Mycobacterium tuberculosis (M.Tb).* From the beginning of seventeenth century until World War-II, several parts of Europe, America and Japan suffered from the TB epidemic with the consequence of death of several million people[1]. However, a major breakthrough in the TB treatment came after introducing streptomycin, followed by *p*-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962) and rifampin (rifampicin; 1963) as an effective primary line of anti-TB drugs. Single drug treatment in TB has however, several drawbacks which include prolonged treatment time to completely eradicate the bacteria as well as increasing the opportunity to develop drug- resistant TB bacilli which has been documented in almost every country where the disease is prevalent. Although pulmonary TB related death has declined sharply in the western world since the beginning of the 20th century, it still causes major health hazards, especially in Asia, Africa and the Western Pacific region. In the beginning of 1980s, increase in TB related mortality raised a global health concern mainly because a) drug-resistant Mycobacterium and b) opportunistic infection of TB bacilli in the immunodeficiency condition as a result of HIV infection. Present gold-standard treatment for TB is a six-month course of rifampicin and isoniazid, supplemented in the initial two months with pyrazinamide in association with either ethambutol or streptomycin. Persons with latent TB infection are treated with isoniazid for six months [2]. The current success rate using cocktail drug is about 95% and is effective on drug-susceptible *M.Tb* provided the patient completes the six to nine month treatment period. Fixed dose combination (FDC) is one of the WHO recommended optimal drug treatments for TB in order to reduce treatment time, cost and perhaps to reduce the risk of emergence of drug resistant mycobacterium [3]. However, any difficulties of FDC treatment can generate chronically contagious cases, which may excrete drug-resistant mycobacteria [4]. The Directly Observed Treatment Short-course (DOTS) and DOTS-Plus programmes are recommended by WHO to control TB by providing a comprehensive organizational and infrastructural framework for the rational use of diagnosis, drug supply, as well as case and programme management services. DOTS-Plus accommodates additional second-line TB drugs to the people presumed to get MDR-TB [5]. Recently, TB due to Multi drug resistant (MDR-TB) and extensively drug resistant (XDR-TB) strains gave a wakeup call to the global health organization agencies to organize the TB programme and reinstate a fresh effort to develop a new drug against the pathogen. According to WHO, about 650,000 cases of MDR-

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TB have been reported worldwide in 2010, among them 9% are estimated to be XDR-TB cases. At present, globally, the fatality rate due to MDR-TB infection is about 30%. A syndemic relationship has been reported in HIV and MDR-TB. In 2010, about 3,50,000 people died of HIV-associated TB and 25% of deaths due to HIV are associated with TB[6]. The global scenario of TB related health hazard is not only pointing to a major loophole of the management of TB programme but also indicating an urgent need to develop new drugs or new combinations of drugs to save the lives of millions of people worldwide. This review aims to shed some light on to redirect our approach to discover a new TB drug by utilizing recent advancements in this field.

CURRENT OPINION ON TB DRUG DEVELOPMENT

TB treatment has been standing in the same strategic point which was started almost five decades ago. Cocktail drug therapy, the most widely accepted TB treatment, though effective, but has a prolonged treatment time and is inefficient to control disease progression in MDR-TB and HIV-TB co-infected patients. Current trends in anti-TB drug development are mainly driven by three major factors: a) development of drugs with long lasting antimycobacterial activity *in vivo* is desirable as because it will be helpful to bypass the problem of non–adherence and thus can reduce the risk of emergence of MDR and XDR-TB cases, b) development of novel compounds to combat MDR-TB and XDR-TB is urgently needed. c) a new class of anti-TB drugs will be very promising for prevention of latent infection or eradication of slowly metabolizing or dormant populations of mycobacterium bacilli [7,8]**.** Companies investigating on new anti-TB drugs have been deterred by a number of constraints which include a) insufficient animal models that closely mimic the human TB; b) difficulties in demonstrating the obvious benefit of a new anti-TB agent over pre-existing drugs, since multi-drug combination therapy is highly effective against ordinary cases of TB; c) perceived lack of commercial return to companies as the disease is mostly prevalent in developing countries and the cost is less than US$13 for the whole course of treatment. Anticipating the success of combination therapy, more reports are coming out in the last few years addressing the efficacy of such therapeutic approach. For example, clinical studies carried out very recently in South Africa with patients suffering from drug sensitive and MDR-TB showed a promising result. A combination of PA-824{(6S)-2-nitro-6-{[4-(trifluoromethoxy)benzyl]oxy}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine}-moxifloxacin-pyrazinamide has been shown to be very effective in reducing the early bacterial count in the sputum of the treated patient[9].Global TB Alliance and Bayer AG very recently replaced Isoniazid by Moxifloxacin in the cocktail of the combination drug therapy which is reported to reduce the treatment time by two months in mice[10]. Currently, this combination is in human clinical trials in several geographical regions around the world including the cases of HIV-TB deadly co-infection and results are expected soon. At present, a combination of antiretroviral therapy and rifampin has been considered as the back bone of treatment of HIV-TB cases[11]. Such efforts to improve the efficacy of combination treatment regimen created a novel example of low-risk drug discovery profile rather than focusing on to develop a completely new stand alone molecule against TB which should be either more cost effective than the existing drug and/or reduce significantly the treatment time. In spite of the success of combination therapy in the field of TB, several new drugs are currently in the development pipeline to come out as a single drug therapy in TB treatment. Among them, novel drugs such as TMC207, SQ109 and sudoterb [LL3858] have produced most promising results. Besides that, gatifloxacin and moxifloxacin; linezolid, PNU100480 and AZD5847; metronidazole, OPC-67683 and PA-824 are currently considered as very promising next generation TB drugs[12,13]. According to the latest WHO global report, list of such drugs and their status in the clinical trials are summarized in Table-1. Though the initial reports of these current pipeline of drugs are very promising, but the increasing global threat of MDR-, XDR-TB and HIV-TB have created a new niche for the development novel drugs in this field. Therefore, updated knowledge in the field on novel compound sources, methods of target validations, molecular models and animal proof of concept, which will be discussed in next several sections, can be implemented to find new drugs in the field of TB.

Table 1: List of latest drugs* and their status in the clinical trials.

Phase-I	Phase-II	Phase-III
AZD5847	TMC-207 OPC-67683 PA-824 Linezolid Rifapentine SQ-109 PNU-100480 Novel Regimens[a]	Gatifloxacin Moxifloxacin Rifapentine

* They are used as single drug or in combination or used as a replacement drug in the existing cocktail therapy.

NOVEL COMPOUND RESOURCE

Classically, any drug discovery process starts with the finding of a set of novel compounds, considered as hits, from a compound collection defined as library. Such a kind of library is built up based on collection of compounds from a wide variety of sources which may include small molecules with novel scaffold and unknown function, molecules originated from natural sources like secondary metabolites of plants and marine habitats. Recently, researchers are utilizing libraries with several thousands molecules of defined structure for the identification of hits in the field of targeted drug discovery. For example, the crystal structure of any key enzyme can be utilized for precise designing or understanding of the scaffold of competitive inhibitors. Similarly, interaction between two proteins either essential for host pathogen interaction or to drive any key metabolic pathway can be targeted to design a molecule which can interfere with the interaction. In silico searching, either competitive inhibitors for any enzyme or inhibitors of protein-protein interaction in any such library is now-a-days a routine job for drug discovery research. Until recently, not too much attention was paid to the natural sources, for example, marine sources, to search for novel compounds against Mycobacteria. There are several unique reasons why marine resources should be explored for searching novel compounds such as a) very wide biodiversity and largely unexplored environment (80% of all life forms on Earth are present only in the oceans), b) uniqueness and diversity at the genetic level (Sponges: >100 000 genes and Humans: ~25 000 genes). Two commercially available antivirals Ara-A (acyclovir) and AZT (zidovudine) were isolated from sponge[14] and c) large chemical diversity (marine organisms often incorporate halogens like F, Cl, Br, I into their chemical structures which are rarely seen in terrestrials). Marine organisms are also a rich source of a very wide variety of bio-reactive molecules like phenols, terpenoids, fatty acids, polysaccharides, proteins, acetylenes, terpenes, indole derivatives, and antimicrobial peptides. Several such compounds like saturated 2-methoxylated FA from sponges and secondary marine microbial metabolites like pseudopyronines were evaluated successfully for growth inhibitory effect of mycobacterium [15, 16]. In collaboration with academic institutions, several companies are searching for novel antimycobacterial agents from a repository built up with the extract of wide variety marine organisms. Besides searching for novel compounds against mycobacterium from natural products and secondary metabolites, genetic chemistry may be a completely new generation source to synthesize novel compounds with unique scaffolds. Several natural products like alkaloids, terpens, alkanes, alkenes, alkynes, phenolics and acetogenic quinones have shown antimycobacterial activity [17]. As a stand alone compound, they showed mild activity but became a potential agent when they were put in combination [18, 19]. Taking the advantage of Genetic Chemistry technology, we can synthesize several of such small molecules which are not exposed to the nature. Genes involved in the pathway of producing small molecules can be collected from a wide variety of different species like sponges, bacteria, human, fungi and plants and will be allowed to go through recombination *in vivo*. By combining and evolving genes from different organisms, genetic chemistry has laid a novel *in vivo* platform of combinatorial chemistry and enables the discovery of unique and novel chemical identity which never existed in nature. Drug discovery companies are currently working on several disease models as a target to utilize this technology[20].

TARGETS IN ANTI-TB DRUG DEVELOPMENT

Very recently, several attempts were made to identify new molecules against TB utilizing a wide variety of model systems which include inhibitors of enzymes critical for bacterial metabolic pathways, cell wall synthesis inhibitors, signaling pathway inhibitors[21-25]**.** However, several such attempts are no doubt far less than sufficient and required more attention to develop a new therapeutic approach utilizing the genomic as well as the systems biology information, as drug resistant TB and TB in a HIV patient created a new paradigm of global health concern. A successful drug discovery programme against TB should include a novel molecular model system which will be unique to the bacteria and should be targeted independently without affecting the host system. Targeting enzymes in a metabolic pathway is a classical approach to inhibit the bacterial growth but the major limitation here is frequent development of resistance. Isoniazid, an inhibitor of the mycolic acid pathway enzyme InhA (an enoyl reductase), is used for the primary line of TB therapy. Patients who undergo treatment for the MDR-TB have shown a very high rate of mutation in this enzyme [26, 27]**.** Other enzymes such as FabH (â-ketoacyl-acyl carrier protein synthase III) that play a very important role in mycolic acid biosynthetic pathway, have drawn recent attention as a fresh drug target[28]. Using structure-based drug designing technology, it is possible to develop very high affinity inhibitors which can be helpful for circumventing the normal mechanism of mutation mediated drug resistance [29]**.** Cyclopropane synthases have been shown to be implicated in pathogenicity and therefore constitute attractive targets for the development of new drugs against TB [30]. In *M.Tb*, genes cmaA2, mmaA2 or pcaA encode enzymes that are involved in the cyclopropanated mycolic acid synthesis, are well characterized and has not been explored in targeted drug discovery[31]. Latent infection is another major health concern in TB prevalent zones and about one third of the world population is estimated to be latently infected. During this asymptomatic phase of infection, *Mycobacterium* is partly capable of by passing the host immune system with the formation of granulomas[32]. Recent data show two enzymes in the glyoxalate shunt pathway, isocitrate lyase and malate synthase play an important role in the growth, persistence and support granuloma formation in host[33, 34]. Subsequently, those enzymes are considered as targets to carry out highthroughput searches to find out potential new enzyme inhibitors. Although many potential new drug targets have been identified and their number is increasing with time therefore, more effort is required in target validation to show unequivocally that they are specifically acting upon the bacterial growth and survival.

APPROACH TO TUBERCULOSIS DRUG DISCOVERY

During the period of the last two decades, the cost of drug discovery has increased several fold partly to meet the increasingly complicated criteria of the regulatory authority. A recent survey on the costs of the research and development of 68 randomly selected new drugs from ten pharmaceutical firms shows an increase in capital costs at an annual rate of 7.4% above general price inflation[35]**.** Even simple arithmetic suggests that the costs of discovering new chemical entities have been climbed up more than four fold in the last two decades. An attempt at TB drug discovery combines several steps and an exit point should be attached to each step to rationalize the cost of the whole discovery process. The three stage approach for drug discovery and development against *M.Tb* should consist of a) highthoroughput, low cost, time saving assay using nonpathogenic mycobacterium, b) lead optimization using pathogenic organism like *M.Tb* and finally, c) test on appropriate animal model to check *in vivo* efficacy and pharmacokinetics before we reach the stage of human clinical trials. Initial screening, in case of targeted drug discovery, should include a collection of several molecules using optimized reporter based assay system and generated hits will follow lead optimization stage and onwards.

Highthoroughput, low cost time saving assay using non-pathogenic mycobacterium

Mycobacterium can be classified into two major categories depending upon their growth *in vitro*, one is fast growing, non-pathogenic organism and another is slow growing, pathogenic organism.

Slow growing pathogenic mycobaterium will be a difficult organism to screen a large number of targets in a very short period of time and particularly at the same time when one of our main objectives is to develop molecules which will preferentially have antimycobacterial activity. Therefore, a preference was given to use *M. smegmatis* mainly because a) it is non-pathogenic and easy to handle b) growth rate of *M. smegmatis* is almost eight times faster than *M.Tb*, and it is widely used to understand the biology of *M.Tb* such as cell culture, gene expression and persistence in the face of nutrient starvation c) most importantly, *M. smegmatis* was found to display a profile similar to MDR *M.Tb*[36-39]**.** Therefore, cell viability assay could serve as a 'surrogate' for MDR *M. TB*. The main goal of this primary assay is to help in prioritizing the compounds which can be tested further in more specific *in vitro* assays on pathogenic *M. TB*, MDR and XDR strains. There are several reports on the use of *M. smegmatis* in primary screening to select compounds which could be active against *M. TB*[40-43]. It was reported that the susceptibility of *M. smegmatis* for the two frontline anti-TB drugs, isoniazid and rifampicin, was identical to that of MDR clinical isolates of *M. tb*. The specificity of the *M. smegmatis* based on screening has to be extremely specific so that hits generated in this assay can be a potential target for both normal as well as MDR strains.

Targeted drug discovery focusing on validation of the molecular models

Classically targeted drug discovery can be subdivided into several major categories which include a) protein-protein interaction, b) inhibitors of key regulatory enzymes, c) antimicrobial peptides, d) antisense RNA, e) siRNA. Our discussion will be focused mostly on first three categories as others have not yet come out as major potential areas, particularly in the field of TB.

a) *Protein-protein interaction*

A network of protein-protein interaction controls a wide variety of biochemical and cellular processes from bacteria to metazoan. Small molecules impair such interactions have emerged as a drug-like inhibitors and recently several such interactions for a variety of disease models going through the screening process[44]**.** A number of protein-protein interactions (PPIs) network have been proposed for *M.Tb*[45]. Disrupting such PPIs using small molecules will create new opportunities for utilizing non-traditional strategies as therapeutics. A fluorescence based highthroughput protein-protein interaction model has been described in Fig. 1. In this assay, the two interacting proteins were taged with two different fluorescence molecules such as Cyan Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP). During interaction, they are close enough to pass the energy from one fluorescent protein to another. In the presence of inhibitors, the energy transfer will be inhibited. This Fluorescence Resonance Energy Transfer (FRET) assay has been considered a routine practice in recent days of drug discovery research.

b) *Inhibitors of enzymes*

As described earlier, enzymes of mycobactrial origin can always be considered as major targets for drug discovery. The major drawback of enzyme inhibitors is that the bacterial cell can easily develop resistance against those inhibitors. At present, several enzymes have been considered as potential targets and some of them are listed in Table-2. Though inhibitors of several enzymes are already tested to check the binding efficiency, however much of the cases can be designed based on the crystal structure of the protein. Active site structure of the enzyme can be determined if the enzyme is crystallized in the presence of a substrate. As well as aiding rational design methods, the detailed structure of the enzyme and the binding position and orientation of ligands allow computer-based drug design methods, such as docking, to be used to screen and identify likely enzyme inhibitors. The basic procedure of computer assisted drug discovery (CADD) has been described in Fig. 2, where virtual hits can be identified by utilizing in silico docking system.

To accomplish docking studies, the native 3D structure of the target protein must be known, typically using X-ray crystallography and virtual

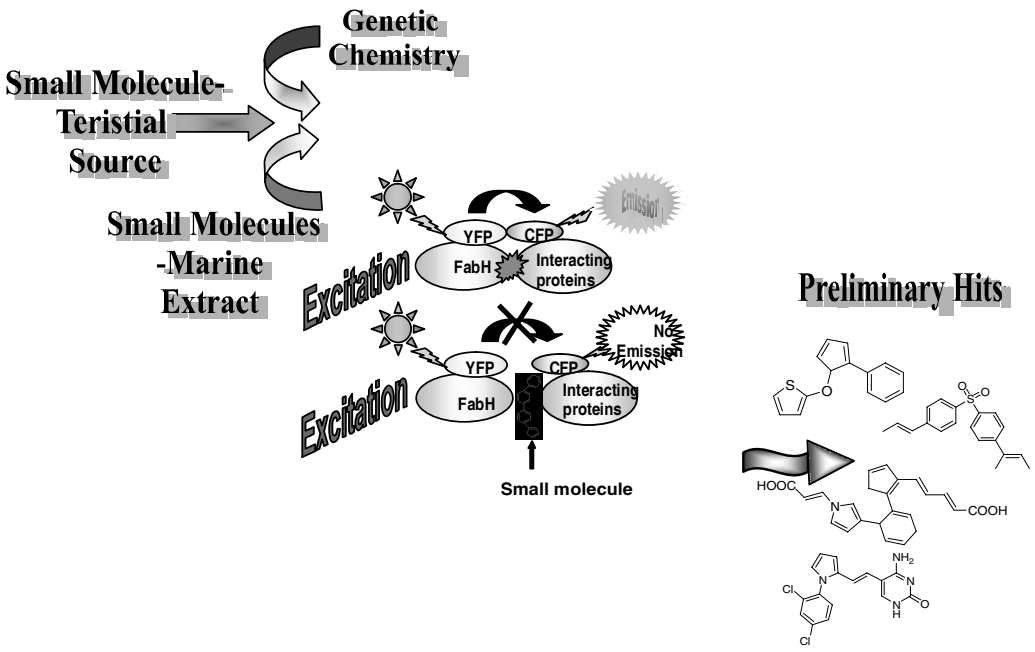

Figure 1: Reverse yeast two hybrid based HT assay models*

*include the mycolic acid biosynthetic pathway enzymes
FRET signal can be generated when two interacting proteins, playing a central role in the mycolic acid biosynthetic pathway, are cloned and overexpressed in the yeast with two different fluorescent tags like YFP and CFP. Such genetically engineered yeast population can be considered as screening machinery to identify novel small molecules originated either from genetic chemistry or from natural sources.

Table 2: List of recent drug targets on mycobacterium

Target	Reference
Maltosyltransferase	Kalscheuer R, *et al*. Nat Chem Biol. May;6(5):376-84. 2010
PhosphoriboPhosphoribosylpyrophosphate Synthetase	Lucarelli AP, *et al*. PLoS One. Nov 15;5(11):e15494, 2010.
ATP phosphoribosyltransferase	ChoY, *et al*, J.lMed. Chem., 51,(19), 2008.
Thymidylate Kinase	Familiar O, *et al*, Chem Med Chem 3,(7),2008
Protein Tyrosine Phosphatase B	Möller-Noren A, J. *et al*, Angewandte Chem. Int. Ed. 47,(32), 2008.
DNA gyrase	Manjunath a, Nuc. Acids Res. 33 (10) 2005.
Cyclopropane synthase	Glickman, MS; Mol. Cell, Vol. 5, April, 2000.
Glycosyltransferases	Lucas, R, Chem Bio Chem, 9, 2008.
Lysine ε-aminotransferase	Dube, D., Med. Chem. Res. 17(2-7) 2008

All recently identified targets and references listed above are enzymes of a wide variety of metabolic activity.

compound libraries can then be docked in the active site. Careful examination of the results will evaluate their binding capacities and allow the discovery of potential inhibitors. An alternate method can be to design inhibitors based on the blue print of the transition state structure. The strongest interaction between the enzyme and the substrate occurs at the transition state of a enzyme substrate reaction and therefore, though it is challenging to identify the intermediate transition state analogues, but it can be an alternative to design a strong binding affinity inhibitor(s) [46,47].

c) *Antimicrobial peptides*

Alveolar macrophages and lung epithelial cells are the first cells that encounter *M. Tb*. Respiratory secretions have microbicidal and microbiostatic properties mediated by their constituent antimicrobial peptides. á- and b-defensins, mainly produced by neuotrophil and various epithelial cells, have been well studied in linking the acquired and innate immunity. The expression of short peptide like defensin is tightly regulated by the NF-*ê*B and requires activation of Toll-like receptor 2 (TLR2)-mediated intracellular signaling pathway. Direct killing of bacilli by antimicrobial peptides as well as to the increased penetration of drugs into the mycobacterium present inside the macrophages, make antimicrobial peptides as potential candidates for the treatment of TB. The effect of combination of antimicrobial peptides and conventional anti-mycobacterial drugs (e.g. isoniazid and rifampicin) has been evaluated against *M. Tb* H37Rv *in vitro*, *ex vivo*, and *in vivo*, and it has been observed that the effective therapeutic dosage of conventional antiTB drugs could be reduced approximately to half by supplementing antimicrobial

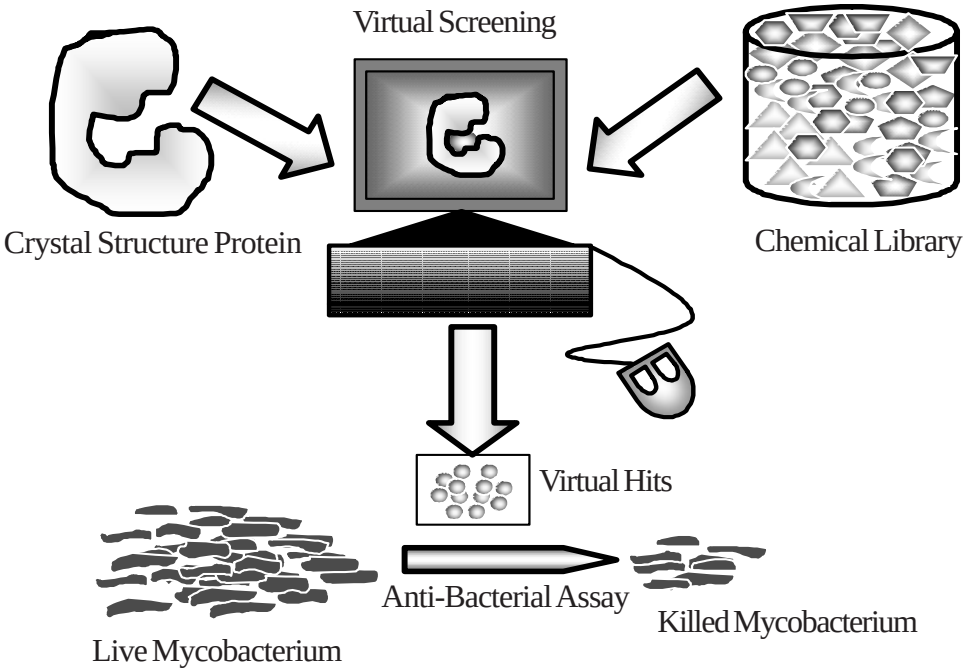

Figure 2: Virtual screening on chemical library using crystal structure of metabolically active proteins

Crystal structure of several crucial proteins of Mycobacterium origin can be used for virtual screening of chemical libraries with indication of antibacterial activity. Such a process, known as molecular docking, may be a novel approach to find out new potential molecules. Screened molecules catalogued based on the binding energy can be tested for antimycrobial activity.

peptides in the therapeutic schedule. Therefore, antimicrobial peptides can be used as adjunct chemotherapy with conventional drugs against human TB[48,49].

LEAD IDENTIFICATION

Monocyte infection Assay

Identification of hits using non-pathogenic mycobacterium should be tested on their pathogenic counterparts to check the efficacy of those compounds. The test is important for lead optimization and validation because several fundamental differences exist between pathogenic and non-pathogenic strains, particularly in metabolic pathways, membrane transportations, and regulation of gene expression as well as more prominently on macromolecules responsible for the pathogenicity. More importantly, all those factors may determine either independently or collectively the response of these organism against a particular molecule with therapeutic potential. For example, *M. Tb* has only five recognizable carbohydrate transporters in the inner membrane, while *M. smegmatis* has twentyeight such transporters at its disposal [50]**.** Similarly, it was found that protein tyrosine phosphatase ptpAt (a virulence factor for *M. tbs* and contributes to its survival within host macrophages) promoter is a highly active in slow-growing species of mycobacteria, such as *M. tb* and *M. bovis BCG*, but inert in fast-growing mycobacterial species, such as *M. smegmatis* [51]. Strong evidence is in favour of the fact that the nature of mycolic acid plays a crucial role in determining the fluidity and permeability of mycobacterial cell wall. *M.tb* modify their mycolic acids by cyclopropanation, whereas fast-growing saprophytic species *M.smegmatis* do not, indicating that this modification may be associated with the adaptation of this organism to oxidative stress[52]. *M. Tb* synthesizes three major types of cyclopropanated mycolic acids through the action of five putative homologous cyclopropane synthases and some of them have been shown to have mycolic acid cyclopropanating activity when introduced into *M. smegmatis*, do not produce cyclopropanated mycolic acids [31,53-54]. The biochemical and the macro molecular differences between the pathogenic and non-pathogenic mycobacterium create a major challenge for taking forward of non-pathogenic hits to the pathogenic test. All evidences, therefore, clearly indicate that compounds which show activity in the *M.smegmatis* may not necessarily work on *M.tb*. The interaction between macrophage and *M.Tb*. is a critical step in the establishment of an early chronic infection. Because of these obvious differences, there is a dire need to confirm the activity of the compounds in pathogenic *M.tb in vitro* using cell based assay such as Monocyte Infection Assay (MIA). MIA is a quick and very established assay model to understand infection mechanism *in vitro* by *M.tb*. Monocytes is a host of both HIV and Mycobacterium and it has been reported that after infection with *M.tb*, monocytes enhanced HIV-1 replication[55,56]. Asymptomatic mycobacterium infection can lead to the disease syndrome under immunodeficiency condition followed by HIV infection. Therefore, hits established in this model can be extrapolated from the single mycobacterium infection to the co-infection model system.

Patient PBMC assay

Efficacy of hits against mycobacterium can be tested further in *ex vivo* before they go through the animal proof of concept. Peripheral Blood Mononuclear Cell (PBMC) isolated from tubercular patients can be considered as a very good *ex vivo* model particularly in a country where TB is widely prevalent. Test on collections of wide variety of patient samples in different stages of the infection with different strains of mycobacterium (MDR or XDR) will reveal the efficacy of the compound in diverse spectrum of disease situation. Several studies in animal models indicate that a series of immuno pathological event happen followed by mycobaterium infection. For example, infected cells from active TB patients showed significant production of nitric oxide as compared to that of uninfected cells[57-59] **.** Elevation in the level of IFN-gamma is also observed in human PBMC infected with *M.Tb.* A gross downregulation of gene expression associated with innate and adaptive immunity are observed in animal model infected with *M.Tb*. A lower relative expression of key innate immunity related genes including the Toll-like receptor (TLR2,4) genes, lack of differential expression of

indicator adaptive immune gene transcripts (IFNG, IL2, IL4) and lower major histocompatibility complex class I (BOLA) and class II (BOLA-DRA) gene expression was consistent with innate immune gene repression in the BTB-infected animals[58]. This wide array of differential gene expression pattern will certainly influence the effect of the functionality of drugs in PBMC isolated from patients in comparison to the normal human counterpart. Therefore, hits tested on MIA followed by the patient PBMC assay before exploring the animal model system will be more informative and cost-effective.

Animal efficacy model

The murine model has been considered as a central tool for the elucidation of protective immune mechanisms that are essential for controlling *M. Tb* infection. Additionally, the study of inbred mice has revealed significant divergence in the susceptibility and disease progression of individual mouse strains to an infection with *M. Tb*[60-61]. The continued study of genetically disparate mouse strains has the potential to identify immune mechanisms that correlate with increasing susceptibility to TB. These mechanisms will be highly applicable to studies in men and will assist in the early detection of individuals that are more vulnerable to the development of reactivation of TB. Also murine models of TB have the advantages of low cost, availability of immunological reagents, and the choice of inbred populations with varying susceptibility to aerosol infection[62-64]. Murine models for TB give the power to detect the differences in duration of therapies. Therefore, considering all those facts, mouse remains the most popular *in vivo* model for testing small molecules for their potential.

DRUG DELIVERY

Research on drug delivery is mainly driven by the two major factors, one is the targeted delivery where drug will be navigated to the diseased tissue and second is sustained release formulation where drug will be released in a controlled fashion. An ideal TB drug formulation should satisfy both of those parameters so that bioavailability of the drug to the targeted tissue in one hand is upregulated and controlled and on the other hand uniform release of the drug will reduce the chance of developing MDR and XDR-TB cases. Liposome encapsulated drug delivery is one of the recent attempts to increase the bioavailability and tissue specific distribution of TB drugs. Single intravenous dose of modified liposomes, loaded with rifampicin and isoniazid, targeted to deliver drugs in alveoli, maintain the plasma/tissue drug level for five-seven days. A significant reduction in bacterial count was reported when mice were going through a weekly dose for six consecutive weeks in comparison to the same dose of free drug treatment. Chemotherapeutic activity against murine TB using once weekly administered drugs such as, isoniazid and rifampicin, encapsulated in liposomes, augment several fold the antibacterial activity of those two drugs[65]. Recent evidence shows that nanoparticle based drug delivery will replace all those existing methods because of high carrier capacity, stability, independence on the route of administration and feasibility of restricted release form the matrix. Frontline TB drugs like rifampicin, isoniazid and pyrazinamide show a significant effect when encapsulated with nanoparticles[66-68].

POST GENOMIC ERA AND SYSTEMS BIOLOGY IN DRUG DISCOVERY

Unveiling genome sequence of *M.tb* H37Rv in 1998 opens altogether a completely new horizon in the field of drug discovery. Though the bacilli harbour an estimated 4000 genes, about 52% of the predicted proteins have known functions and 376 putative proteins can be considered as unique to the organism because there is no apparent sequence homology with the existing data bases[69] . With the help of functional genomics, if some of them are detected as essential for the survival, then those macromolecules can be considered as a novel drug target for *M.tb*. Since the complete genetic information is also available for the non-pathogenic strains like *M.smegmatis* therefore, comparative genomics will provide a new outlook on the differential gene expression patterns across the strains. Comparison of gene expression arrays among the drug sensitive and drug resistance organism would reveal a significant clue regarding macromolecules responsible for the phenotype of

the organism and thus can generate new targets for anti-TB drug discovery[70-72]. Databases like GenoMycDB, comprise pair-wise sequence alignments of the protein coding sequences collected from five different pathogenic mycobacterium. A comparison of such data bases would be an added advantage to identify pathogen specific genes and can be used as a drug target for any particular class of mycobacterium[73] . Identification of genes supports the latency of the organism and can also have a significant implication in TB drug development. Recent studies on genes related to the latency of the organism identified isocitrate lyase and gene regulator ó-factor (SigF). Another study on amphibian TB model shows that genes with polymorphic PE-PGRS repeats are related to the granuloma formation, a stage associated with the chronic mycobacterium infection. Genes with the tandem PE-PGRS repeat encode a set of unique, exceptionally glycine and alanine rich protein located mostly on the surface of the mycobacterium and thought to be responsible for the pathogenesis. However, vaccination using those proteins failed to protect mice against TB infection most likely indicating the antigenic variation of PE-PGRS repeat genes[74]. Therefore, pathogenicity of mycobacterium does not solely depend on the regulation of a particular class of gene expression rather it may be a cumulative effect of several classes of stage specific gene expressions and thus justifies a highthoroughput global gene search to identify novel genes related to determine virulency and latency of the organism.

Recent introduction of systems biology in the field of drug discovery created a new niche to understand the overall regulatory network at the level of cells, tissues, organs and the organism. This emerging field has the potential to generate a specific molecular model as a target. Based on the computational modeling and predictive simulation, it will be very effective for identifying the control point of a molecular network which can be targeted for the drug discovery[75-77] . Integration of genome wide data on several molecules like mRNA, proteins and metabolites will provide a logical picture of their cellular behaviour. *M.tb* transcriptome complies the information of global analysis of gene expression under different conditions which would enable us to identify expression of stage specific gene and the products of those differentially expressed genes can also be considered as a subject of drug targets. Protein-protein interaction network, interactome, encompasses a variety of interaction like physical association of two proteins critical for the metabolic pathway or interaction needed to make biologically active complex. After analysis of the interaction network, Raman K *et al*[78] identified several critical drug targetable protein-protein interactions based on novel algorithm. One of the classical examples is the interaction of proteins drive the mycolic acid biosynthetic pathway. Presence of mycolic acid, arabinogalactan-mycolate covalently linked with peptidoglycan and trehalose dimycolate in the cell wall provides an extra protection to this organism to survive in the hostile phagolysosomal environment within macrophage, antibiotic treatment as well as help them to evade the host immune system. In silico analysis based on the recent biochemical as well as genetic information identifies InhA, AccD3, Fas, FabH, Pks13, DesA1/2, and DesA3 proteins are competent for the drug targets and it was also reported that FabH-InhA interaction is critical in the FAS-II biosynthetic pathway. A highthoroughput co-immunoprecipitation assay or Yeast-two-hybrid based assay, such as FRET assay can be very effective model for target assisted drug discovery. Therefore, system analysis of interaction network and global analysis of comparative gene expression using omics (Transcriptome, Reactome, Interactome) can highlight potential novel targets in TB drug discovery[79].

CONCLUSION

Development of new drugs in the field of TB continues to be more challenging because of the emergence of drug resistance organisms as well the cases of the opportunistic mycobacterium infection in HIV patients. On the other hand, crystal structures of proteins or enzymes, unrevealing mycobacterium genome sequence as well as systems biology have opened a new horizon in the field of drug discovery. Researchers, at this cross road, are facing an additional challenge in the field of the cost of new drug discovery, finding new therapy cheaper

than the existing treatment regimen and developing any new drug which will significantly reduce the treatment time. On the other hand, the molecular basis of drug discovery in the field of TB is very much in the rudimentary stage. In this review, focus was given on the molecular basis of drug discovery based on the recent information available in the field of molecular, cell and systems biology in the field of TB. As an example, a unique model of drug discovery based on protein-protein interaction was proposed where interacting proteins as well as the interaction is playing a critical role in the synthesis of cell surface molecule, mycolic acid, required for the pathogenesis of this organism. Target selection, conceptualized on the recent systems biology information, includes docking on unique enzymes related to pathogenicity such as cyclopropane synythetase, would be another potential model in the field of anti-TB drug discovery.

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REFERRENCES

1. Johnston, W. The Modern Epidemic: A history of TB in Japan. 432 pp. Volume 334:739,11 Cambridge, Mass., Harvard University Press, 1996 .
2. Collaborating Centre for Chronic Conditions. Tuberculosis Guidelines, Royal College of Physicians, London, 2006.
3. Laing RO, McGoldrick KM. Tuberculosis drug issues: prices, fixed-dose combination products and second-line drugs. *Int J Tuberc Lung Dis* 2000; **Dec;4(12 Suppl 2)**: S194-207.
4. Blomberg B, Fourie B. Fixed-dose combination drugs for tuberculosis: application in standardised treatment regimens. *Drugs* 2003; **63(6)**: 535-53.
5. Garner P, Alejandria M, Lansang MA. Is DOTS-Plus a Feasible and Cost-Effective Strategy? *PLoS Medicine* September, 2006; **3(9)**: e350.
6. WHO Report: Global tuberculosis control - surveillance, planning, financing, 2011.
7. Balganesh, TS., Balasubramanian, V. and Anand Kumar, S. Drug discovery in tuberculosis: Bottlenecks and pathforward. *Current Science* 2004; **86(1)**: 166-76.
8. Balganesh TS, Furr BJ. Molecular approaches to target discovery:evaluating targets for anti- tuberculosis drug discovery programmes. *Infect Disord Drug Targets* 2007; **7(2)**: 120-6.
9. Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet* 2012; **Jul**: 20.
10. A Krattiger, RT Mahoney, L Nelsen, *et al*. MIHR: Oxford, U.K., and PIPRA: Davis, U.S.A. . Reduced-Duration tuberculosis Treatment: Tuberculosis Alliance and Bayer HealthCare. In Executive Guide to Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices, 2007.
11. Havlir DV, Kendall MA, Ive P, *et al*. Timing of antiretroviral therapy for HIV-1 infection and TB. *N Engl J Med* 2011; **365**:1482.
12. Ginsberg AM. Drugs in development for TB. *Drugs* 2010 Dec 3; **70(17)**: 2201-14.
13. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, Venter A, Allen J, Palomino JC, De Marez T, van Heeswijk RP, Lounis N, Meyvisch P, Verbeeck J, Parys W, de Beule K, Andries K, Mc Neeley DF. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 2009 Jun 4; **360(23)**: 2397-405.
14. Laport, M. S.; Santos, O. C.; Muricy, G. "Marine sponges: Potential sources of new antimicrobial drugs". *Current pharmaceutical biotechnology* 2009; **10(1)**: 86-105.
15. Giddens, A. C., Nielsen, L., Boshoff, H. I., Tasdemir, D., Perozzo, R., Kaiser, M., Wang, F., Sacchettini, J. C., and Copp, B. R. Natural product inhibitors of fatty acid biosynthesis: synthesis of the marine microbial metabolites pseudopyronines A and B and evaluation of their anti-infective activities. *Tetrahedron* 2008; **64**: 1242-9.
16. Carballeiraa, NM. , Cruz, H., Kwong, CD., Wan, B and Franzblau, S. 2-Methoxylated fatty acids in marine sponges: Defence mechanism against mycobacteria? *Lipids* 2004; **39**: 675-80.
17. Spigelman MK. New TB therapeutics: a growing pipeline. *J Infect Diseas* 2007; **S28**: 196.
18. Copp BR, Pearce AN. Natural product growth inhibitors of *Mycobacterium tuberculosis*. *Nat Prod Rep* 2007; **24(2)**: 278-97.
19. Copp BR. Antimycobacterial natural products. *Nat Prod Rep* 2003; **20(6)**: 535-57.
20. Naesby M, Nielsen SV, Nielsen CA, Green T, Tange TO, Simón E, Knechtle P, Hansson A, Schwab MS, Titiz O, Folly C, Archila RE, Maver M, van Sint Fiet S, Boussemghoune T, Janes M, Kumar AS, Sonkar SP, Mitra PP, Benjamin VA, Korrapati N, Suman I, Hansen EH, Thybo T, Goldsmith N, Sorensen AS. Yeast artificial chromosomes employed for random assembly of biosynthetic pathways and production of diverse compounds in Saccharomyces cerevisiae. *Microb Cell Fact* 2009 Aug 13; **8**: 45.

21. Choia, K-J, Yub, YG, Hahnc, HG, Choid, J-D, Yoona, M-Y.Characterization of acetohydroxyacid synthase from *Mycobacterium tuberculosis* and the identification of its new inhibitor from the screening of a chemical library. *FEBS Letters* 2005; **579(2)1**: 4903-10.
22. Scherman, MS., Winans, KA., Stern, RJ., Jones, V., Bertozzi CR., and McNeil MR. Antimicrobial Agents and Chemotherapy, Drug Targeting *Mycobacterium TB* Cell Wall Synthesis: Development of a Microtiter Plate-Based Screen for UDP-Galactopyranose Mutase and Identification of an Inhibitor from a Uridine-Based Library. Antimicrob. *Agents Chemother* 2003; **47(1)**: 378-82.
23. Boyne ME, Sullivan TJ, amEnde CW, Lu H, Gruppo V, Heaslip D, Amin AG, Chatterjee D, Lenaerts A, Tonge PJ, Slayden RA. Targeting fatty acid biosynthesis for the development of novel chemotherapeutics against *Mycobacterium tuberculosis*: evaluation of A-ring-modified diphenyl ethers as high-affinity InhA inhibitors. *Antimicrob Agents Chemother* 2007; **51(10)**: 3562-7.
24. Eoh, H., Brennan, PJ., Crick, DC. The *Mycobacterium tuberculosis* MEP (2C-methyl-d-erythritol 4-phosphate) pathway as a new drug target. *Tuberculosis* 2009; **89(1)**: 1-11.
25. Hegymegi-Barakonyi, B., R. Szekely, Z. Varga, R. Kiss, G. Borbely, G. Nemeth, P. Banhegyi, J. Pato, Z. Greff, Z. Horvath, G. Meszaros, J. Marosfalvi, D. Eros, C. Szantai-Kis, N. Breza, S. Garavaglia, S. Perozzi, M. Rizzi, D. Hafenbradl, M. Ko, Y. Av-Gay, B. M. Klebl, L. Orfi & G. Keri. Signalling inhibitors against *Mycobacterium TB* - early days of a new therapeutic concept in tuberculosis. *Curr Med Chem* 2008; **15**: 2760-70.
26. Marrakchi H., Lanéelle G., and Quémard A. InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II have to find out. *Microbiology* 2000; **146**: 289-96.
27. Mendez, JC. Multi dug resistance in TB and the use of PCR for defining molecular markers of resistance. *Jacksonville Madicine* 2001; **52(2)**.
28. Sachdeva,S. and Reynolds, KA. *Mycobacterium TB* â-Ketoacyl Acyl Carrier Protein Synthase III (mtFabH) Assay: New Antibiotic Targets. Principles and Methods in Molecular Medicine, Vol. 142, 205-213 Edited by: W. Scott Champney © Humana Press Inc., Totowa, NJ.
29. Todd, SJ, Sullivan TJ, Truglio JJ, Boyne ME, Novichenok P, Zhang X, Stratton CF, Li HJ, Kaur T, Amin A, Johnson F, Slayden RA, Kisker C, Tonge PJ. High affinity InhA inhibitors with activity against drug-resistant strains of *Mycobacterium tuberculosis. ACS Chem Biol* 2006; **1(1)**: 43-53.
30. Glickman MS., Cox JS. and Jacobs Jr. WR. A Novel Mycolic Acid Cyclopropane Synthetase Is Required for Cording, Persistence, and Virulence of *Mycobacterium tuberculosis. Mol Cell* 2000; **5**: 717-27.
31. Alahari A, Trivelli X, Guérardel Y, Dover LG, Besra GS, Sacchettini JC, Reynolds RC, Coxon GD, Kremer L. Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. *PLoS ONE* 2007; **2(12)**: e1343.
32. Manabe YC, Bishai WR. Latent *Mycobacterium tuberculosis*-persistence, patience, and winning by waiting. *Nat Med* 2000; **6(12)**.
33. Purohit HJ, Cheema S, Lal S, Raut CP, Kalia VC. In Search of Drug Targets for *Mycobacterium TB*. *Infect Disord Drug Targets* 2007; **(3)**: 245-50.
34. zu Bentrup KH., and. Russell DG. Mycobacterial persistence: adaptation to a changing environment. *Trends in Microbiol* 2001; **9(12)1**: 597-605.
35. DiMasi JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. *J Health Econ* 2003; **22(2)**: 151-85.
36. Cunningham, A. F., and C. L. Spreadbury. Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. *J Bacteriol* 1998; **180**: 801-08.
37. Jacobs, W. R., Jr., M. Tuckman, and B. R. Bloom.. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. *Nature* 1987; **327**: 532-5.
38. Smeulders, M. J., J. Keer, R. A. Speight, and H. D. Williams. Adaptation of Mycobacterium smegmatis to stationary phase. *J Bacteriol* 1999; **181**: 270-83.
39. Chaturvedi V, Dwivedi N, Tripathi RP, Sinha S.J. Evaluation of Mycobacterium smegmatis as a possible surrogate screen for selecting molecules active against multi-drug resistant *Mycobacterium tuberculosis*. *Gen Appl Microbiol* 2007; **53(6)**: 333-7.
40. Wang, R. and Marcotte, EM. The Proteomic Response of Mycobacterium smegmatis to Anti- tuberculosis Drugs Suggests Targeted Pathways. *J Proteome Res* 2008; **7(3)**: 855-65.
41. Andries, K., Verhasselt, P., Guillemont, J., Göhlmann, H. W. H.,Neefs, J-M., Winkler, H., Van Gestel, J., Timmerman, P.,Zhu, M., Lee, E., Williams, P., de Chaffoy, D., Huitric, E.,Hoffner, S., Cambau, E., Truffot-Pernot, C., Lounis, N., and Jarlier, V. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005; **307**: 223-7.
42. Lu, T. and Drlica, K. *In vitro* activity of C-8-methoxy fluoroquinolones against mycobacteria when combined with anti- tuberculosis agents. *J Antimicrob Chemother* 2003; **52**:1025-8.
43. Murdock, K. C. Lin,Y.,Thomas, J.P., and Langs, S.A., Jr. Antitubercular 2,8- bis(alkylaminomethyl)phenazines. *J Med Chem* 1978; **21**: 403-05.
44. Fry DC. Current Trends in Peptide Science. Protein-protein interactions as targets for small molecule drug discovery. *Peptide Science* 2006; **84(6)**: 535-52.
45. Mawuenyega, K.G., Forst, C.V., Dobos, K.M., Belisle, J.T., Chen, J., Bradbury, E.M., Bradbury, A.R. and Chen, X. *Mycobacterium tuberculosis* functional network analysis. *J Bacteriol* 2000; **182(20)**: 5771-8.
46. Tuck K.L. Chemical biology: tools for advancing drug design. *Chemistry in Australia* 2005; **72(10)**: 7-9.
47. Deschamps, JR. The Role of Crystallography in Drug Design. *AAPS Journal* 2005; **7(4)**: Article 78.
48. Ganz T. Antimicrobial polypeptides in host defense of the respiratory tract. *J Clin Invest* 2002; **109**: 693-7.

49. Me´ndez-Samperio P. Role of antimicrobial peptides in host defense against mycobacterial infections. *Peptides* 2008; **29(10)**:1836-41.
50. Niederweis, M. Nutrient acquisition by mycobacteria. *Microbiology* 2008; **154**: 679-92.
51. Lei J, Zhang H, Wu C, Wang X, Yang Y, Zhang X, Huang Y, Wang H. The Influence of *Mycobacterium tuberculosis* sigma factors on the promotion efficiency of ptpAt promoter in Mycobacterium smegmatis. *Curr Microbiol* 2005; **51(3)**: 141-7.
52. Yuan Y, Lee RE, Besra GS, Belisle JT, Barry CE 3rd. Identification of a gene involved in the biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 1995 Jul 3; **92(14)**: 6630-4.
53. Rao V, Gao F, Chen B, Jacobs WR Jr, Glickman MS. Trans-cyclopropanation of mycolic acids on trehalose dimycolate suppresses. *Mycobacterium tuberculosis* - induced inflammation and virulence. *J Clin Invest* 2006; **116(6)**: 1660-7.
54. Glickman MS, Cox JS, Jacobs WR Jr. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of Mycobacterium TB. *Mol Cell Apr* 2000; **5(4)**: 717-27.
55. Collins K.R., Quinones-*Mateu* M.E., Toossi Z., Arts E.J. Impact of tuberculosis on HIV-1 replication, diversity and disease progression. *AIDS Rev* 2002; **4**: 165-76.
56. Goletti D, Carrara S, Vincenti D, Giacomini E, Fattorini L, Garbuglia AR,Capobianchi MR, Alonzi T, Fimia GM, Federico M, Poli G, Coccia E. Inhibition of HIV-1 replication in monocyte-derived macrophages by Mycobacterium. *J Infect Dis* 2004; **189(4)**: 624-33.
57. Singh R, Manjunatha U, Boshoff HI, Ha YH, Niyomrattanakit P, Ledwidge R, Dowd CS,Lee IY, Kim P, Zhang L, Kang S, Keller TH, Jiricek J, Barry CE 3rd. PA-824 kills non-replicating *Mycobacterium tuberculosis* by intracellular NO release. *Science* 2008; 28 **2(5906)**: 337-8.
58. Sharma, S., Sharma, M., and Bose M. *Mycobacterium tuberculosis* infection of human monocyte-derived macrophages leads to apoptosis of T cells. *Immunology and Cell Biology* 2009; **87**: 226-34.
59. Voskuil MI., Schnappinger D., Visconti KC., Harrell MI., Dolganov GM., Sherman DR., and Schoolnik GK. Inhibition of Respiration by Nitric Oxide Induces a *Mycobacterium tuberculosis* Dormancy Program. *J Exp Med* 2003; **198(5)**: 705.
60. Baldwin SL, D'Souza C, Roberts AD, Kelly BP, Frank AA, Lui MA, Ulmer JB, Huygen K, McMurray DM, Orme IM. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. *Infect Immun* 1998 Jun; **66(6)**: 2951-9.
61. Beamer GL, Turner J. Murine models of susceptibility to tuberculosis. *Arch Immunol Ther Exp* (Warsz) 2005 Nov-Dec; **53(6)**: 469-83.
62. Turner OC., Basaraba RJ., and Orme IM. Immunopathogenesis of Pulmonary Granulomas in the Guinea Pig after Infection with *Mycobacterium tuberculosis. Infect Immun* 2003; **71(2)**: 864.
63. McMurray DN. Disease model: pulmonary tuberculosis. *Trends Mol Med* 2001; **7(3)**: 135-7.
64. Orme, I. M., McMurray, D. N. & Belisle, J. T. Tuberculosis vaccine development: recent progress. *Trends Microbiol* 2001; **9**: 115-8.
65. Khuller GK, Pandey R. Sustained release drug delivery systems in management of *tuberculosis*. *Indian J Chest Dis Allied Sci* 2003; **45(4)**: 229-30.
66. Labana S, Pandey R, Sharma S, Khuller GK. *Int J Antimicrob Agents* 2002; **20** :301-04.
67. Gaspar MM, Cruz A, Fraga AG, Castro AG, Cruz ME, Pedrosa J. Developments on drug delivery systems for the treatment of mycobacterial infections. *Curr Top Med Chem* 2008; **8(7)**: 579-91.
68. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. *Am J Respir Crit Care Med* 2005; **172(12)**: 1487-90.
69. Catanho M, Mascarenhas D, Degrave W, Miranda AB. GenoMycDB: a database for comparative analysis of mycobacterial genes and genomes. *Genet Mol Res* 2006; **5**: 115-26.
70. Arcusa VL., Lottb JS., Johnstonb JM and Bakerb EN. The potential impact of structural genomics on TB drug discovery. *Drug Discovery Today* 2006; **11(1-2)**: 28-34.
71. Hasan S, Daugelat S, Rao PS, Schreiber M. Prioritizing genomic drug targets in pathogens: application to *Mycobacterium tuberculosis*. *PLoS Comput Biol* 2006 9; **2(6)**: e61.
72. Ioerger TR, Sacchettini JC. Structural genomics approach to drug discovery for *Mycobacterium tuberculosis*. *Curr Opin Microbiol* 2009; **28**.
73. Cole ST. Comparative mycobacterial genomics as a tool for drug target and antigen discovery. *Eur Respir J Suppl* 2002; **36**: 78s-86s.
74. Tian C, Jian-Ping X. Roles of PE_PGRS family in *Mycobacterium tuberculosis* pathogenesis and novel measures against tuberculosis. *Microb Pathog* 2010 Dec; **49(6)**: 311-4.
75. Young D, Stark J, Kirschner D. Systems biology of persistent infection: Tuberculosis as a case study. *Nat Rev Microbiol* 2008; **6(7)**: 520-8.
76. Kumble, K.D. Anti-bacterial drug discovery using systems biology. *Mini Rev Med Chem* 2006; **6(11)**: 1275-83.
77. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB. Tuberculosis drug resistance mutation database. *PLoS Med* 2009 10; **6(2)**: e2.
78. Raman K, Rajagopalan P, Chandra N. Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. *PLoS Comput Biol* 2005; **1(5)**: e46.
79. Raman K, Chandra N. *Mycobacterium tuberculosis* interactome analysis unravels potential pathways to drug resistance. *BMC Microbiol* 2008; **23(8)**: 234.